Novel Disposable Bioreactor for Mammalian Cell Cultures and Virus Production

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ABSTRACT

A novel disposable BelloCell® bioreactor has been developed and successfully applied for cultivation of Vero cells and production of Japanese encephalitis virus (JEV). A cell density of 5 $x10^7$ and total JEV titer of $2.5x10^{12}$ pfu/l have been achieved in this bioreactor.

BelloCell® bioreactor has also been successfully applied to grow many cell lines including BHK, C127 and CHO cell lines. For all cell lines a cell density of >5 $x10^7$ or a total cell number of >4.5x $x10^9$ has been obtained in BelloCell ® 300.

Many advantages of this simple, inexpensive laboratory bioreactor system including no requirement of agitator, pump, air sparging or oxygen enrichment, low shear stress, high oxygen transfer rate, no foaming problem, low risk of contamination have been demonstrated.

INTRODUCTION

Oxygen transfer limitation is always the threshold of achieving high density in cell culture. In the stirring tank bioreactor system with or without use of microcarriers, it requires high air sparging and agitation rates to achieve high oxygen transfer but it also generates high shear stress and foaming problem, which are detrimental to animal cells. The hollow fiber bioreactor system is designed to generate lower shear stress and perform perfusion culture, but it requires exterior oxygenation system and high circulation rate to provide sufficient oxygen supply. Furthermore the axial non-uniform distribution of cells and proteins along extracapillary (EC) space because of convective flow in EC may result in serious sedimentation and poor oxygen transfer.

Some commercial packed bed bioreactor system is designed on basis of airlifting principle combined with modified impeller to promote gentle liquid circulation through annular packed bed with low shear stress but good oxygen transfer . However, the height of packed bed limits the industrial scale-up capability for this type of bioreactors. In order to minimize the disadvantages and retain the advantages of the commercialized bioreactors aforementioned, CESCO Bioengineering Inc has developed BelloCell® and TideCell® bioreactors.

In BelloCell® and TideCell®, a massive oxygenating surface area was created with simple medium movement relative to the cell embedded matrix for highly efficient oxygen transfer without any agitation and air sparging. BelloCell® is a disposable bioreactor mainly designed for simple, efficient and economical laboratory use while TideCell® was for pilot and production plant application. In this study the application of BelloCell for growth of Vero, BHK, CHO and C127 cells and production of JEV were illustrated

WHAT IS *BelloCell*® ? Basic Principle

The BelloCell® as shown in Figure 1&3 contains two chambers: the upper chamber contains a porous fiber matrix mounted inside where the cells are embedded; the lower chamber contains a compressible bellows and has culture medium inside. The BelloStage®, a driving device as shown in Figure 2&3, compresses the lower compressible bellow in order to expel the culture medium out of the lower chamber and raise the culture medium level to submerge the matrix in the upper chamber. Alternatively, the BelloStage® lowers compressible bellow in order to lower the culture medium level to expose the matrix to the air. The matrix containing carriers packed in the culture vessel is thus exposed and submerged in the culture medium alternatively controlled by the moving rate of the platform of BelloStage®. During the exposing phase, the cells embedded in the porous carriers will not expose directly to the gaseous environment but through a thin liquid film. Not only this would not cause the damage of the culture cells, but also facilitate the most efficient oxygen transfer from air to the cells. During the submerging phase, the cells expose to the new liquid surface and facilitate the uptake of nutrients from the medium (such as glucose, glutamine and growth factors, *etc*.) and removal of the wastes (such as ammonium, lactic acid *etc*) from the cells.

MATERIALS and METHODS

Vero cell, a continuous African Green Monkey kidney cell line, was purchased from ATCC CCL-81. The cells were grown in a BelloCell 300 using a 300 ml of M199 medium with 5% FBS in an CO2 incubator controlled at 37 C. Semi-batch feeding strategies were adopted by changing 1/3 medium of working volume each time to maintain glucose concentration above 1 g/l. At the end of log growth phase, the growth medium was replaced with a fresh medium.

The culture was then infected by JEV at MOI (Multiplicity of infection) of 0.1 and followed by a semi-batch feeding as done in the growth phase. During the infection period, pH was controlled above 6.8 by CO2 concentration inside of the incubator and glucose concentration maintained above 1 g/l by the medium replacement. The ratio of matrix bed volume and medium volume was predetermined so that the feeding frequency will not be more than twice a day for convenience of operation. The cell density was estimated by dividing glucose uptake rate (GUR) by specific glucose uptake rate, which was predetermined by T-flask studies. The final cell density was measured by a DNA staining method. The JEV titer was measured by a standard plaque assay using BHK-12 cells.

For growth of various cell lines including Vero, BHK, C127 and CHO, the same simple operation procedure was employed as above except different media and carriers as noted in the Table 1 were used.

RESULTS AND DISCUSSION

In Table 1 is summarized the cell densities of various common cell lines grown in BelloCell® 300 bioreactors using various media and fibrous carriers. The growth profiles are shown in Fig. 4. Results indicated that most of animal cells can grow satisfactorily in BelloCell® 300 to a high cell density of >$6x10^7$ and a total cell number of >$4.5x10^9$. In this study a bed volume of 72 cm^3, tide rate of 1 mm/sec with delay time of 10 sec and the maximum frequency of medium exchange of two times a day were applied. No attempt was made to maximize the capacity of the bioreactor.

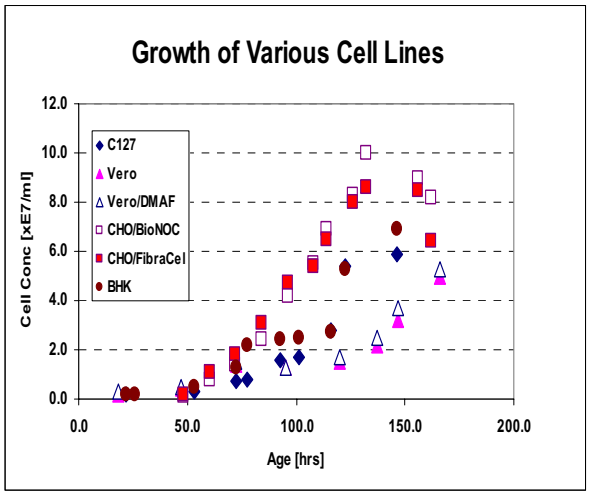
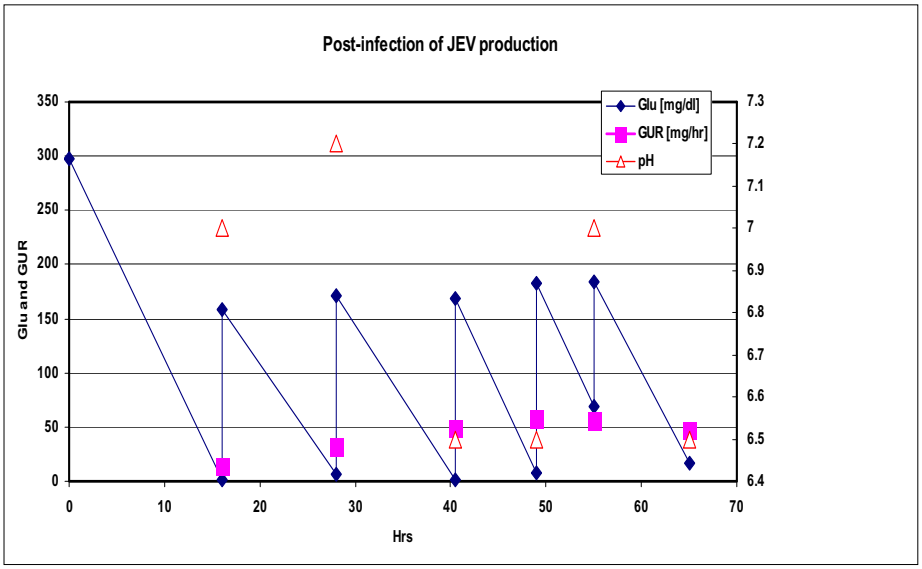


TABLE 1 Summary of Cell densities of Various Cell Lines Grown in BelloCell 300

	Vero [a]	Vero [b]	BHK [c]	C127 [d]	CHO [e]	CHO [f]
Sp. growth rate [hr^{-1}]	0.032	0.031	0.031	0.023	0.036	0.038
Doubling time [hrs]	21.6	22.3	22.3	30	19.2	18.2
Cell density [cells/cm3] [g]	$6.5x10^7$	$6.1x10^7$	$7.5x10^7$	$6.4x10^7$	$9.1x10^7$	$9.8x10^7$
Cell density [cells/cm3] [h]	$5.3x10^7$	$5.4x10^7$	$6.8x10^7$	$6.0x10^7$	$8.6x10^7$	$10.1x10^7$
Total cell number [cells/unit] [g]	$4.7x10^9$	$4.4x10^9$	$5.4x10^9$	$4.6x10^9$	$6.6x10^9$	$7.1x10^9$

[a] cultured in M199 w/ 5% FCS
[b] cultured in DMAF
[c] cultured in DMEM
[d] cultured in MEM w/ 5% FCS
[e] cultured in MEM w/ 5% FCS
[f] cultured in MEM w/ 5% FCS
[g] counted by DNA staining method and based on the total bed volume (72 cm^3)
[h] based on GUR (s-GUR was assumed to be $2x10^{-8}$ mg/cell/hr for all cells) and based on the total bed volume (72 cm^3)

Fig. 5-7 are the growth profile of Vero cells and JEV production profile after virus infection. The same operating parameters mentioned above were applied in this study. The Vero cells were grown to the end of log phase at a cell density of $1.5x10^7$ and then infected with viruses at MOI of 0.1. A total virus particle of $1.07x10^{12}$ was obtained in 65 hrs after infection. Further optimization study will be conducted to investigate the effect of cell density, MOI and post-infection medium on JEV production.



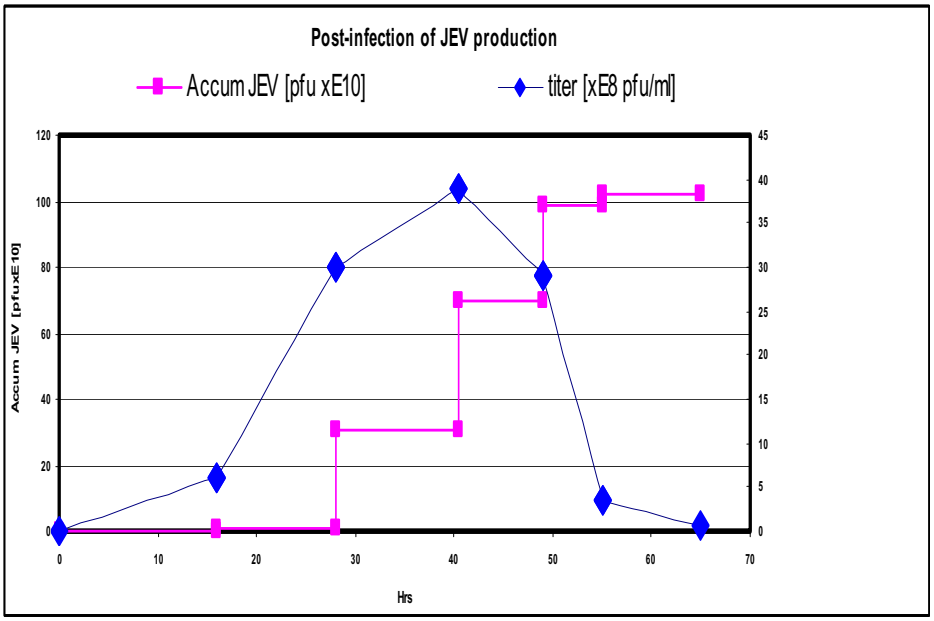


TABLE 2. Comparison of Vero Cell Growth and JEV Production in Various Lab Bioreactors

	T flask	Spinner flask /microcarrier	Roller bottle	BelloCell
Description	T-25 flask /5 ml medium	250 ml flask / 125 ml medium /0.375g Cytodex 1	2000 cm^3 bottle /850 cm^2 /200 ml medium	500 ml bottle/ 300 ml medium / 72 cm^3 carriers
Total surface area (cm^2)	25	2250	850	8640
Mode of operation	Semi-batch	Semi-batch	Semi-batch	Semi-batch
Medium for growth	Plus-Vero III	DMAF / Plus-Vero III	DMAF	DMAF
Cell density (cells/ml of medium)	8×10^5	2.5×10^6 /6.96×10^5	1.48×10^6	1.5×10^7
Total cell number	4×10^6	3.13×10^8 /8.7×10^7	2.96×10^8	4.4×10^9
Medium for virus production	Plus-Vero III	MFKB /Plus-Vero III	MFKB	MFKB
Multiplicity of infection (MOI)	0.1	0.1	0.1	0.1
Total virus production (pfu)	5×10^8	2.5×10^{10} /1.91×10^{10}	5.8×10^{10}	1.07×10^{12}

In Table 2 is the comparison of various lab bioreactor systems for Vero cell growth and JEV production. It shows that one BelloCell® 300 can have a surface area of the carriers equivalent to that of 10-15 roller bottles or 10-15 spinner flasks (250ml) with microcarriers. Accordingly, equivalent amount of cells and JEV were produced

CONCLUSION

∗ BelloCell® is a simple, efficient, disposable laboratory bioreactor for animal cell culture. No moving parts, no air sparging, no pumping, no agitation is required. Easy and convenient to use.

∗ BelloCell® yields high OTC, low shear rate and high cell density for animal cell culture. It has been successfully applied for growth of Vero, BHK, C127, CHO cells and production of Japanese encephalitis virus.

∗ BelloCell® can be used for batch and semi-batch cultures.

∗ BelloCell® can be easily scaled up to TideCell® bioreactor. TideCell ® bioreactor was derived from the same principle and designed for large scale of production.