SCALABLE EXPANSION OF HUMAN EMBRYONIC STEM CELLS IN A MICROCARRIER SUSPENSION CULTURE SYSTEM

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INTRODUCTION

Human embryonic stem (hES) cells offer great potential for cell therapy and regenerative medicine due to their pluripotency and high capacity for self-renewal. The challenge is to produce these cells in large enough numbers for such therapies, whilst retaining their characteristics.

Historically, hES cells have been grown using largely undefined media on mouse or human feeders in cell culture flasks or dishes - a labour intensive system with limited scalability. We have assessed a microcarrier suspension system for the culture of various cell lines, including hES cells.

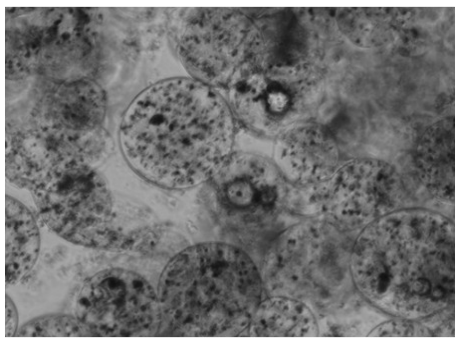
MATERIALS AND METHODS

Initial assessment of hESC (H9) on gelatin, laminin & BM coated GEMs (Stem Cell Starter Kit, Global Cell Solutions) in static culture

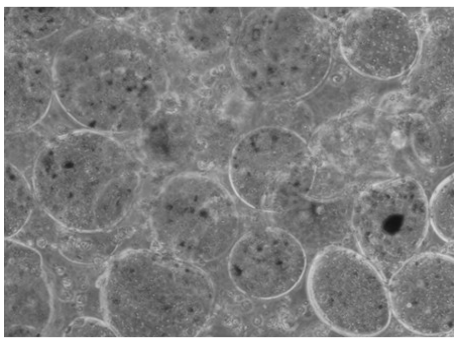
Following the manufacturer's protocol, H9 stem cells were added to GEMs (Global Eukaryotic Microcarrier) alginate microcarriers. Cells were assessed for ability to attach to the GEMS and then serially subcultured for up to 5 passages.

Good cell attachment to beads for all conditions:

- Gelatin coated GEMS/KO-SR hESC medium
- Laminin coated GEMS/KO-SR hESC medium
- BM (basement membrane) coated GEMS/KO-SR hESC medium
- Gelatin coated GEMS/mTeSR medium
- Laminin coated GEMS/mTeSR medium
- BM coated GEMS/mTeSR medium



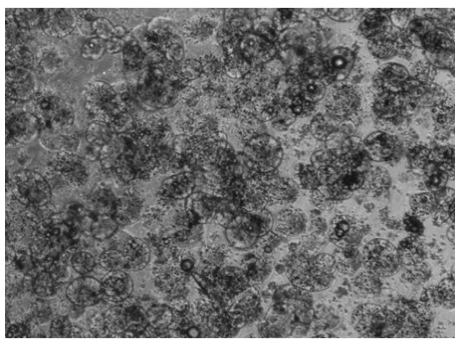
Low density cells on GEMs



High density cells on GEMs

H9 cells showed better growth on all 3 types of GEMs (gelatin, laminin & BM) when maintained in KO-SR medium than for mTeSR defined medium. These KO-SR cultures were serially subcultured for 5 passages, subculturing according to manufacturer's protocol as they reached high density on the beads (approx 4-5 days). Good cell growth was maintained for all 3 types of GEMS.

H9 cells showed poor growth and started to detach from GEMS when maintained in mTeSR medium and only achieved 2 subcultures.



Cells detaching from beads

Assessment of GEMS in suspension culture using prototype Wiggler (Global Cell Solutions)

The Wiggler is a control unit that fits inside an incubator and holds up to 8 LeviTubes (an impeller -free 50ml vessel that allows gentle lifting & mixing of microcarrier and suspension cell cultures). The growth of various mammalian cell lines was assessed using this system.

Growth of human & murine fibroblasts

Murine fibroblasts were seeded at $8x10^4$ cells in DMEM +10% FCS medium to a T75 flask, a LeviTube (no GEMS), and to gelatin coated GEMS in a LeviTube.

After 5 days in culture, the following cell numbers were achieved:

- Static culture (T75 flask) - $6x10^5$ cells
- Suspension culture/no GEMs - $1x10^6$ cells
- Suspension culture/gelatin coated GEMs - $1.3x10^6$ cells

Cells appeared healthy & viable (no non-viable cells) for all conditions. Higher cell numbers achieved for suspension culture, with the greatest increase in cell numbers on GEMs in suspension culture.

Suspension culture/gelatin coated GEMs – cells were maintained in culture for 20 days, subculturing at a 1/10 split ratio every 4-5 days. 10-fold increase in cell numbers was maintained over this period.

Human fibroblasts seeded at $1x10^5$ cells in DMEM +10% FCS medium to a T75 flask, a LeviTube (no GEMS), and to gelatin coated GEMS in a LeviTube.
After 5 days the following cell numbers were achieved:

- Static culture (T75 flask) - $4.5x10^5$ cells
- Suspension culture/no GEMs - $5x10^5$ cells
- Suspension culture/gelatin coated GEMs - $5.5x10^6$ cells

Cells appeared healthy & viable (no non-viable cells) for all conditions. Similar cell numbers achieved for all conditions, with no significant increase in cell numbers or growth rate for suspension culture compared to static culture.

Growth of human embryonic stem cell lines

OXF2 human embryonic stem cell line was seeded at $1.5x10^5$ cells in KO-SR hES medium.
After 5 days the following cell numbers were achieved:

- Suspension culture/laminin coated GEMs - $1.9x10^6$ cells
- Suspension culture/Matrigel coated beads - $1.8x10^6$ cells

Approximately 10-fold increase in cell numbers every 5 days compared to a 6-fold increase in cell numbers in static culture (6-well plates, hESCs grown on iMEF feeders).

Cells were maintained in culture for 5 serial subcultures with a 10-fold increase in cell numbers every 5 days over this period. Cells were plated back to iMEF feeders at the end of the test period – normal hESC morphology maintained.

Similar results were obtained for other hESC lines – 20-25% greater increase in cell numbers in suspension culture compared to static culture system.

Cell line	*Suspension culture +GEMS*	*Static culture (6-well plate)*
EDI-2	8-fold increase every 5 days	6-fold increase every 5 days
KCL001/ WT-3	10-fold increase every 5 days	8-fold increase every 5 days
NCL-2	16-fold increase every 5 days	12-fold increase every 5 days
OXF3	8-fold increase every 5 days	6-fold increase every 5 days
H9	16-fold increase every 5 days	12-fold increase every 5 days

All hESC lines showed slightly better growth on laminin compared to Matrigel coated GEMs, but this may be different for other defined media.

All cells appeared healthy & no non-viable cells were detected in the suspension cultures. In static culture systems, there are generally a few (1-2%) non-viable/apoptotic cells present.

Freezing of hES cells on laminin coated GEMs

OXF2 hES cells were grown in suspension on laminin coated GEMs. When the cells reached high density, but still actively growing, they were cryopreserved using conventional freezing (+10% DMSO).

Half of the cells were frozen on the beads, whilst the rest were dissociated from the beads and then frozen as normal. Frozen samples were thawed and assessed for viability and their ability to establish growing cultures.

Cells frozen on beads showed >80% viability (compared to 84% for cells dissociated from the beads prior to freezing) and healthy hESC cultures were established in suspension. After 5 days post-thaw, these cultures were subcultured and maintained in serial subculture for a total of 5 passages.

DISCUSSION

The scale-up of various mammalian cell lines, including hESCs, using this suspension culture system has been demonstrated. Further work needs to be done to determine whether pluripotency & differentiation potential is maintained for hESCs grown in this system.

The total cell yield for a 50ml suspension culture of hESCs on laminin coated GEMs was $>10^8$ cells, enough for the production of a 100 vial cell bank. This currently requires the generation of up to 20 6-well plates of hESCs over a number of weeks.

This suspension culture system could potentially be used for the complete cell banking process, with scale-up to high cell numbers & then freezing of a cell bank, with the key benefits of significant reductions in both handling time and media consumption.

Advantages of the Wiggler/GEMs system over static culture systems

- Supports the growth and allows scale-up of a range of mammalian cell lines
- hES cell culture in feeder-free, defined media conditions
- Reduced media usage & handling time

ACKNOWLEDGEMENTS

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