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 Micocarrier) 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

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 the 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.

**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 8 x 10^4 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) - 6 x 10^8 cells
- Suspension culture/no GEMS - 1 x 10^7 cells
- Suspension culture/gelatin coated GEMS - 1.3 x 10^6 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.

**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, 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.

**Cell line** | **Suspension culture +GEMS** | **Static culture (6-well plate)**  
--- | --- | ---  
ED1-2 | 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.

**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 >10^6 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

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