Time Dependent Virus Replication in Cell Cultures

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Abstract. We present in this report a stochastic model for the virus replication of influenza A in a cell culture. We consider not only the infection process of individual cells but also the number of intracellular components expressed in virus equivalent. Given that this expression is non constant in time we suggest a variable threshold, related to a viral resistance in the cell population, that could explain the time variation in the viral expression in the cell seen in experiments.

Keywords: Virus Replication, Influenza A, Cellular viral Resistance.

1 Introduction

The inoculation of pathogens has been used for a long time in several cultures around the world as a method to boost the resistance of the population against viral infections. For instance, Voltaire describes how the inoculation of pustules in small children has been used by Circassian womans as protection against small-pox, a method that was later introduced in England in the XVIII century [1]. Since its introduction and further development in Europe, this method has become a fundamental element in modern medicine. A vaccine consists of a weak form of a given pathogen that later is inoculated to an individual that has not been infected. As a consequence the individual is infected in a controlled way, inducing a reaction of the individual's immune system, which not only attacks the virus but also learns to recognize such kind of pathogens. This makes this individual immune against this pathogen.

The use of vaccines requires its efficient production. In order to optimize such production process it is necessary to understand how the infection in a cell culture works. However, there is no enough information about virus-host cell interaction in a cellular level and virus spreading in populations of cells in bioreactors. In this report we describe the replication dynamics of influenza A virus in mammalian cell cultures.

The replication of Influenza A virus has been extensively described in several works [2,3]. In this frame the infection of the cell population, and not the virus

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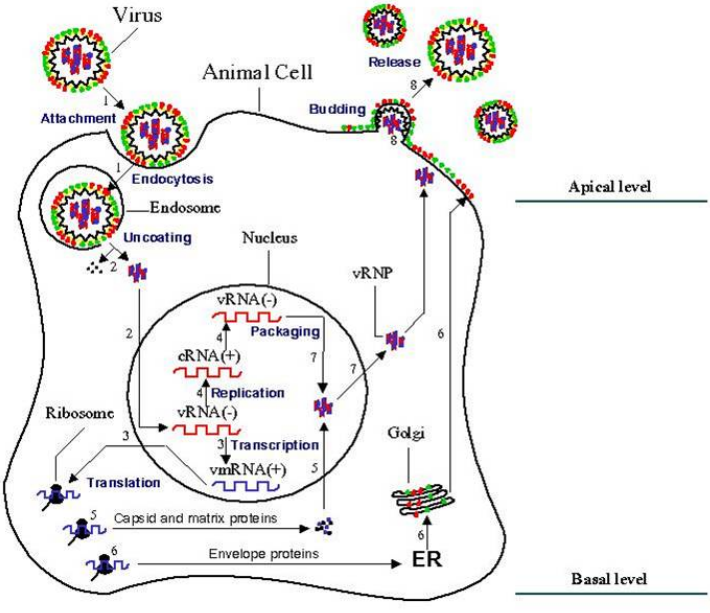

Fig. 1. Replication cycle of Influenza A Virus.Following virus attachment to the surface's receptos of the cell, virions are incorporated to the cell. In diferent steps the genome is transfered to the nucleus, virus protein syntesis and virus genome replication starts and the virus life-cycle ends with the budding and generating of new virus particles (Figure from Sidorenko et al. [6]).

propagation inside a given human population (and the interaction of the virus with the immune system) -see for instance [4,5]-, is the relevant question of this investigation. The studied virus basically consists of a polar DNA molecule encapsulated in a protein membrane forming two basic structures; hemaglutinin and neuramidase. After virus attachment to the cell membrane the genome is transferred to the nucleus. Thereafter virus protein synthesis and virus genome replication starts and the virus life cycle ends with the release of newly generated virus particles (See Fig. 1).

In some works the intracellular process of the infection cycle was considered [6], whereas in other approaches the initial steps of infection and endocytosis were implemented [9]. Other investigations have showed, how the ratio in the production rates of different viral strains is, a relevant problem in order to analyze drug resistance of different viral variants [8,7]. However, the present model describes systems where there is only one class of viral species, focusing more on the specific infection mechanism of the cell rather than on the concurrence with different viral variants. In a nut shell, the virus spreading in populations as well as the differentiation of infected cells is not well understood (those are two important factors for the optimization of vaccine production). In the present

work we extend the investigation with the development of different variants of adequate stochastic models for the replication of a virus population in a cell culture based on a model sugested by Sidorenko et. al. [10].

Given that such model is not able to reproduce a time variation in the cells infection grade, observed by means of experimental techniques, we propose in the present approach mechanisms that could explain this variation. In particular we consider the variability of the resistance of the infected cells, represented by a non constant probability of infection, as a plausible mechanism explaining the non monotone infection behavior of the cells. In the next section we introduce the fundamental assumptions of the model and the implementation strategies.

2 Model and Strategies

The present approach considers a distributed balance model, accounting for the stochastic nature of the infection process (See Fig. 1). In previous works a similar approach were adopted, allowing a qualitative and quantitative description of the replication process [10,11]. In such model the interaction between virus and host cells is explicitly represented. It is also assumed that the infection process takes place depending on the concentration of virions. Hence, the fundamental assumption is that the virus infection and replication in the cell culture takes place as a consequence of the adsorption of free virions and not simply as the direct contact among cells. This assumption additionally implies that the spatial distribution of virions and cells is not explicitly considered (from the experimental point of view the spatial distribution is not relevant, because the cell culture is well mixed in a bioreactor). The basic formulation of the cell infection and degradation is similar to the mathematical basis of virus population dynamics introduced by Nowak and May [12], where the population dynamics is represented as a balance of infected and degraded cells. However, here the virus expression is heterogeneous among the cell population. Therefore it is neccesary to introduce an internal coordinate J that corresponds to the intracellular number of viral components expressed in virus equivalents (VE). For the sake of the modeling of the system, this internal coordinate gives the different possible reproduction pathways of the virus inside the cell. Experimentally this number of VE is equivalent to the fluorescence intensity of the expressed cells.

The dynamics is modeled by means of a kinetic Monte Carlo method [13], which requires different transition probabilities for each simulation step. An individual cell can be infected, remain uninfected or suffer for degradation. One process is the degradation of individual cells. If the cell survives then the internal coordinate can adopt either the value $J + 1$, for virus replication, or $J - 1$, for virus release. The change of this internal coordinate represents a change of the class of the cell. Naturally it is assumed that a virus release is only possible if $J > 0$. If the internal state reaches a maximal state J_{max} then the cell does not releases new viruses. The total population of free virions is again described using a population balance equation.

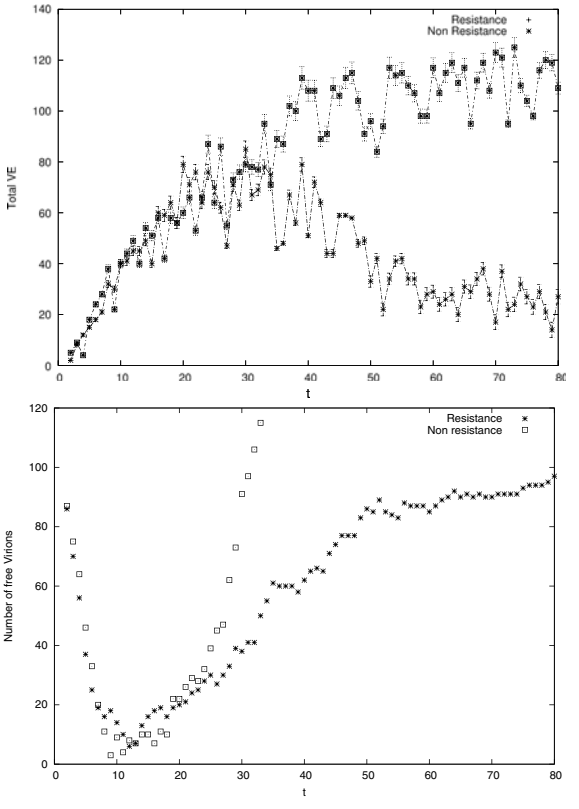

Fig. 2. Frequency of virus replication in the cell population (upper figure) and mean number of free virions (lower figure) as a function of the time for two scenarios: when the cell population develops a kind of resistance agains the virus and when there is no resistance. In the second scenario the frequency of virus replication decrease after some initial time regime (the lines are guides for the eyes).

This basic approach qualitatively (and quantitatively) represents the virus spread in a culture. However, this approach does not account the time variation of the cells infection grade, which has been experimentally obtained using flow calorimetry and fluorescence techniques. Essentially it appears to be that constant infection and release rates do not correctly represent the cellular dynamics. In particular, the initial implementation assumes that the cellular culture is simply infected depending on the concentration and a constant penetration mechanism of the virus into the cell. However, it is also possible to assume that the cells, after some time regime, are able to develop some incipient form of resistance. Naturally, this resistance is a cellular mechanism is not related to the action of an antibody system. In particular, cell signaling mechanism and media mediated transport allow the growth of the concentration of interferons in the cell culture, which have an antiviral function in the cellular host [14].

In order to test this idea we propose that the expression of virus into the cell depends on some threshold related to the whole population of infected cells, assuming that some signaling mechanism advertise the population of non infected cell, increasing their resistance

$$R = \Theta(\Sigma_i V_i - Pr) \tag{1}$$

where $\Theta(x)$ is the step function, R is the resistance probability, Pr the resistance threshold, where $Pr = (0, V_{Max}]$, with V_{Max} the minimal viral population when there is no cell resistance; V_i are the free virions in the reactor. With this assumption, at some particular time the expression process is stopped, reducing the number of free virions. This simultaneously reduces the number of cells in the population able to express the virus, producing a time variation in the VE. This time variation qualitatively shows that a similar mechanism should be taking place into the cell culture (In [10] experimental results are reported).

The present investigation will be extended to a more detailed description of the role of the host defence system in the dynamics of the virus population; a detailed comparison with experimental results will be shown in future works. Aditionally, a spatial distribution is not considered in this report. However,the present results will help to consider if the the spatial distribution of the virus particles, in particular if the formation of a kind of biofilm, in the microcarriers play also a role in the regulatory process of the virus production.

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