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Passaging Methods for hPSCs Under Feeder-Free Conditions

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Abstract

From basic science to translational medicine, human pluripotent stem cells (hPSCs) are a valuable yet delicate resource. Unlike their robust mouse counterparts, hPSCs are highly susceptible to cell death and differentiation. Research shows that hPSC culture conditions and dissociation methodology significantly impact cellular characteristics (i. e., growth rate), and potential in downstream applications (i. e., differentiation capacity). In addition, the long-term goal of therapeutic stem cell use requires special consideration, including culture scalability and avoidance of xenogenic-factors.

Introduction

hPSCs have been shown to grow well when supported by paracrine factors found either in fetal bovine serum, or secreted from mitotically inactive mouse or human fibroblasts, known as a "feeder layer"[1,2]. However, variability in these animal-derived products necessitated the development of feeder-free culture systems, which rely on chemically defined basal media, recombinant growth factors, and components of extracellular matrix to support growth and adhesion of hPSCs[3]. Matrigel® is a widely used and referenced hPSC culture substrate for feeder-free culture. However it contains xenogenic factors and is not suitable as a clinical-grade cell culture compound [4,5]. Other products of solely human origin like purified recombinant matrix proteins (i.e., Vitronectin ACF, cat.# 05-754-0002, and LaminStem™ 521, cat.# 05-753-1F) are excellent alternatives for feeder-free hPSC culture.

To facilitate nutrient availability, continued growth, and expansion of hPSC lines under feeder-free conditions, large colonies require routine dissociation and replating. This process, known as passaging or subculturing, can be performed through a variety of well-defined methods. Large aggregate passaging utilizes manual dissociation, alone or in combination with enzymes, such as dispase and collagenase. Small aggregate passaging utilizes non-enzymatic solutions like EDTA, while trypsin-based products or non-enzymatic reagents can be used in combination with apoptosis inhibitors to facilitate single cell dissociation. The choice of dissociation methodology often varies, depending on downstream experiments. Factors including experiment length, scalability, and cost must be balanced with overall culture health.

Materials

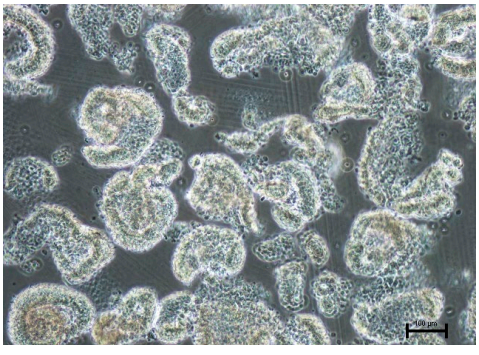
Product	Cat. #	Size
NutriStem® hPSC XF Medium	05-100-1A	500 mL
Vitronectin ACF	05-754-0002	200 µg
0.5M EDTA solution	01-862-1B	100 mL
Recombinant Trypsin EDTA Solution	03-079-1B	100 mL
LaminStem™ 521	05-753-1F	1 mL

Passaging Methods

Passaging hPSCs as Large Aggregates

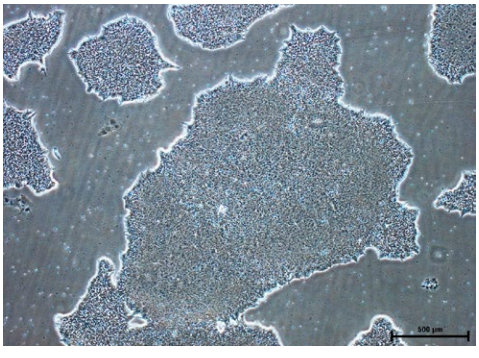
Passaging of hPSC as large aggregates was once considered standard practice for hPSC culture. Although cellular damage and death occur (<50% viability), this method maintains healthy cultures and preserves, normal karyotype, pluripotency markers, and differentiation capacity for numerous passages. To generate large aggregates, a skilled technician may manually remove differentiated colonies by micro-dissection. The remaining colonies are scored and | or scraped with a glass pipette and collected via gentle centrifugation. Manual passaging of large aggregates can also be combined with enzymatic dissociation using dispase or collagenase (recommended at a concentration of 1 mg/mL in medium) to facilitate colony detachment and increase the yield of viable cells for expansion[7]. While these methods are compatible with Matrigel®, or other protein matrices, they cannot be used on cultures grown in large-scale, multi-layer plates. As such, large aggregate passaging is most commonly used for low-throughput research experiments. Large aggregates are also poorly suited for transfection experiments, where access to the cell surface facilitates efficient transfer of plasmids or small RNAs.

Figure 1: *hPSCs Cultured in NutriStem® hPSC XF Medium and Passaged as Large Aggregates (Clumps)*



Note. Image shows cells at 100 × magnification

Figure 2: *Typical Recovery of hPSCs From Collagenase Dissociation (Day 3 Post-Passage)*

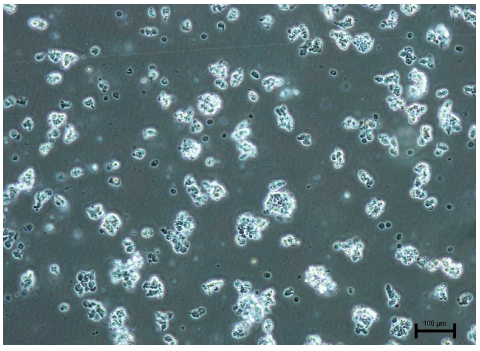


Note. Image shows cells at 40 × magnification

Passaging hPSCs as Small Aggregates (Enzyme-Free Passaging)

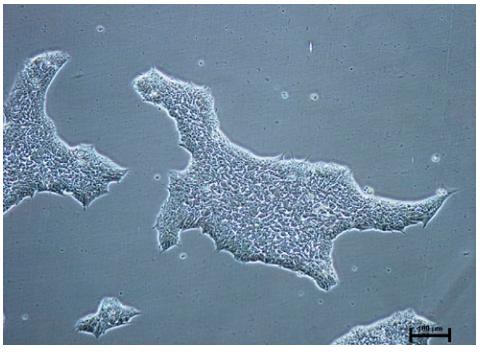
In contrast to large aggregate and single cell methods for hPSC dissociation, small aggregate dissociation is a gentle, enzyme-free method of passaging cells grown in feeder-free conditions. 0.5 mM EDTA (cat.# 01-862-1B) mediates rapid cell dissociation by chelating the calcium and magnesium ions that facilitate cell adhesion. While this method is associated with high cell viability (>60%), the use of high concentrations or extended incubation periods will result in the generation of single cells, followed by apoptosis. Studies suggest aggregates between 50–100 µm in size are optimal for maintaining cell-to-cell connections and balancing efficiency of transfection or other the experimental methods. Because EDTA has a high affinity for calcium ions, careful timing of dissociation with EDTA is critical.

Figure 3: *hPSCs Cultured in NutriStem® hPSC XF Medium and Passaged as Small Aggregates (Mini Clumps)*



Note. Image shows cells at 40× magnification

Figure 4: *Typical Recovery of hPSCs From Enzyme-Free Dissociation Using 0.5 mM EDTA Solution (Day 3 Post-Passage)*

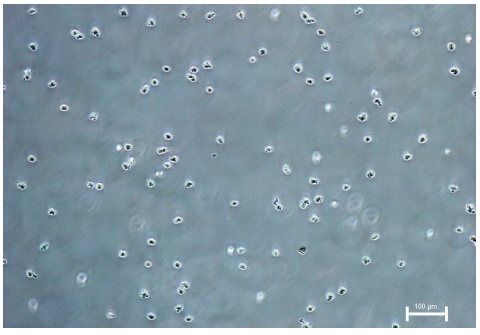


Note. Image shows cells at 40× magnification

Passaging hPSCs as Single Cells

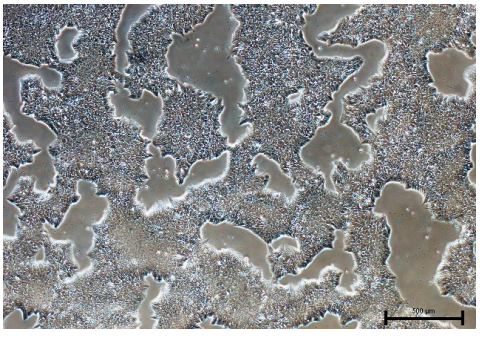
With the growth of genetic manipulation of cells, genetic editing, and CRISPR/Cas9 applications, single cell passaging is rising. The use of entirely dissociated single-cell suspension for passaging generates a monolayer culture that benefits from higher culture scalability, rapid expansion, and high efficiency. The introduction of recombinant protein matrices (i.e., LaminStem™ 521, #cat 05-753-1) and recombinant trypsin solutions (i.e., Recombinant Trypsin EDTA Solution, cat.# 03-079-1) now support the expansion of hPSCs in a monolayer. This makes single-cell passaging more efficient and viable (without the addition of ROCKi) while maintaining cell integrity and characteristics. With the support from the laminin-521 matrix, cells can be passaged at very low densities and can be cultured to high confluence without phenotypic alterations[6]. Laminin-521 also supports efficient clonal culture and is an excellent substrate for iPSC reprogramming. When used with NutriStem® hPSC XF medium (cat.# 05-100-1A), LaminStem™ 521 has been proven to promote cellular survival and expansion of hPSCs after plating from a single-cell suspension. When cultured with LaminStem™ 521, hPSCs grow as a monolayer and remain pluripotent without spontaneous differentiation.

Figure 5: *hPSCs Cultured in NutriStem® hPSC XF Medium and Passaged as Single Cells*



Note. Image shows cells at 100× magnification

Figure 6: *Typical Recovery of hESCs From Single-Cell Passage Using Recombinant Trypsin EDTA Solution (Day 4 Post-Passage)*



Note. Image shows cells at 40× magnification

Conclusion

Comparison Table

Dissociation Method	Passaging Large Aggregates	Passaging Small and Large Aggregates	Passaging Small Aggregates	Single Cell Passaging
Cell Dissociation Reagent	Collagenase	Accutase	0.5 M EDTA solution	Recombinant Trypsin EDTA Solution
Intended Matrix	▪ Matrigel®	▪ Matrigel®	▪ Matrigel® ▪ Vitronectin ACF	▪ Laminstem™ 521
Benefits	Widely referenced and well established dissociation method	Gentle dissociation method	▪ High cellular viability ▪ Non-enzymatic dissociation reagent ▪ Cost effective ▪ Does not require ROCK inhibitor	▪ Animal component free dissociation reagent ▪ Does not require ROCK inhibitor
Drawbacks	Animal-derived dissociation reagent (lot-to-lot variability)	Animal-derived dissociation reagent (lot-to-lot variability)		
Detach time	5–10 min.	5–45 min.	Time sensitive: only 3–4 min. detach time	Time-sensitive: only 2–4 min. detach time
Recommended culture medium	NutriStem® hPSC XF Medium	NutriStem® hPSC XF Medium	NutriStem® hPSC XF Medium	NutriStem® hPSC XF Medium

References

[1] Thomson, J. A., J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, and J. M. Jones. 1998. "Embryonic Stem Cell Lines Derived from Human Blastocysts." Science 282 (5391): 1145–47.

[2] Richards, Mark, Chui-Yee Fong, Woon-Khiong Chan, Peng-Cheang Wong, and Ariff Bongso. 2002. "Human Feeders Support Prolonged Undifferentiated Growth of Human Inner Cell Masses and Embryonic Stem Cells." Nature Biotechnology 20 (9): 933–36.

[3] Lambshead, Jack W., Laurence Meagher, Carmel O'Brien, and Andrew L. Laslett. 2013. "Defining Synthetic Surfaces for Human Pluripotent Stem Cell Culture." Cell Regeneration (London, England) 2 (1): 7.

[4] Kleinman, Hynda K., Mary L. McGarvey, Lance A. Liotta, Pamela Gehron Robey, Karl Tryggvason, and George R. Martin. 1982. "Isolation and Characterization of Type IV Procollagen, Laminin, and Heparan Sulfate Proteoglycan from the EHS Sarcoma." Biochemistry 21 (24). American Chemical Society: 6188–93.

[5] Xu, C., M. S. Inokuma, J. Denham, K. Golds, P. Kundu, J. D. Gold, and M. K. Carpenter. 2001. "Feeder-Free Growth of Undifferentiated Human Embryonic Stem Cells." Nature Biotechnology 19 (10): 971–74.

[6] Rodin, Sergey, Anna Domogatskaya, Susanne Ström, Emil M. Hansson, Kenneth R. Chien, José Inzunza, Outi Hovatta, and Karl Tryggvason. 2010. "Long-Term Self-Renewal of Human Pluripotent Stem Cells on Human Recombinant Laminin-511." Nature Biotechnology 28 (6): 611–15.

[7] Loring, Jeanne Frances, and Suzanne Peterson. 2012. Human Stem Cell Manual: A Laboratory Guide. Academic Press.

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