A NEW CGMP COMPLIANT ADHERENT PSC CULTURE MEDIUM THAT ENABLES ROBUST PERFORMANCE IN CELL THERAPY DEVELOPMENT

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Abstract

Culture systems for human pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells capable of differentiation to all three germ lineages with potential for cell-based regenerative therapies. To advance PSC therapy research to the clinic, there is a need for high-quality ancillary materials with GMP-compliant manufacturing, quality and safety testing, raw material traceability and supporting regulatory documentation. To ease this transition, we have developed Gibco™ Cell Therapy Systems (CTS™) StemFlex™ Medium based on research use StemFlex™ Medium, but without raw materials directly of human or animal origin. CTS StemFlex Medium supports expansion of high-quality PSCs as well as challenging applications in the PSC workflow. Here we demonstrate PSCs cultured long-term in CTS StemFlex Medium maintain typical PSC characteristics including >95% expression of OCT4/NANOG pluripotency markers, normal morphology, trilineage differentiation potential and karyotype. This new medium allows for versatility in culture conditions: a flexible feed schedule with a weekend-free option and compatibility with multiple defined matrices and passaging reagents. CTS StemFlex Medium is shown to provide critical support of stressful PSC applications including single-cell passaging, with consistent cumulative fold expansion over 10 passages, maintenance of normal karyotype and >95% OCT4/NANOG expression. This new medium supports PSC expansion post electroporation-based delivery of the CRISPR Cas9-gRNA complex as well as downstream clonal expansion of edited PSCs. Adherent PSCs cultured in CTS StemFlex Medium can be transitioned to 3D culture using CTS StemScale™ PSC Suspension Medium resulting in high-quality PSC spheroids.

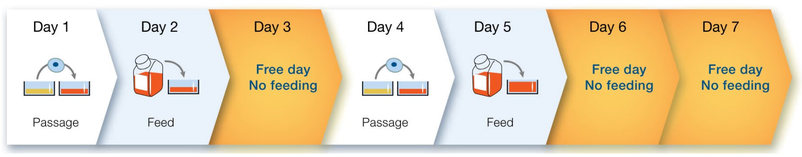
Introduction

Advancing pluripotent stem cell (PSC) therapy research to clinical applications requires researchers to carefully select materials, as the quality of starting materials has a significant impact on the properties of the final stem cell therapy product. Gibco™ Cell Therapy Systems (CTS™) products are available throughout the PSC therapy workflow, aiming to help facilitate the transition from discovery to commercial manufacturing. These materials are extensively qualified, traceable and accompanied by regulatory documentation. Here, we introduce Gibco™ CTS™ StemFlex™ Medium, developed to support expansion of high-quality PSCs as well as challenging applications in the PSC workflow.

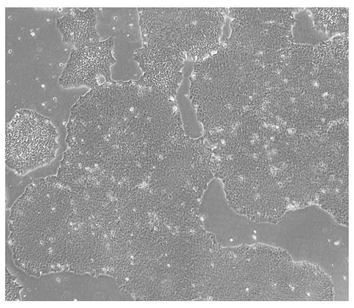
Results

Figure 1. CTS StemFlex Medium supports long-term maintenance of high-quality PSCs (>10 passages)

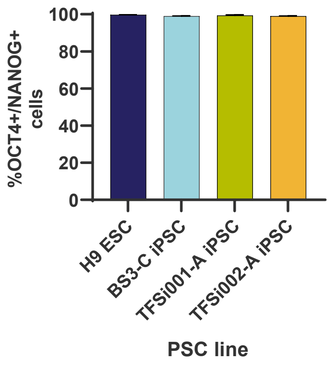
(A) Weekend-Free Flexible Feed Schedule



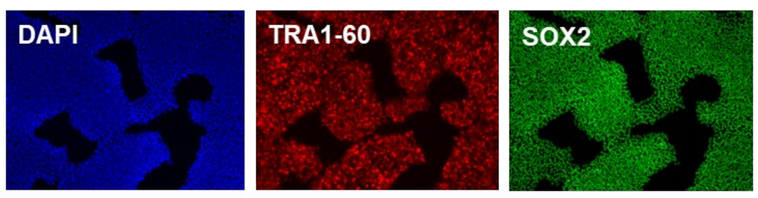
(B) Normal Morphology

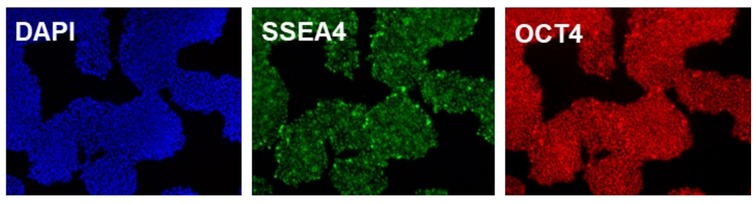


(D) Pluripotency by Flow Cytometry



(C) Pluripotency by Immunocytochemistry





(E) Genomic stability by KaryoStat™ Assay

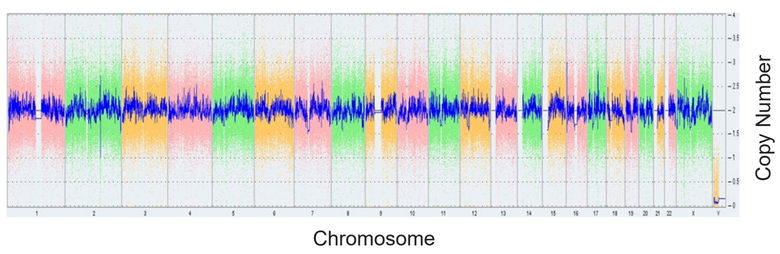


Figure 1 PSCs were expanded in CTS StemFlex Medium on CTS™ Vitronectin Recombinant Human Protein (rhVTN-N) matrix for ≥10 passages using CTS™ Versene Solution for clump passaging while following the (A) weekend-free flexible feed schedule. PSCs were shown to maintain (B) normal morphology, pluripotency as assessed by (C) immunocytochemistry and (D) flow cytometry and (E) normal karyotype as assessed by the KaryoStat™ Assay.

Figure 2. CTS StemFlex Medium supports maintenance of trilineage differentiation potential

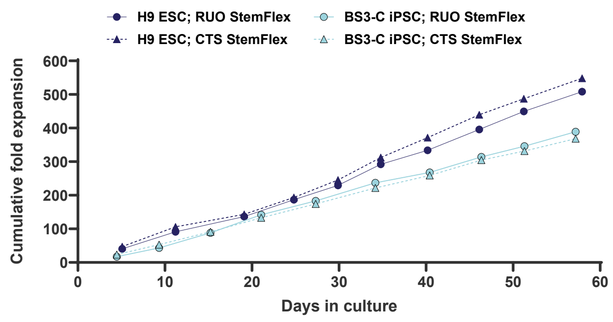
Figure 2 PSCs grown in CTS StemFlex Medium were spontaneously differentiated as embryoid bodies using Gibco™ CTS™ Essential 6™ Medium. Trilineage differentiation potential was confirmed by gene expression analysis using the Applied Biosystems™ TaqMan™ hPSC Scorecard™ Assay.

Figure 3. CTS StemFlex Medium supports directed differentiation to the three germ layers

Figure 3 PSCs grown in CTS StemFlex Medium transitioned to Gibco™ PSC differentiation kits for directed differentiation Surface marker expression was determined via flow cytometry. (A) PSC Dopaminergic Neuron Differentiation Kit (B) PSC Cardiomyocyte Differentiation Kit (C) PSC Definitive Endoderm Induction Kit.

Figure 4. CTS StemFlex Medium supports expansion comparable to RUO StemFlex Medium with single-cell passaging

(A) Expansion Post Single-Cell Passaging



(B) Maintenance of Pluripotency

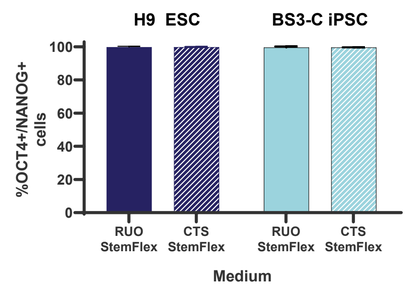
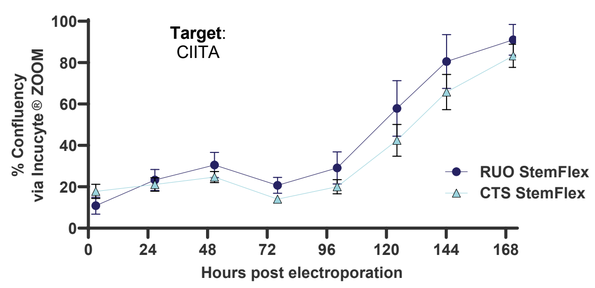


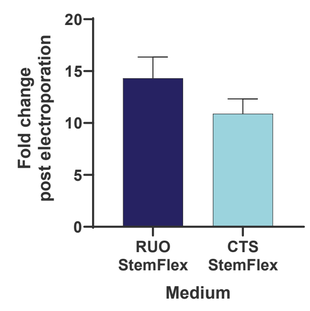
Figure 4 PSCs were singularized with CTS™ TrypLE™ Select and seeded at 12.5K cells/cm^2 on iMatrix-511 MG with CTS™ RevitaCell™ Supplement for 10 passages in RUO StemFlex Medium or CTS StemFlex Medium. CTS StemFlex Medium was shown to support (A) comparable expansion and (B) maintenance of pluripotency to RUO StemFlex Medium over 10 single cell passages in 2 PSC lines.

Figure 5. CTS StemFlex Medium supports iPSC expansion post electroporation-based delivery of CRISPR Cas9-gRNA complex

(A) Confluency Post Electroporation



(B) Fold Change



(C) Editing Efficiency

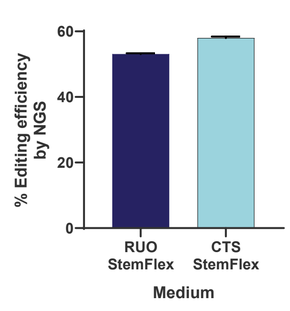
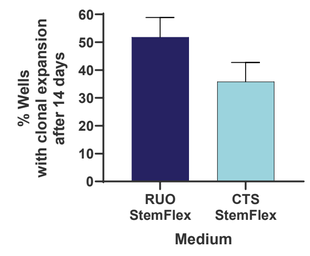


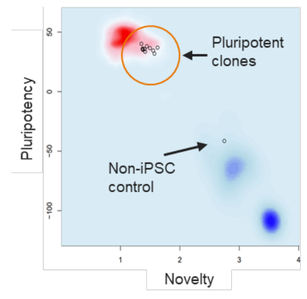
Figure 5 TFSi001-A iPSCs in CTS StemFlex Medium or RUO StemFlex Medium underwent electroporation to deliver the CTS™ HiFi Cas9 Protein complexed with TrueGuide™ Synthetic gRNA targeting the CIITA gene, and were seeded onto iMatrix-511 MG with CTS RevitaCell Supplement. CTS StemFlex Medium was shown support iPSC expansion following this challenging application in the gene editing workflow, with (A,B) expansion and (C) editing efficiency comparable to RUO StemFlex Medium.

Figure 6. CTS StemFlex Medium supports clonal expansion of edited PSCs

(A) Clonal Expansion Efficiency



(B) Clone Pluripotency by PluriTest



(C) Clone Genomic Stability by KaryoStat

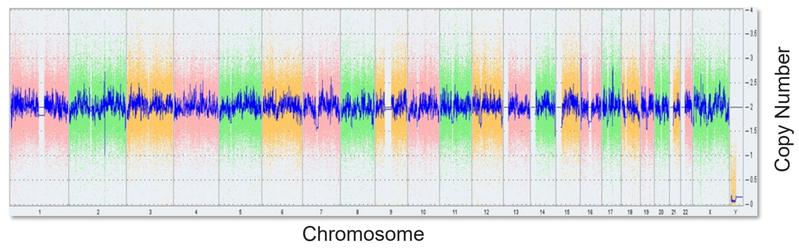
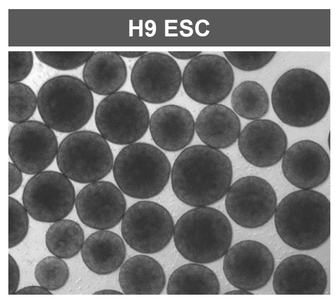


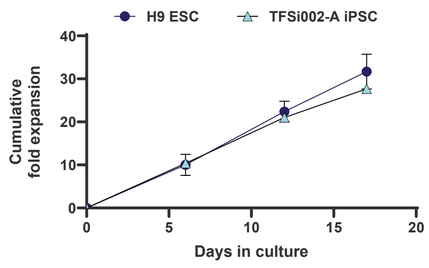
Figure 6 TFSi001-A iPSCs were seeded in CTS StemFlex Medium or RUO StemFlex Medium at a clonal seeding density of 1 cell per well onto iMatrix-511-coated 96-well plates with CTS RevitaCell Supplement. CTS StemFlex Medium was shown to support (A) clonal expansion at 70% of RUO StemFlex Medium in this iPSC line, with ~35% of wells per 96-well plate showing clonal expansion. Furthermore, individual clones were selected and expanded further in CTS StemFlex Medium and were shown to maintain (B) pluripotency via the PluriTest Assay and (C) genomic stability via the KaryoStat Assay.

Figure 7. Adherent PSCs cultured in CTS StemFlex Medium can be transitioned to 3D culture using CTS™ StemScale™ PSC Suspension Medium

(A) PSC Spheroid Morphology



(B) PSC Spheroid Expansion



(C) Pluripotency after 5 passages

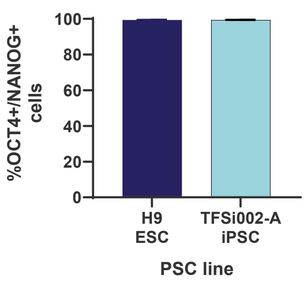


Figure 7 Adherent PSCs adapted to CTS StemFlex Medium were dissociated with CTS TrypLE Select and seeded for 3D spheroid initiation in CTS StemScale PSC Suspension Medium. This resulted in high-quality PSC spheroids with (A) rounded morphology (B) consistent expansion (C) and maintenance of pluripotency.

Conclusions

- CTS StemFlex Medium supports long-term maintenance of high-quality PSCs maintaining normal morphology, karyotype, pluripotency & trilineage differentiation potential.
- CTS StemFlex Medium supports challenging applications in the PSC therapy workflow including single-cell passaging, electroporation & clonal expansion.
- Adherent PSCs cultured in CTS StemFlex Medium can be transitioned to 3D culture using CTS StemScale PSC Suspension Medium.

Acknowledgements

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