mTeSR®1 and TeSR™2: The Advancement of Culture Media for Undifferentiated Pluripotent Stem Cells towards Greater Regulatory Compliance

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Introduction
Defined and feeder-independent cell culture systems provide a platform for greater reproducibility and standardization in human pluripotent stem cell (hPSC) research. mTeSR®1 has become the most widely used medium for feeder-free culture of hPSC. As the field develops potential therapeutic applications for hPSC-derived cells, it is increasingly important that media products are manufactured that meet applicable regulatory compliance standards. Therefore, we have developed two new products for the expansion of undifferentiated hPSCs: mTeSR®1 manufactured in GMP facility (mTeSR®1-GMP) and Animal Protein-free TeSR™2. We show data to support their use in maintaining hPSC in the pluripotent state.

Materials and Methods
The hESC lines and H1 and H9 were cultured in mTeSR®1-GMP or TeSR™2 on Matrigel™ (BD). Cells were passaged every 5 to 7 days using dispase (1mg/mL, STEMCELL). Minimal selection of differentiated colonies (<10%) was performed. Cell expansion was evaluated using a developed clump enumeration method. Maintenance of pluripotency marker expression was measured by FACS analysis for Oct3/4 and/or SSEA-3. In addition, cells cultured in TeSR™2 were evaluated for chromosomal stability by G-band karyotype analysis and functional pluripotency using an in vivo teratoma assay. To investigate growth on a more defined matrix, hPSC were grown in TeSR™2 on rhVitronectin (R&D Systems). In these experiments, cells were passaged using a scraping method without the use of enzymes and cell expansion and expression of Oct3/4 and SSEA-3 markers were measured as described above.

Results and Discussion
Cells were grown in mTeSR®1-GMP on Matrigel™ for 5 passages. Expansion in mTeSR™1-GMP averaged 4.9 and 8.3-fold per passage for H1 cells and H9 cells respectively. After 5 passages expression of Oct3/4 was 92.7% for H1 and 96.5% for H9. These results confirm that the mTeSR®1 media manufactured under GMP compliance yields a product with equivalent performance to the standard mTeSR®1 product. hPSC could be maintained in TeSR™2 on Matrigel™ for > 25 passages. Expansion in TeSR™2 was 7.0 and 6.2-fold per passage for H1 and H9 cells respectively. When H9 cells were isolated after 11 passages in TeSR™2 and were injected into mice, they generated teratomas over a period of 9 weeks. Tissues representing all 3 germ layers could be identified in histological sections of the teratomas confirming the pluripotency of the cultured cells. FACS analysis of Oct3/4 and SSEA-3 expression after >25 passages in TeSR™2 was >95% for both H1 and H9. G-band analysis of cells from 4 separate experiments 11 to 22 passages in length confirmed karyotypic stability of cells cultured in TeSR™2. rhVitronectin used in combination with TeSR™2 supported the undifferentiated growth of both H1 and H9 cells (>90% Oct3/4+ by FACS after 5 passages) with a 3 to 5-fold expansion per passage on this matrix. Other defined surfaces for robust expansion of hPSC in TeSR™2 are currently being investigated.

Conclusions
These new products represent STEMCELL’s ongoing commitment to provide regulatory compliant reagents for the translation of basic research to pre-clinical studies.

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Disclosures
The authors have nothing to disclose.