# The Effect of Microwell Size and Inoculation Density on the Automated Processing of Stem Cells

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# Introduction

Stem cells have the ability to self renew and differentiate into one or more type of specialised cell. They have a number of potential applications in the clinic including the generation of replacement tissue for treating degenerative diseases and damaged organs. However, there are a number of processing hurdles associated with delivering stem cell derived treatments to the clinic. Simply maintaining cell type during expansion has become a major stumbling block. Adult stem cells can only be passaged for a finite number of population doublings whilst human embryonic stems are notoriously difficult to culture reproducibly. These are major concerns as the safety and efficacy of cell-based therapies will be directly related to the way in which they are processed. An environmentally controlled platform for the automation of stem cell expansion and differentiation would significantly enhance the reproducibility of current processes. The work presented in this poster covers the investigation of key parameters for the expansion of stem cells in an automated micro-well format. The effect of inoculation seeding density (ICD), well size, passage number and feeding strategies on the proliferation of human mesenchymal stromal cells (hMSC) was investigated. In parallel we examined the effect of well size and seeding density on the proliferation of mouse embryonic stem (mES) cells. The studies represent the first steps in defining critical parameters for the automation of stem cell cultures.

# Results

## Mouse Embryonic Stem Cells

Oct4GiP cells, derived from the strain 129Ola mice carrying an Oct4-GFPiresPac transgene was kindly donated by Stem Cell Sciences (Edinburgh, UK). These cells were routinely cultured in GMEM (Sigma) supplemented with non-essential amino acids, β-mercaptoethanol, glutamine, pyruvate, foetal calf serum (all Invitrogen) and LIF (Chemicon). Iwaki tissue culture plate were coated with gelatin (Sigma) prior to use. Cells were cultured in 6 well plates unless stated otherwise.

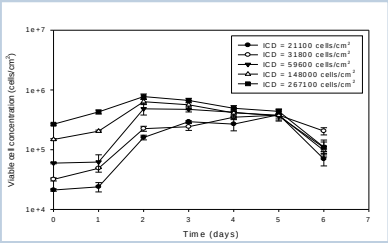



**Figure 1. The effect of inoculation seeding density (ICD) on mES cell growth**

- Higher inoculation densities resulted in an increase in maximum viable cell concentration
- Early Induction of death phase at high ICD

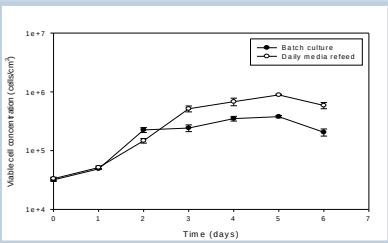



**Figure 2. The effect of feeding strategy on mES cell growth**

- Refeeding resulted in an extension of the lag phase
- Refeeding increased the maximum viable cell concentration by 133%

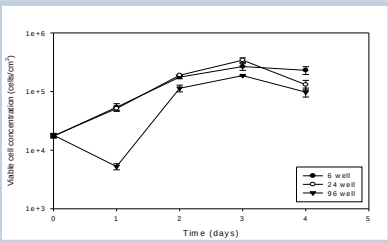



**Figure 3. The effect of well size on mES cell growth**

- Loss of viable cells during lag phase in 96 well format
- Cells were capable of proliferating once they have recovered from the lag phase in 96 well format
- There was a reduction in the maximum viable cell concentration achievable in 96 well format

## Mesenchymal Stromal Cells

Frozen human bone marrow samples were thawed in a water bath at 37°C. Mononuclear cells were isolated using Ficoll density gradient centrifugation and cultivated in low-glucose DMEM (Invitrogen) supplemented with 10% foetal bovine serum (Stem Cell Technologies), 1 ng/ml FGF (R & D systems) and 2mM glutamine (Invitrogen). Once the attached cells had reached 80% confluency they were passaged, replacing the media every 4 days. Cells were cultured in 6 well plates unless stated otherwise.

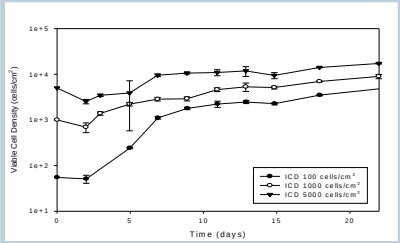



**Figure 4. The effect of ICD on hMSC growth**

- Cell growth is dependent on cell density at any given time point
- Shorter expansion times can be achieved by decreasing the inoculation cell density

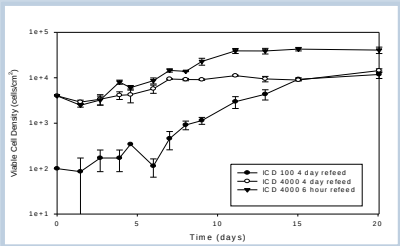



**Figure 5. The effect of feeding strategy on hMSC cell growth**

- The growth rate at low ICDs can be reproduced at high ICDs by increasing the frequency of media exchange
- Maximum viable cell concentration increases with increasing frequency of media exchanges

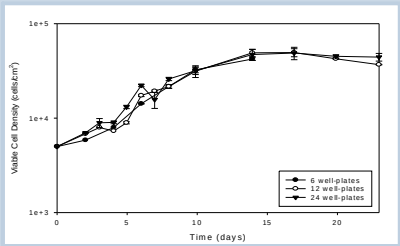



**Figure 6. The effect of well size on hMSC cell growth**

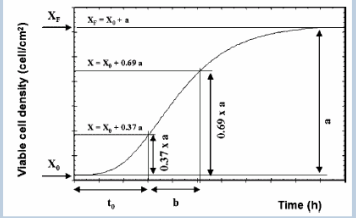
- Cell growth was independent of well size

## Modelling Stem Cell Growth

The effect of media feeding strategy on the cell growth characteristics of adult and embryonic stem cell growth was quantified using the Gompertz equation:

**Gompertz equation**

$$X = Xo + a \cdot Exp(-Exp(\frac{-(t-to)}{b}))$$




**X**: Cell Density (cells/cm²)
**t**: Time (days)
**Xo**: Inoculation cell density (cells/cm²)
**a**: Increase in cell density (cells/cm²)
**to**: Duration of lag and early growth phase (days)
**b**: Duration of exponential phase growth (days)
μ: apparent maximum growth rate
$= \frac{\ln(X_d/X_0)}{e^1 \cdot b}$ (days$^{-1}$)

**Figure 7. Graphical representation of the variables generated by the Gompertz equation (Martin et al. (2005) Biotechnol. Prog.)**

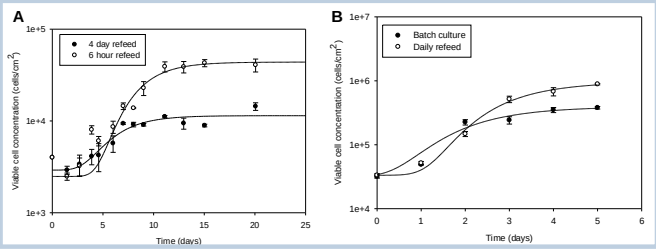



**Figure 8. Modelling cell growth characteristics using the Gompertz equation: The effect of feeding strategy on (A) hMSC and (B) mES cell growth**

| | Mesenchymal stromal cells | | | Embryonic stem cells | | |
|---|---|---|---|---|---|---|
| | 4 day RF | 6 hr RF | % change | Batch | Daily RF | % change |
| to (days) | 5.60 | 7.84 | + 40.0 | 1.86 | 2.69 | + 45.0 |
| μ (days$^{-1}$) | 0.16 | 0.42 | + 155.1 | 0.93 | 1.15 | +23.6 |
| b (days) | 2.42 | 2.49 | + 2.9 | 1.14 | 1.07 | - 6.5 |
| a (cells/cm²) | 8498 | 41284 | + 385.8 | 361706 | 930802 | + 157.3 |

**Table 1. The variables generated by the Gompertz equation for refeeding (RF) of hMSC and mES cultures**

(i) to was increased by medium refeeding in hMSC and mES cells
- The exponential growth phase was delayed by replacing the medium on a daily basis
- Components secreted by stem cells are required for cell growth during the lag phase
- Lag phase could be shortened by minimising refeeding during the lag phase
- Lag phase could be shortened by adding conditioned medium to media refeeds

(ii) Regular refeeding caused an increase in specific growth rate and maximum viable cell concentration in hMSC and mES cells
- Exponential phase cell growth was not inhibited by the removal of growth factors secreted by the mES and hMSC stem cells

(iii) The length of the exponential phase of hMSC and mES cells was not substantially affected by refeeding

# Summary and Future Directions

Increasing the inoculation cell density of mES cell resulted in a rise in the maximum viable cell concentration and a decrease in the length of the lag phase. Increasing the seeding density of hMSCs caused a decrease in growth rate. Further analysis highlighted that the growth of hMSCs was dependent on the cell density of the culture at any given time. For example, unlike mES cells, high ICD hMSC cultures entered into the stationary phase immediately after inoculation. Our data highlights the need for different strategies for the expansion of embryonic and adult stem cells. Embryonic stem cells seeded at high densities were capable of moving into the exponential phase relatively quickly. In contrast, the highest growth rate of hMSCs was achieved at the lowest ICD. Cultures seeded at or near confluency were not capable of entering an exponential phase of cell growth. These cultures did not exhibit an exponential growth phase. Modelling cell growth illustrated that the exponential growth phase was delayed by regular refeeding for both hMSC and mES cells. These results imply that factors secreted by cells were essential for cell growth immediately after inoculation. In contrast regular refeeding increased the specific growth rates and maximum viable cell concentrations of both hMSC and mES cells. These results represent the preliminary characterisation of stem cell growth and feeding for the automation of high-throughput applications. The screening potential of these technologies will be reliant upon the ability to reproduce medium scale cell cultures (e.g. 6 well) in higher density micro-well format (e.g. 96 and 384 well).

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