Validation of Defined Feeder-Free Culture Systems for the Maintenance of EUTCD-Compliant Human Embryonic Stem Cell Lines

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Introduction

Validated UK Stem Cell Bank (UKSCB) protocols for the expansion of EUTCD– compliant human embryonic stem cells (hESCs) include the use feeders as well as feeder-free alternatives that contain the use of animal-derived products, undefined serum and other components. This may pose a risk of microorganism transmission as well as carrying inherent batch-to-batch variability, which may ultimately result in undesirable changes in cellular function. These issues become even more pertinent when hESCs with potential clinical applications are being expanded, and therefore the development of a more standardised and well-defined approach to culturing hESCs with product safety and reliability in mind becomes crucial.

Since the UKSCB is currently preparing to derive seed stocks of clinical-grade hESCs, a study to generate feeder-free protocols for the cultivation of EUTCD-compliant hESCs has been performed comprising of 3 phases:

- ⇒ Phase I - Meta-analysis of published findings
- ⇒ Phase 2 - Assessment of media/matrix combinations on a well-characterised cell line
- ⇒ Phase 3 - Assessment of selected media/matrix combinations on clinical-grade cell lines

Methods and Materials

PHASE I

A total of 14 commercially available media and matrix combinations commonly used to culture hESCs were identified during a meta-analysis of current published protocols. These combinations were then taken forward into Phase II of the media/matrix project.

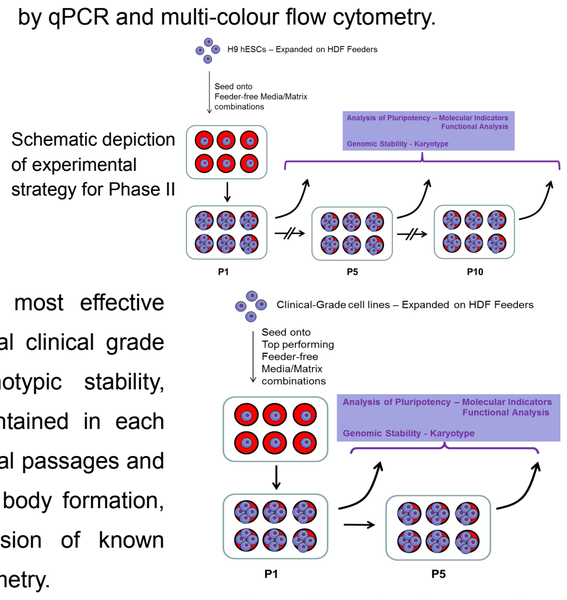
Media	Matrix
NutriStem™	Laminin 511
TeSR™-E8™	Laminin 521
mTeSR™2	Vitronectin
mTeSR™1	Matrigel®

Culture media and matrix components most abundantly used for the culture of feeder-free hESC culture.

PHASE II

Using the well-characterised cell line H9 (WiCell, USA), the 14 combinations identified in phase I were assessed for their ability to maintain viable and functional hESCs, which will retain their stem cell characteristics over multiple passages. Cells were morphologically examined for 10 serial passages and were assessed for differentiation potential by embryoid body formation, proliferative capacity, karyotypic stability and expression of known pluripotency markers by qPCR and multi-colour flow cytometry.

Schematic depiction of experimental strategy for Phase II



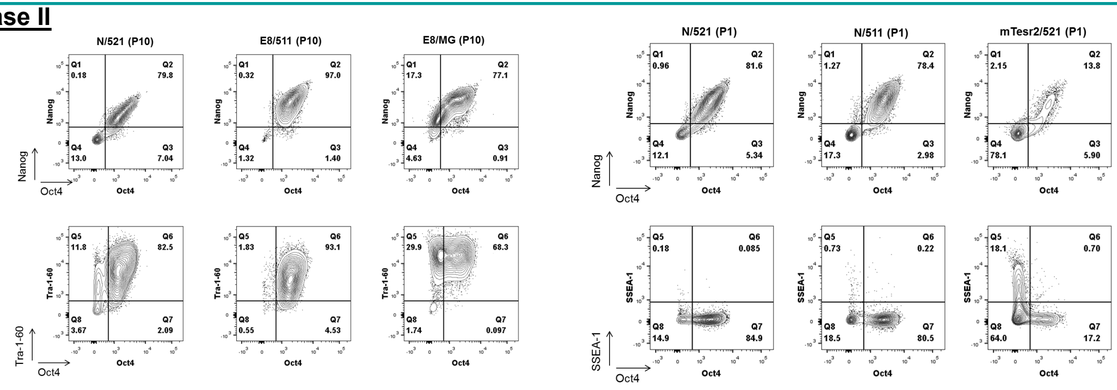
Schematic depiction of experimental strategy for Phase III

PHASE III

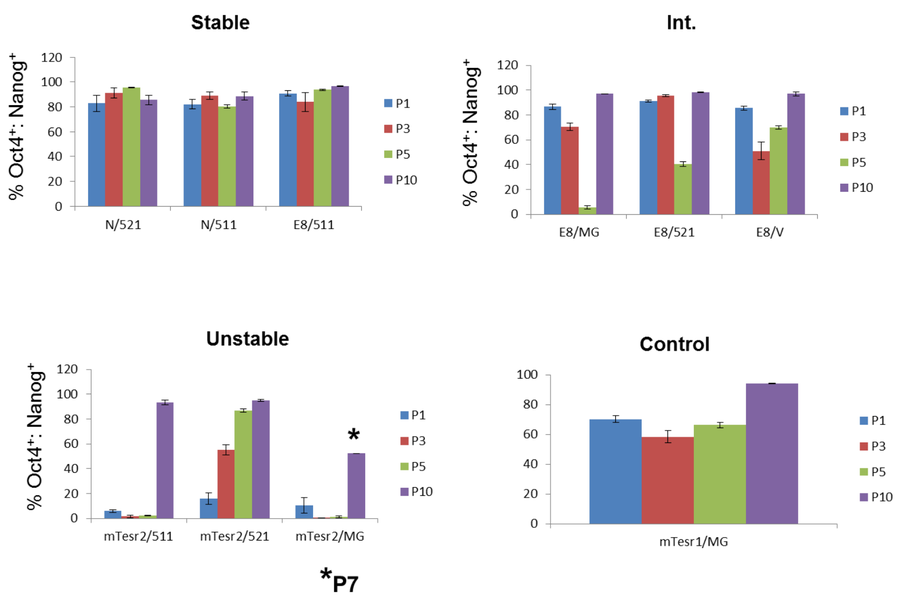
Phase III is currently in progress whereupon the 3 most effective conditions from phase II are being assessed on several clinical grade hESCs based on criteria of phenotypic and genotypic stability, morphology and growth rate. As such, the cells maintained in each condition were morphologically examined for multiple serial passages and were assessed for differentiation potential by embryoid body formation, proliferative capacity, karyotypic stability and expression of known pluripotency markers by qPCR and multi-colour flow cytometry.

Results

Phase II

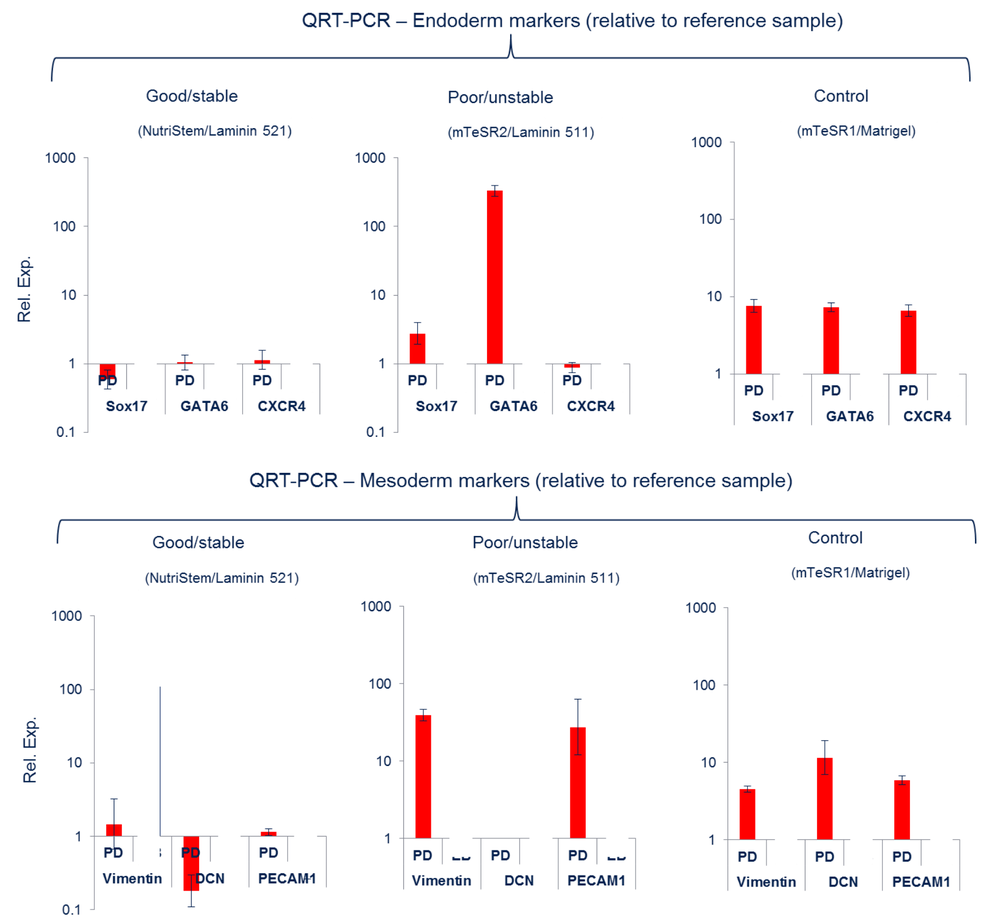


Representative flow cytometry for the pluripotency markers Oct-4 and Nanog performed on H9 hESCs cultured in the media/matrix combinations indicated. Examples shown are of cells isolated at P1.

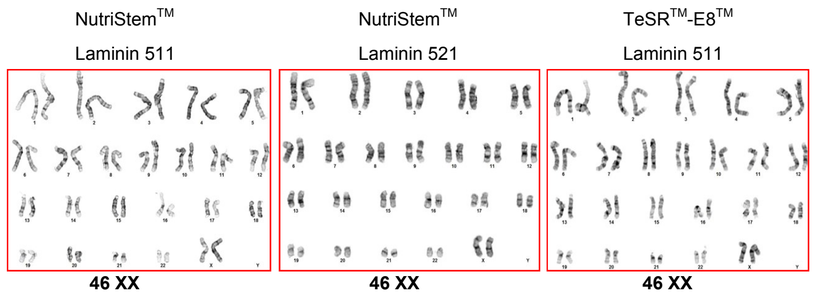


The proportion of Oct4$^+$:Nanog$^+$ cells, determined by flow cytometry, in hESCs cultured in the indicated media/matrix combinations (analysis performed at P1, P3, P5 and P10).

Results



Functional potency of hESCs maintained in each 'stable' media/matrix combination was assayed by spontaneous differentiation in Embryoid Bodies (EBs). Differentiation into endoderm, mesoderm and ectoderm was determined by qPCR analysis of a variety of genes associated with lineage specification. The expression of the pluripotency markers Oct-4 and Nanog was also analysed as an additional measure of exit from the hESC state. Data shown are for cells isolated at P10. Expression levels are expressed relative to undifferentiated H9 hESCs. PD = Pre-differentiation, EB = Embryoid Body.



Karyograms of H9 hESCs grown in the 'stable' media/matrix combinations. Images show Giemsa stained metaphase chromosomes of cells isolated at P10 (NutriStem™/Laminin 511 and NutriStem™/Laminin 521) and P5 (TeSR™ E8™/Laminin 511). The numerical value below each karyogram indicates the karyotype determined from >20 cells for each condition. Cells grown in each condition exhibit a normal (46 XX) karyotype.

Phase III

Results from phase III are still being generated however, early indications show that all clinical-grade cell lines cultured in the 3 most effective conditions identified in phase II are able to adapt quickly to feeder-free conditions without loss of potential pluripotency or stability. However, the preferred combination is cell line specific.

Conclusions

Of the 14 different culture systems analysed, three were identified to consistently outperform all other combinations in the maintenance of undifferentiated hESCs without loss of functional characteristics. These conditions were also able to successfully maintain the growth of clinical-grade hESCs. Furthermore, hESCs maintained on these systems were able to quickly transition from feeder-dependent to feeder-free conditions as well as maintaining a shorter doubling time without altering chromosomal stability and loss of potential pluripotency. Optimal media/matrix combinations identified in this study will subsequently be utilised for the maintenance of EUTCD compliant hESC lines, thus providing a better defined, feeder-free system for culturing clinical grade cell products.

Acknowledgements

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