Culturing MDBK Cells in the BioLevitator™

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Application Note

Abstract

Mardin-Darby Bovine Kidney (MDBK) cells were successfully cultivated in the BioLevitator. The ideal inoculation density was determined to be 2 million cells per 0.5 mL Global Eucaryotic Microcarriers™ (GEM™). Cell viability was higher that 90%. After amplification yields higher than 75 million cells in 30 mL cultures were reached. This represents the amount of cells obtained from seven to eight T75-flasks.

Introduction

The BioLevitator is a unique benchtop incubator and bioreactor hybrid. The unit is a self-contained, all-in-one incubator and centrifuge. It utilizes a unique microcarrier called the GEM, developed by Global Cell Solutions, to facilitate cell growth and expansion of adherent cell lines. Here we report the successful culture of Mardin-Darby Bovine Kidney (MDBK) cells in the BioLevitator.

Method

MDBK cells were cultured in DMEM/F12 (BioConcept AG) with 10% newborn calf serum (Sigma-Aldrich) and 2 mM L-glutamine (BioConcept AG). 0.5 to 4 million MDBK were inoculated and cultured in the BioLevitator on 0.5 mL pre-washed GEM substrate (Global Cell Solutions). The BioLevitator settings used are shown in Table 1. Inoculation was done in 5 mL conditioned medium from a 4 day old culture and 5 mL fresh medium. 10 mL fresh culture medium was added on day 2 and day 3. From day 4 to the end of the culture, 20 mL culture medium was changed daily.

Inoculation Parameters	
Inoculation time	12 h
Rotation type	2-way
Rotation period	1 sec
Rotation pause	0 sec
Rotation speed	60 rpm
Agitation period	2 min
Agitation pause	40 min

Culture Parameters	
Inoculation time	infinite
Rotation type	2-way
Rotation period	1 sec
Rotation pause	0 sec
Rotation speed	70 rpm

Table 1. BioLevitator parameters for inoculation and culture.

The cultures were sampled daily (sample size was 500 µL) for microscopic observation and growth monitoring. Cell counts and viability were determined using the Nucleocounter (ChemoMetec). Nuclei were stained with Hoechst 33342 (Invitrogen).

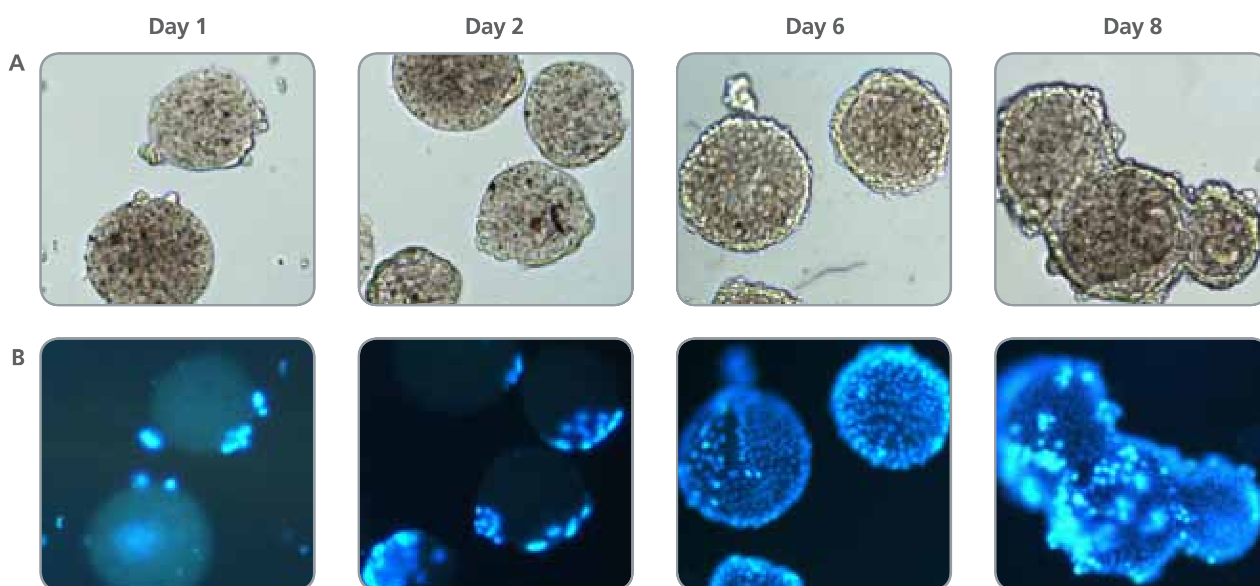

Figure 1. Microscopic observation of loading and growth of MDBK cells on GEM. 2 million cells were inoculated on 0.5 mL GEM substrate and observed under light microscopy (A) and after Hoechst staining (B).

Results

Loading and amplification of MDBK cells was visually monitored by microscopic analysis (Figure 1). 24 hours after inoculation (day 1), successful loading was observed with 3 to 7 cells attached to one GEM. The rounded shape of the cells observed on day 1 disappeared on day 2, indicating that cells were spreading. A layer of cells completely covered the surface of the GEM on day 6. On day 8, the GEM seemed to aggregate with cells growing between two beads. It may be possible to reduce this aggregation by increasing the rotation speed and hence the homogenisation of the GEM suspension at the end of the culture. Alternatively, we suggest to stop the cultures before aggregation starts on day 6 or 7.

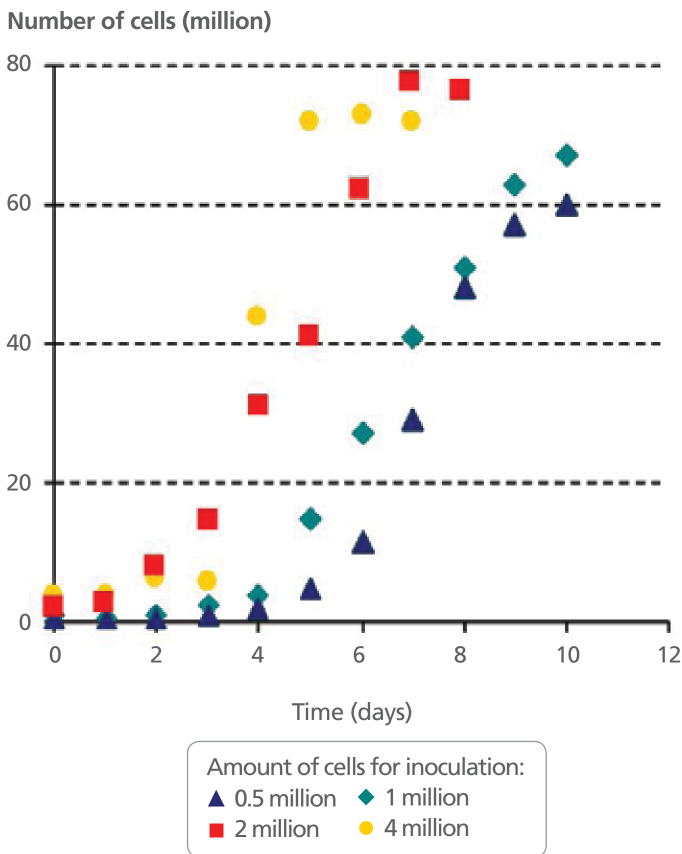

Figure 2: MDBK growth in the BioLevitator. Cultures were inoculated with 1, 2 and 4 million cells and yielded more than 75 million cells.

In addition, growth curves were established (Figure 2). Growth curves obtained after culture in the BioLevitator have a standard shape with 3 phases: a lag-phase, an exponential growth phase and a stationary phase. Four inoculation conditions were compared. The amount of cells for inoculation on 0.5 mL GEM substrate was increased from 0.5 to 4 million cells.

Inoculation with 2 million cells provided optimal growth with a limited lag-phase and a high yield. Using only 0.5 million cells resulted in an extended lag-phase whereas 4 million cells did not provide any improvement in growth or yield. Starting with 2 million cells, a maximal growth rate μmax of 0.049 h-1 was observed, which represents a minimum doubling time of 14 hours. The average growth rate μ was 0.021 h-1 which corresponds to a doubling time of 33 hours. Viability higher than 90% was consistently monitored.

Final yields reached on average 75 million cells from a 30 mL culture. Standard yields from a T75-flask were about 10 million cells. Hence a 7.5-fold yield increase was obtained with the BioLevitator. In other words, one 30 mL culture in the BioLevitator is equivalent to seven to eight T75-flasks. Besides, two passaging steps are required to obtain seven T75-flasks starting with one flask. Hence, cells were trypsinized about 3 times less often when cultured in the BioLevitator, saving time, consumables and reagents while providing healthier cells.

Conclusion

The BioLevitator appeared to be an efficient and straightforward tool for the culture of MDBK cells. Standard yields reached were about 75 million cells from a 30 mL culture, which represents the amount of cells obtained from seven to eight T75-flasks. In addition, we were able to reduce manipulation time, reagents and consumables required as cells were passaged about 3-times less often.

For further information on the BioLevitator, visit: www.biolevitator.com

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