Modifications Designed to Stabilize the mTeSR™1 Medium Formulation Do Not Impact Downstream Differentiation

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INTRODUCTION

The ultimate goal of human pluripotent stem cell (hPSC) research is to generate mature cell types for use in regenerative medicine, drug screening, or disease modeling. The field has invested years developing robust and efficient protocols for hPSC differentiation to a wide variety of cell types, however, reproducibility can suffer if part of the workflow changes. mTeSR™ Plus is a new hPSC maintenance medium formulation based on mTeSR™1, which enables reduced feeding by maintaining a more consistent pH and stabilizing components including FGF2. We investigated the impact of these modifications on downstream differentiation protocols by investigating 10 separate cell types across the 3 germ layers, without modifying the original differentiation protocols. Overall, we observed little to no impact on the efficiency of downstream differentiation protocols developed for mTeSR™1 when used with hPSCs maintained in mTeSR™ Plus.

METHODS

Cells were maintained in mTeSR™ Plus for ≥ 5 passages with a reduced feeding schedule or in mTeSR™1 with daily feeding. We used STEMdiff™ kits for all downstream differentiation. For ectoderm differentiation, we generated neural progenitor cells using an EB-based protocol with the SMADi Neural Induction Kit, and further differentiated cells using the Astrocyte Differentiation and Maturation Kit or the Neuron Differentiation and Maturation Kit. We also verified that the Cerebral Organoid Kit could be used to differentiate hPSCs maintained in mTeSR™ Plus. For mesoderm differentiation, we generated early mesoderm cells using Mesoderm Induction Medium and more mature cell types using the Cardiomyocyte Differentiation Kit and the Hematopoietic Kit. For endoderm differentiation, definitive endoderm cells were generated using the Definitive Endoderm Kit, and more mature cells were generated using the Pancreatic Progenitor Kit. We also verified that the Intestinal Organoid Kit could be used to differentiate hPSCs maintained in mTeSR™ Plus. For each differentiation protocol, efficiency was assessed by staining for appropriate differentiated cell markers and analyzed by either flow cytometry or immunocytochemistry.

RESULTS

ECTODERM

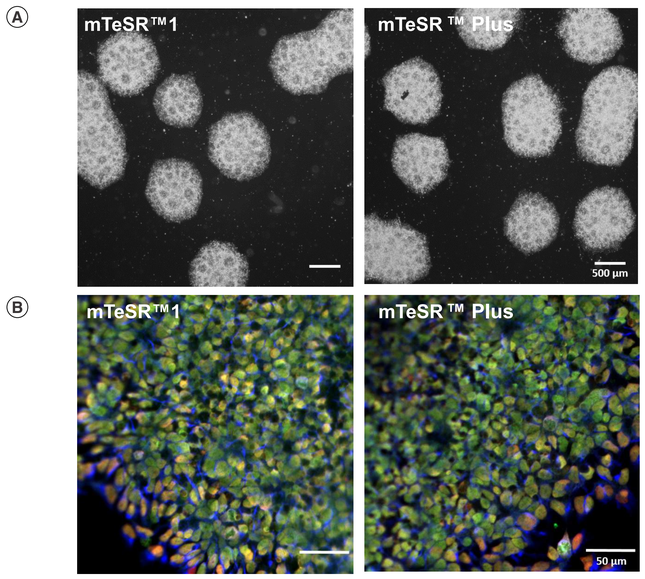

FIGURE 1. Differentiation to Neural Progenitor Cells
(A) H9 hES cells maintained in mTeSR™1 or mTeSR™ Plus clearly display neural rosettes after replating EBs.
(B) Neural rosettes from STiPS-M001 hiPS cells show neural progenitors stained with Nestin (blue), SOX1 (red), and PAX6 (green).

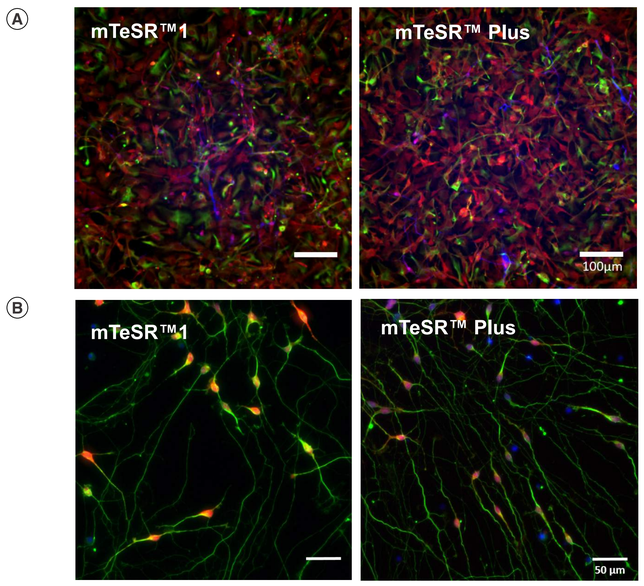

FIGURE 2. Differentiation to Astrocytes and Neurons
(A) H9 hES cells maintained in either mTeSR™1 or mTeSR™ Plus generated astrocytes expressing GFAP (green), S100B (red), and DCX (blue).
(B) STiPS-M001 hiPS cells maintained in either mTeSR™1 or mTeSR™ Plus generated neurons expressing Tuj1 (green) with a subset also expressing GABA (red). Nuclei are counterstained with DAPI (blue).

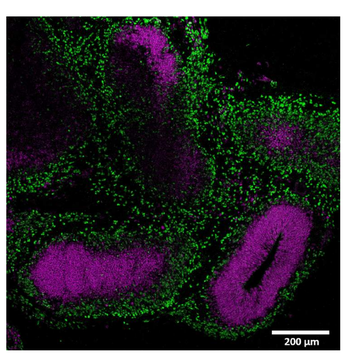

FIGURE 3. Cerebral Organoid Differentiation
H9 hES cells were cultured with mTeSR™ Plus and directed to cerebral organoids using STEMdiff™ Cerebral Organoid Kit. Cerebral organoids at day 40 show apical progenitor marker SOX2 (purple) and neuronal marker TBR1 (green).

MESODERM

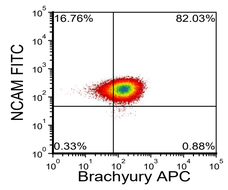

FIGURE 4. Differentiation to Early Mesoderm
Representative plot showing Brachyury (T) and NCAM expression analyzed by flow cytometry. The average percent T⁺ cells obtained from mTeSR™ Plus cultures was 80 ± 2% (mean ± SEM; n = 4 hPSC lines).

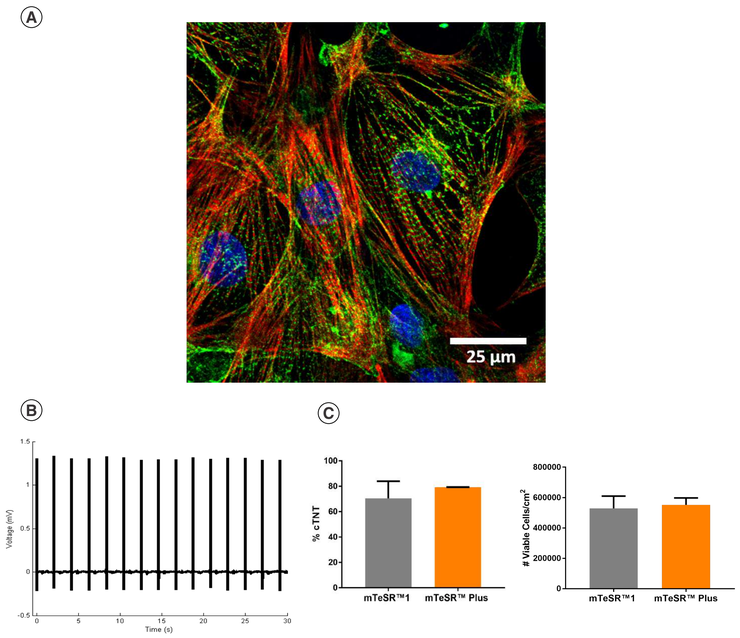

FIGURE 5. Differentiation to Cardiomyocytes
(A) STiPS-M001 hiPS cells maintained with mTeSR™ Plus were directed to cardiomyocytes and stained with markers cTnT (red) and α-actinin (green). Nuclei are counterstained with DAPI (blue).
(B) Representative microelectrode array (MEA) voltage recordings of cardiomyocytes (day 20) show a characteristic electrical profile and stable beat rate.
(C) Summary of the average percentage cTnT⁺ cells and total number of viable cells. Data expressed as mean ± SEM; n = 2 hPSC lines.

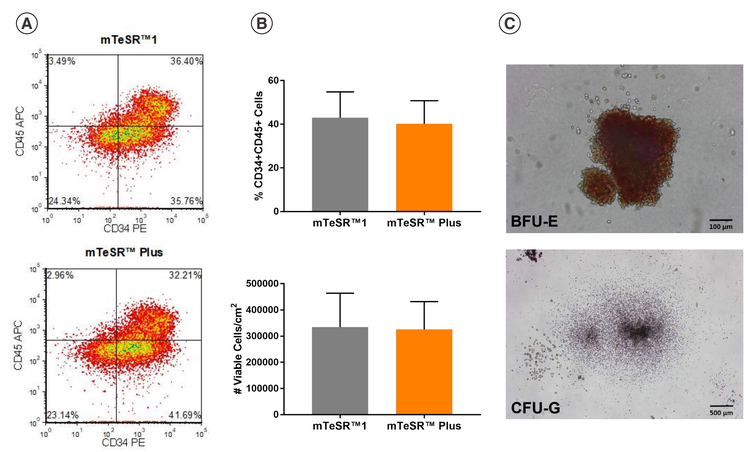

FIGURE 6. Differentiation to Hematopoietic Progenitor Cells
(A) Representative plots of differentiated H1 hES cells showing CD34 and CD45 expression analyzed by flow cytometry.
(B) Summary of the average percentage of CD34⁺CD45⁺ cells and total number of viable cells. Data expressed as mean ± SEM; n = 4 hPSC lines.
(C) Hematopoietic progenitor cells differentiated from H9 hES cells maintained in mTeSR™ Plus form multiple types of colonies in the colony-forming unit (CFU) assay. Representative BFU-E and CFU-G shown.

ENDODERM

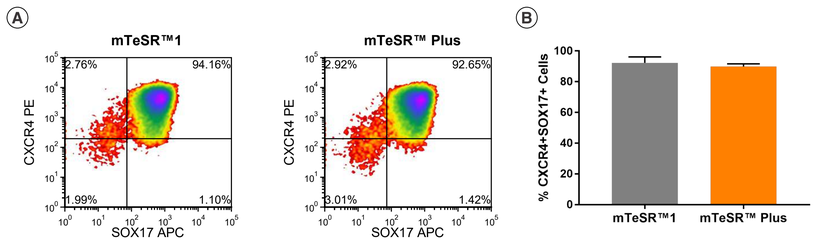

FIGURE 7. Differentiation to Definitive Endoderm
(A) Representative density plots of differentiated STiPS-M001 hiPS cells showing CXCR4 and SOX17 expression analyzed by flow cytometry.
(B) Summary of the average percentage of differentiated cells co-expressing CXCR4 and SOX17. Data are expressed as the mean ± SEM; n = 3 hPSC lines (H9, STiPS-M001, WLS-1C).

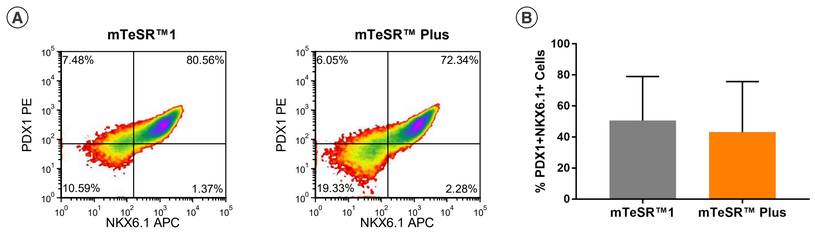

FIGURE 8. Differentiation to Pancreatic Progenitor Cells
(A) Representative density plots of differentiated STiPS-M001 hiPS cells showing PDX-1 and NKX6.1 expression analyzed by flow cytometry.
(B) Summary of the average percentage of differentiated cells co-expressing PDX-1 and NKX6.1. Data are expressed as the mean ± SEM; n = 3 hPSC lines (H9, STiPS-M001, WLS-1C).

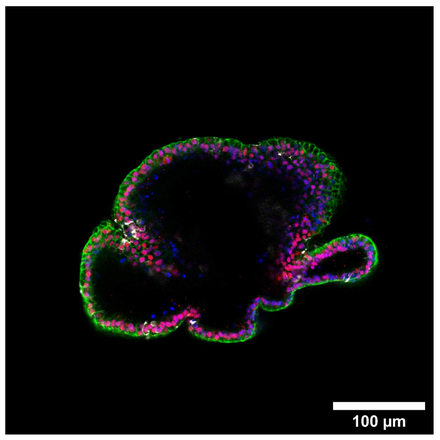

FIGURE 9. Intestinal Organoid Differentiation
Human ES (H9) cells were cultured with mTeSR™ Plus and directed to intestinal organoids using the STEMdiff™ Intestinal Organoid Kit. Intestinal organoids at day 28 show markers of the intestinal epithelium EpCAM (green) and CDX2 (red), and intestinal mesenchyme marker vimentin (white). Nuclei are counterstained with DAPI (blue).

Summary

- mTeSR™ Plus is a new hPSC maintenance medium formulation based on mTeSR™1, which enables reduced feeding by maintaining a more consistent pH and stabilizing components including FGF2
- Maintaining hPSCs in mTeSR™ Plus does not change or bias hPSC differentiation capacity compared to cells maintained in mTeSR™ 1 when tested in 10 separate differentiation protocols spanning all 3 germ layers