BioResearch

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Lonza L7™ hPSC Culture System
for Research and Future Clinical Applications

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Introduction

Chemically defined and feeder-independent cell culture systems have provided a superior platform for reproducibility and standardization of human pluripotent stem cell (hPSC)-based research. As the field advances towards potential clinical applications involving hPSC-derived cell progenies, hPSC culture systems compliant with regulatory standards are necessary. Lonza has developed a robust xeno-free and defined system that could be translated for use in GMP and clinical-grade manufacturing. L7™ hPSC Culture System is a culture platform (medium, matrix, passaging solution and cryosolution) that supports every-other-day feeding of hPSCs. We evaluated Lonza L7™ hPSC Culture System for maintenance and expansion of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs).

Methods

Human ESCs and hiPSCs[1] were maintained in L7™ hPSC Medium and passaged every 5–7 days using L7™ hPSC Passaging Solution. Dissociated hPSC colonies were seeded on L7™ hPSC Matrix. After four passages, molecular characterization for pluripotency was performed using immunostaining and FACS. The differentiation potential of hPSCs into three primary germ lineages was characterized through embryoid bodies (EBs)[2].

Advantages of L7™ hPSC Passaging Solution

1. Attachment ratio post-passaging is high (>90% cell aggregates attach in 16 hours)
2. High post-detachment viability (>90%)
3. Generates uniform-sized aggregates
4. Increased split ratios (1:8 to 1:10)

Results

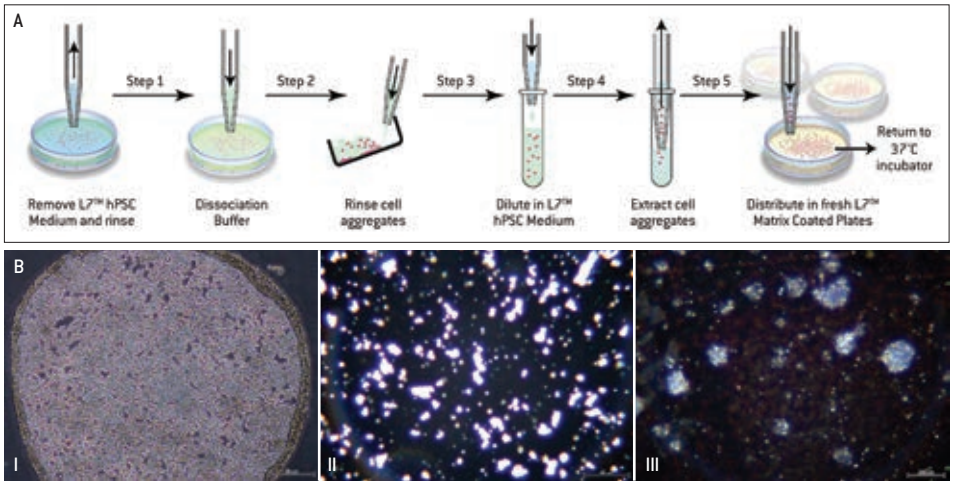

Figure 1
(A) Schematic representation of dissociation protocol using L7™ hPSC Passaging Solution. (B) Phase contrast images showing stages of hPSC after addition of L7™ hPSC Passaging Solution for 5 minutes (I) note disruption of hPSC colonies (II) uniform small cell aggregates after Step 2 (III) and colonies after 16 hours of seeding on a fresh L7™ hPSC Matrix Coated Plate in L7™ hPSC Medium.

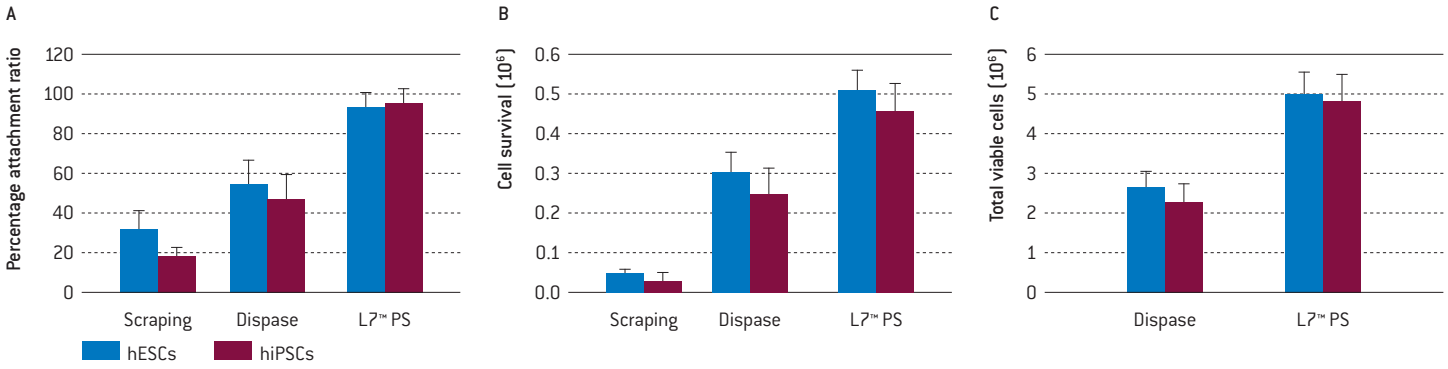

Figure 2
(A) Graph shows the percentage attachment ratio of cell aggregates that attach at 16 hours post passaging using various methods. Note the marked difference between the three methods. (B) Graph represents number of viable cells after 24 hours of passaging with various methods. (C) Graph shows the total viable cells after 6 days of culture, comparing two different passaging methods. Data presented are mean ± s.e.m. of three experiments. Abbreviation: L7™ PS = L7™ hPSC Passaging Solution.

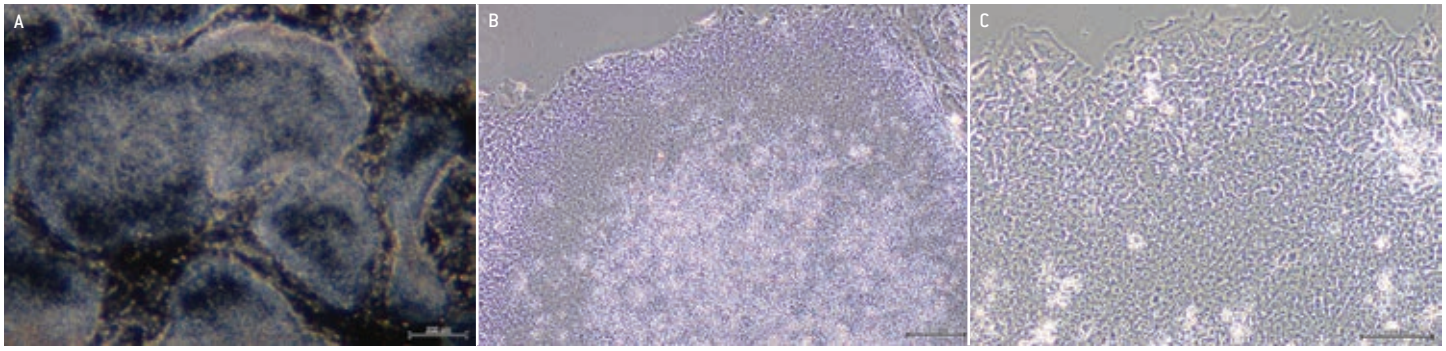

Figure 3
(A) Low magnification of hPSC culture on day 6, ready for passaging. (B) Phase contrast image of an hPSC colony showing compactness. (C) Magnified phase contrast image of hPSCs maintained in L7™ hPSC Medium. Note hPSCs show high nucleus to cytoplasm ratio and are highly compact in morphology.

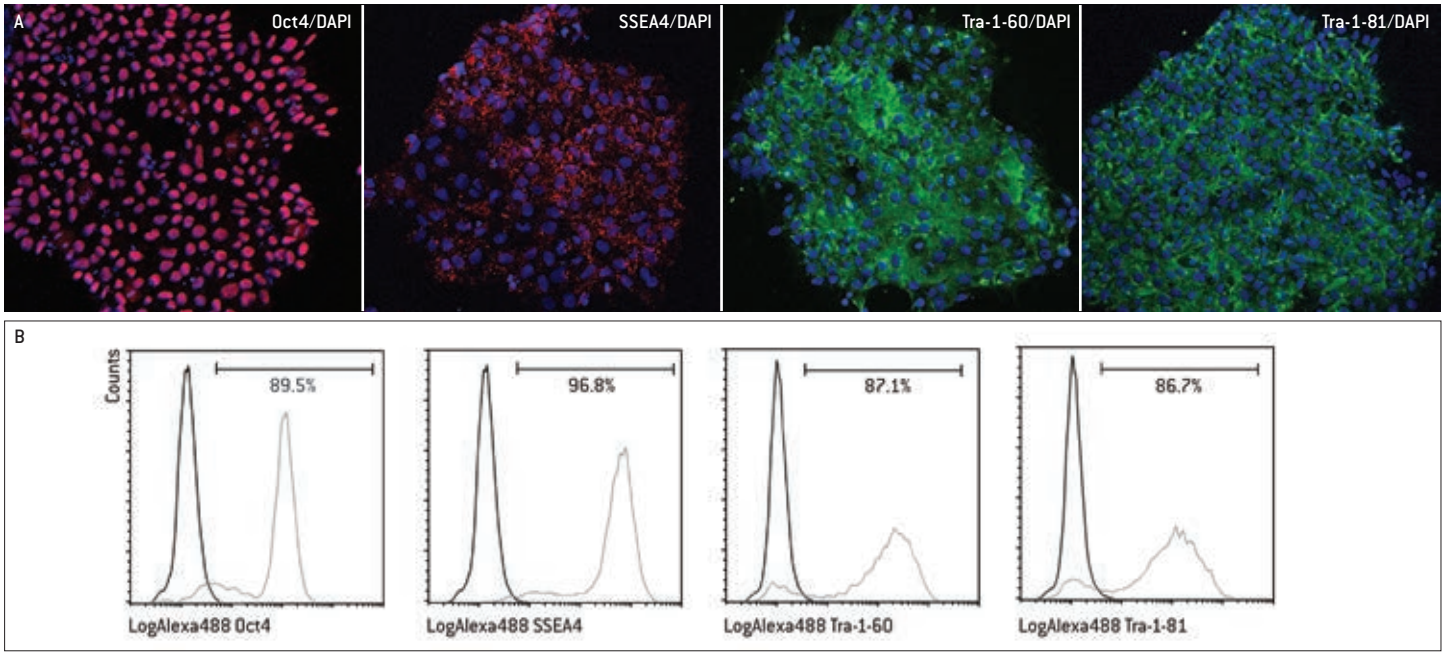

Figure 4
(A) Immunostaining of hPSC colonies grown on L7™ hPSC Medium in combination with L7™ hPSC Matrix exhibiting pluripotency markers, Oct4, SSEA4, Tra-1-60 and Tra-1-81. Scale bar: 100 µm. (B) Flow cytometric analyses demonstrate more than 85% cells express pluripotency markers. A total of 10,000 events were counted and evaluated.

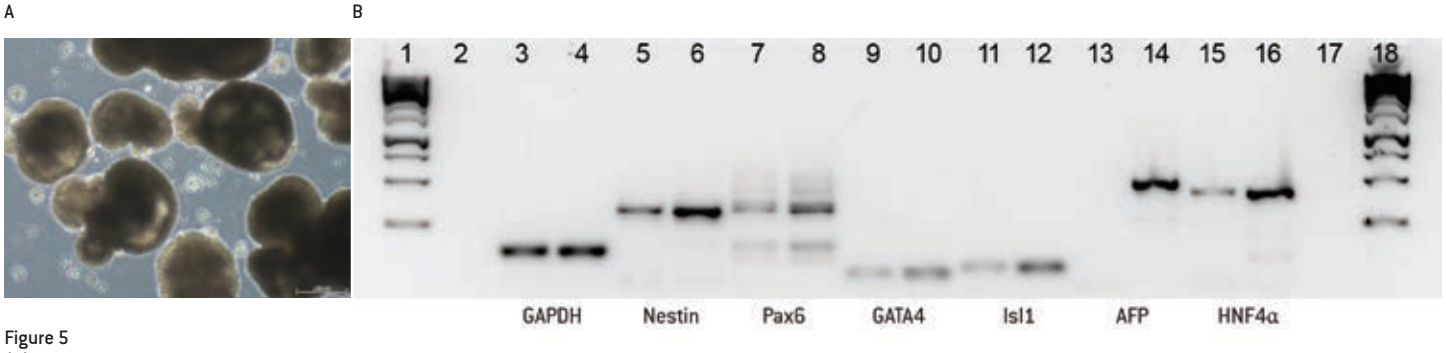

Figure 5
(A) Phase contrast image of 5-day-old EBs showing ability of hPSCs grown in L7™ hPSC Culture System for differentiation. (B) Semi-quantitative RT-PCR for two lines showing the ability of the cells to differentiate into three germ layers, ectoderm (Nestin-lane 5, 6; Pax6-lane 7, 8), mesoderm (GATA4-lane 9, 10; Isl1-lane 11, 12) and endoderm (AFP-lane 13, 14; HNF4α-lane 15, 16), with GAPDH (lane 3, 4) as housekeeping gene. hiPSC line (lane 3, 5, 7, 9, 11, 13, 15) and hESC line (lane 4, 6, 8, 10, 12, 14, 16). Molecular weight ladder (lane 1, 18)

Conclusion

We evaluated L7™ hPSC Culture System to passage 4 and found it to be a robust and efficient system for expansion and growth of hPSCs in a xeno-free, regulatory-compliant environment for use in both research and clinical applications involving hPSC-derived cell derivatives. For long-term data, please see ISSCR poster # T-2173, "Robust Generation and Maintenance of Human Induced Pluripotent Stem Cells Under Defined Conditions".

References

1. Mehta A et al., Cardiovasc Res. 2011; 91: 577–586.
2. Mehta A et al., Toxicol Sci. 2013; 131: 458–469.

Acknowledgements

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