Expansion of High-Quality Human Pluripotent Stem Cells (hPSCs) Using a Novel Animal Origin-Free and Stabilized hPSC Maintenance Medium

Kimberly A. Snyder[1], Olivia J. Neef[1], Thuy T. Hoang[1], Allen C. Eaves[1,2], Sharon A. Louis[1], and Arwen L. Hunter[1]

[1]STEMCELL Technologies Inc., Vancouver BC, Canada; [2]Terry Fox Laboratory, BC Cancer, Vancouver BC, Canada

INTRODUCTION

As the number of clinical trials and approved therapies in the field of regenerative medicine increases, it is important to ensure that hPSC culture media are not only compliant with current required manufacturing and quality control processes but also ease the path toward regulatory approval. To simplify traceability and viral safety concerns, we have developed an animal origin-free (AOF) hPSC maintenance medium—TeSR™-AOF—using animal origin-free raw materials with traceability to the secondary level of manufacturing. hPSCs require specialized culture media to promote expansion while maintaining self-renewal and pluripotency. TeSR™-AOF, based on the TeSR™ formulations, was developed to ensure versatile feeding schedules while maintaining high-quality hPSC cultures. We investigated key cell quality parameters of hPSCs cultured for at least 10 passages in TeSR™-AOF, and found that hPSCs cultured in TeSR™-AOF have higher expansion and plating efficiency without affecting cell quality or downstream applications.

METHODS

Human embryonic stem (ES) cells (H9 & H1) and induced pluripotent stem (iPS) cells (STiPS-F016 & STiPS-M001) were cultured on Vitronectin XF™ for up to 10 passages in TeSR™-AOF with restricted feeding schedules or in TeSR™-E8™ with daily feeding. In addition, H9 ES cells were maintained on Corning® Matrigel® for 10 passages in the above conditions. hPSC cultures were passaged as clumps using ReLeSR™ passaging reagent on a 6- or 7-day passaging schedule.

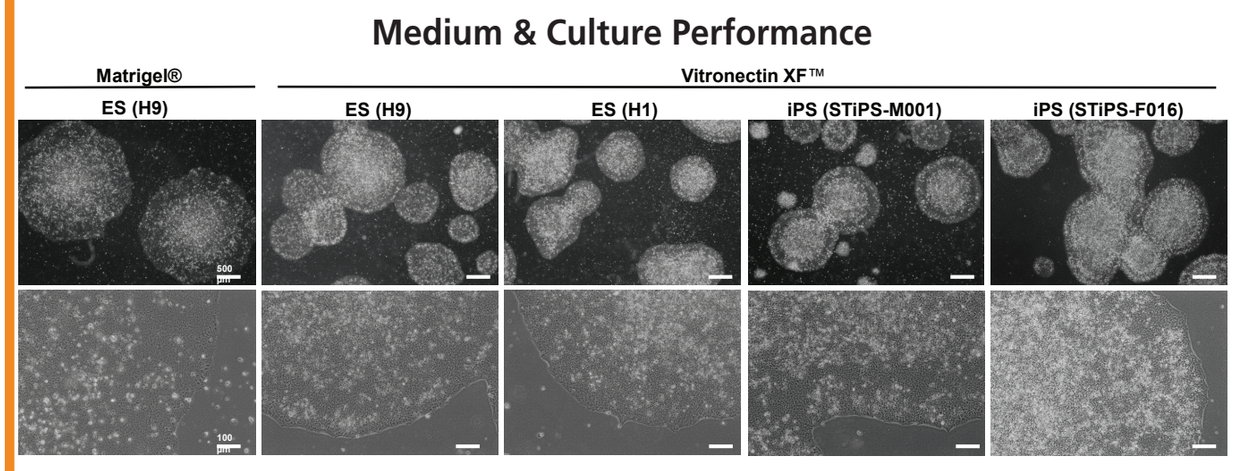


FIGURE 1. hPSCs Cultured in TeSR™-AOF with Restricted Feeding Maintain Excellent Colony Morphology

hPSCs maintained in TeSR™-AOF exhibit hPSC-like morphology forming densely packed, round colonies with smooth edge morphology. Homogeneous cell morphology characteristic of hPSCs are observed, including large nucleoli and scant cytoplasm.

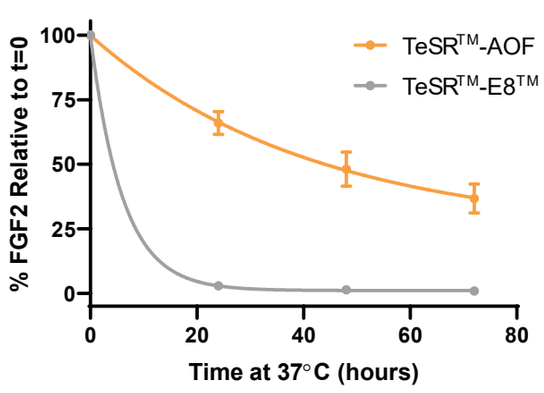
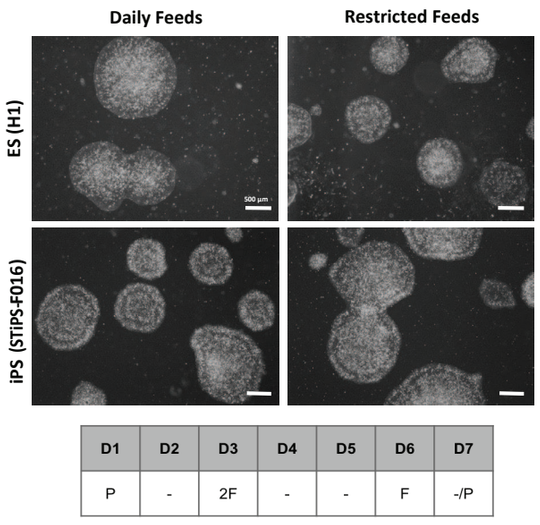


FIGURE 2. Native bFGF Levels are Stabilized at 37°C in TeSR™-AOF

TeSR™-AOF and TeSR™-E8™ were incubated at 37°C for 24, 48, and 72 hours. FGF2 levels were measured by Meso Scale Discovery (MSD) immunoassay; data were normalized to t = 0 levels for TeSR™-E8™ and TeSR™-AOF, respectively. FGF2 levels in TeSR™-AOF remain at 36.7 ± 5.61% of t = 0 levels at 72 hours when incubated at 37°C. Data representative of n = 3 biological replicates ± SD.



D1	D2	D3	D4	D5	D6	D7
P	-	2F	-	-	F	-/P

FIGURE 3. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Exhibit Comparable Colony Morphology

hPSCs were maintained on Vitronectin XF™ for five passages. Phase-contrast images were taken on day 7 after seeding. For restricted feeds, hPSCs were fed with a double volume (4 mL) of medium on day 2 after passage, followed by two consecutive skipped days of feeds, with a final single-volume feed (2 mL) on day 5, prior to passaging on day 6 or 7.

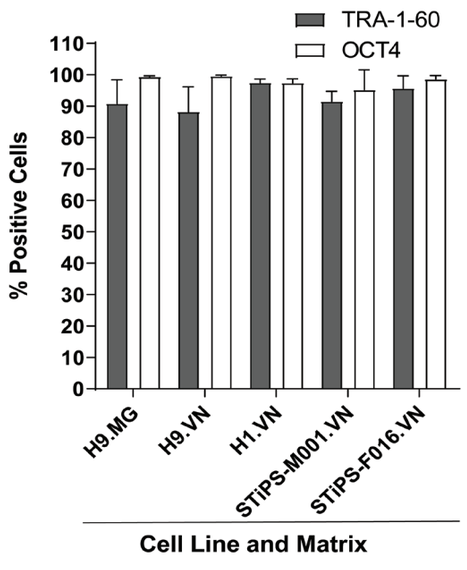


FIGURE 4. hPSCs Cultured in TeSR™-AOF Express Markers of the Undifferentiated State

hPSCs maintained in TeSR™-AOF exhibit high levels of TRA-1-60 and OCT4 by flow cytometry at passage 5 and 10. Across n = 4 cell lines, the average TRA-1-60 expression was 92.8 ± 3.77%, and percent OCT-4 positive cells were 98.1 ± 1.79%. Data shown represent an average of passage 5 and 10 flow results for each cell line. MG = Matrigel®; VN = Vitronectin XF™.

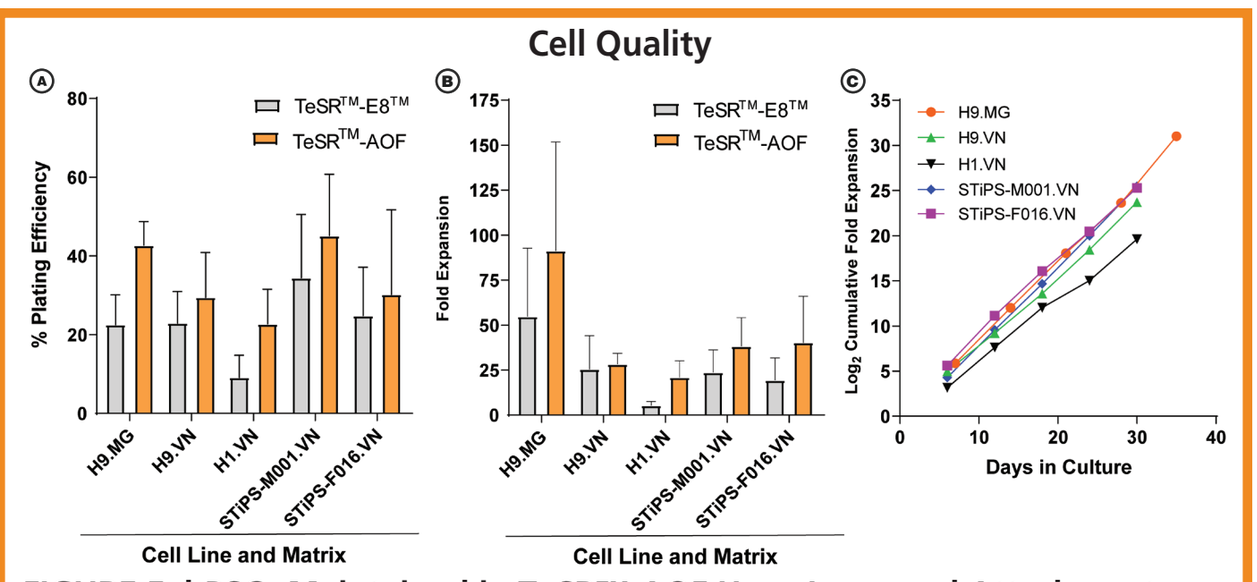


FIGURE 5. hPSCs Maintained in TeSR™-AOF Have Improved Attachment and Higher Overall Expansion Compared to Low-Protein Medium

(A) hPSCs cultured in TeSR™-AOF demonstrate a higher plating efficiency compared to hPSCs maintained in low-protein medium (TeSR™-E8™). Plating efficiency is calculated by seeding a known number of aggregates and comparing to the number of established colonies on day 7. **(B)** hPSCs maintained in TeSR™-AOF exhibit a higher average fold expansion per passage compared to TeSR™-E8™. **(C)** hPSCs cultured in TeSR™-AOF demonstrate consistent expansion and minimal cell line-to-cell line variability among ES and iPS cell lines assessed. Cumulative fold expansion was measured from passage 1 to 5. Data represented as mean plating efficiency or fold expansion across 10 passages ± SD. MG = Matrigel®; VN = Vitronectin XF™.

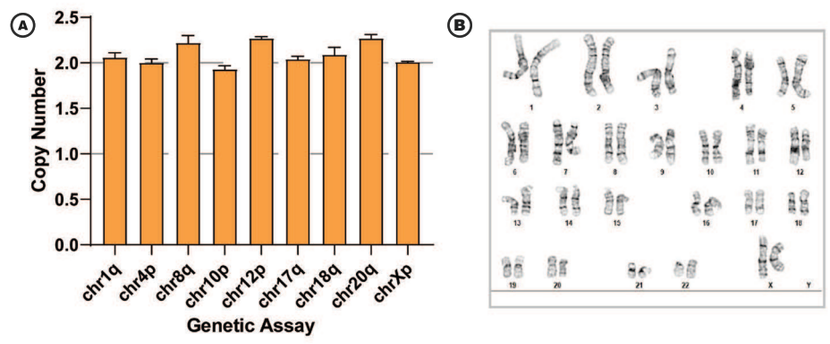


FIGURE 6. hPSCs Cultured in TeSR™-AOF with Restricted Feeding Maintain a Normal Karyotype

ES (H9 & H1) and iPS (STiPS-M001 & STiPS-F016) cell lines cultured in TeSR™-AOF were screened for chromosomal abnormalities using the hPSC Genetic Analysis Kit and by G-banding at ≥ 10 passages. Representative data are shown for **(A)** H9 ES cultures at passage 10; no common chromosomal abnormalities were detected using the hPSC Genetic Analysis Kit, and **(B)** H9 ES cultures; these displayed a normal karyotype by G-banding at passage 13.

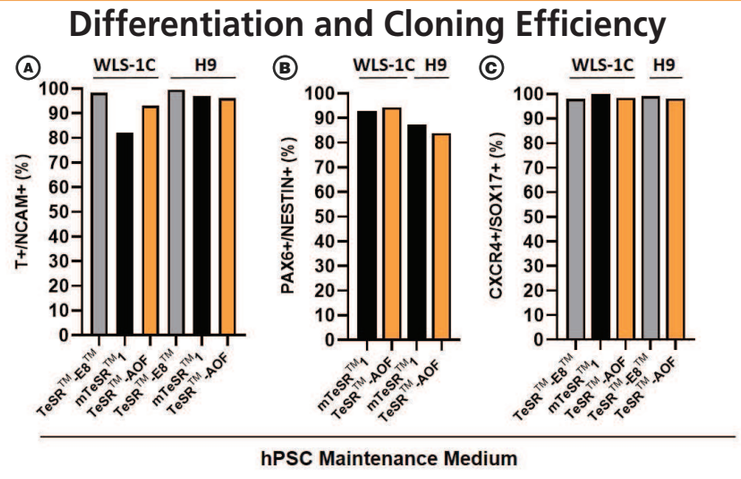


FIGURE 7. hPSCs Maintained in TeSR™-AOF with Restricted Feeding Differentiate to the Three Germ Layers

Efficient differentiation to the three germ layers was demonstrated in one ES and one iPS cell line maintained for > 5 passages in TeSR™-AOF compared to mTeSR™1 or TeSR™-E8™ controls. **(A)** Cultures were processed for flow cytometry and assessed for PAX6⁺/Nestin⁺ cells on day 7 following monolayer differentiation using STEMdiff™ Neural Induction Medium. **(B)** Cultures were processed for flow cytometry and assessed for Brachyury (T)⁺/OCT4⁻ cells on day 5 following differentiation in STEMdiff™ Mesoderm Induction Medium. **(C)** Cultures were processed for flow cytometry and assessed for CXCR4⁺/SOX17⁺ cells on day 5 following differentiation using STEMdiff™ Definitive Endoderm Kit.

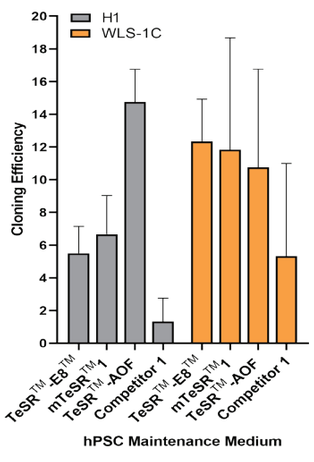


FIGURE 8. hPSCs Exhibit Enhanced Cloning Efficiency in TeSR™-AOF Compared to GMP Competitor Medium

hPSCs were seeded at clonal density (20 cells/cm²) in TeSR™-E8™, mTeSR™1, TeSR™-AOF, and a GMP competitor hPSC maintenance medium (Competitor 1) onto Vitronectin XF™. H1 ES cultures cloned in TeSR™-AOF had significantly higher cloning efficiencies compared to TeSR™-E8™ and Competitor 1 ($p < 0.05$; Paired Student's t test). WLS-1C iPS cells seeded in TeSR™ media trended toward having a higher cloning efficiency compared to Competitor 1. Furthermore, reduced variability in cloning efficiency was observed across the H1 ES and WLS-1C iPS cell lines cloned in TeSR™-AOF. Data representative of n = 2 biological replicates ± SD.

Summary

- TeSR™-AOF is an animal origin-free hPSC culture medium, manufactured with raw material traceability to the secondary level of manufacturing.
- hPSCs maintained in TeSR™-AOF exhibit increased attachment and more consistent expansion when compared with hPSCs maintained in low-protein medium.
- Cell quality is maintained in TeSR™-AOF, even when using reduced feeding schedules. Gene and marker expression, genetic stability, and differentiation potential are unaltered when compared with hPSCs maintained in low-protein medium.
- Higher cloning efficiency is observed when hPSCs are seeded in TeSR™-AOF with CloneR™ at clonal density compared to competitor media.