Flow Cytometry as a Tool for Process Monitoring of Virus-cell Systems

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Abstract: Physiological and morphological changes upon infection by viruses have been observed in many cell lines used for the production of proteins and viruses. Here we demonstrate the utility of single-cell analysis to monitor various cellular parameters in insect cells-baculovirus and HEK293-adenovirus systems, including DNA content, cell size and cell granularity and their correlation with the propagation of virus and production of recombinant protein. Analysis of infected cells at the single-cell level by flow cytometry was shown to be an effective method to monitor the changes in virus titre and intracellular protein. The results show that flow cytometry is an effective tool for process monitoring of virus-cell systems that allows the design of optimum harvest strategies.

Key words: Flow Cytometry, Adenovirus, Baculovirus, HEK293, Sf9, GFP, Cell size, Granularity, DNA.

1. INTRODUCTION

Physiological and morphological changes upon infection by viruses have been observed in many cell lines used for the production of proteins and viruses. Measurements of morphological and physiological parameters, primarily based on measuring fluorescence can be simply achieved by flow cytometry. The technique has the facility for detection and quantification of specific changes in each cell within population. This may provide powerful evidence in the assessment of effects of viral infection on cellular activity

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and enable the identification and characterisation of heterogeneous cell populations. Here we utilise single-cell analysis to monitor DNA content, cell size and cell granularity in HEK293-adenovirus and insect cells-baculovirus systems and correlate these parameters with the propagation of virus and production of recombinant protein.

2. RESULTS AND DISCUSSION

2.1 Animal cell (HEK293)-Adenovirus(Ad5GFP) results

The relationships between Ad5GFP titres & relative DNA content, relative cell size & relative granularity in the cells after infection with MOI's 1, 10, and 50 are shown in Table 1. The correlation coefficients (R^2) indicate that best correlation is obtained with DNA content.

Table 1. The correlation parameters between cellular attributes and virus titre.

Parameter correlated with Ad5gfp titre	Equation	R^2
DNA content	Y=3E-08x +486.13	0.910
Cell Size	Y=3E-08x +307.08	0.840
Granularity	Y=3E-08x +246.07	0.748

2.2 Insect cell (Sf9)-baculovirus(AcNPV-lacZ) results

Table 2 shows a decrease in viable cell number with increase in MOI. However, there is an increase in %DNA content of cells proportional to the increase in MOI. Similar increase was found in cell size and granularity as well as in β-galactosidase production, all of which are proportional to the increase in MOI. The results suggest that flow cytometric monitoring of cellular parameters can give a good indication of productivity. FC could detect the increasing DNA content as the virus multiplies with time relative to the MOI and in a manner that would predict infectivity and productivity level.

Table 2. Changes in cellular attributes at various MOI's with time as a % of MOI 50 value.

Parameter measured at 27 hr pi	MOI 0.1	MOI 1	MOI 10	MOI 50
DNA content	66	74	90	100
Cell Size	79	82	94	100
Granularity	58	71	91	100
Production of B-galactosidase	25	37	62	100
Viable Cell Number X10E5/ml	10.9	9.3	6.7	5.3

3. CONCLUSION

Analysis of infected cells at the single-cell level by flow cytometry was shown to be an effective method to monitor the changes in virus titre in HEK293 cells and intracellular recombinant protein in insect cells.

The results show that flow cytometry can be an effective tool for process monitoring of virus-cell systems that may allow the design of optimum harvest strategies. Understanding and prediction of the virus infection and multiplication process are possible with the aid of DNA, cell size and cell granularity analysis which can also provide the data necessary to address the problem of modelling cell population-virus infection dynamics. For example by measuring the DNA content, the replication of adenoviruses and baculoviruses in cells can be predicted and specific control actions recommended.

We also found that the naturally fluorescing reporter gene product, GFP showed a good correlation with viability, virus titre and the proportion of GFP positive cells in infected HEK293 heterogeneous populations (data not shown) which supports the evidence that FC is clearly important technique for cell culture process identification and a sophisticated early process sensor.