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A multiscale model of influenza A virus replication covering highly different infection conditions in cell cultures

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Abstract: The multiplicity of infection (MOI) is an important parameter for influenza A virus replication in animal cell culture. Depending on the respective question addressed in research or vaccine production, a range comprising several orders of magnitude is typically covered. Mathematical models, however, have paid little attention to the impact of MOI on virus replication dynamics so far. To overcome this limitation, we implemented kinetics that consider the time point of cell infection in an existing model of influenza A virus replication. We find that the extended model closely captures infection dynamics for MOI conditions ranging from 10^{-4} to more than one infectious virion per cell. Additionally, this model allows the reproduction of both the concentration of infectious virions and the total number of virus particles. Therefore, the extended model is well suited to predict process performance and to optimize influenza A virus production in cell cultures.

Keywords: Modeling, Biotechnology.

1. INTRODUCTION

Influenza A virus infection poses a permanent risk to human health due to seasonal epidemics causing up to 500,000 deaths every year. The common strategies of prevention and intervention, vaccination and antiviral treatment, can be complemented substantially by insights obtained from mathematical modeling. Previously, model-based analyses were performed to investigate viral infection spread (Lee et al., 2015) and cell culture-based influenza vaccine production (Heldt et al., 2013). Yet, these models were lacking a key feature, the ability to describe a broad range of multiplicities of infection (MOI). Experimental studies showed that the MOI has a significant impact on several critical aspects of influenza A virus replication including infection spread dynamics and virus titres. Additionally, process yields in cell culture-based vaccine production can be greatly increased by MOI optimization (Aggarwal et al., 2011). A major challenge for constructing a mathematical model capable of capturing viral dynamics in both low and high MOI infections results from the interaction of this parameter, typically chosen as a fixed initial condition, with several kinetic expressions and state variables of the model.

2. RESULTS

To enable the description of cultivations using adherent MDCK cells for a broad range of MOIs, we modified a multiscale model of influenza A virus infection in cell cultures recently developed in our group (Heldt et al., 2013). This model combines a detailed description of intracellular virus replication dynamics with a segregated cell population. The intra- and the extracellular level are linked by a virus particle release that is dependent on the time elapsed since a cell was infected, i.e. the infection age τ, which allows the reproduction of experimental results for low MOI infection conditions (MOI<10^{-1}). However, model predictions for a high MOI=10 failed to capture cell infection dynamics (Fig. 1A) and viral mRNA accumulation (Fig. 1D) (Frensing et al., 2016). To adjust the model for the distinct dynamics exhibited by medium to high MOI infections, we introduce an additional "infection age-dependent" (IAD) kinetics on the extracellular and a state variable on the intracellular level.

The initial step was the implementation of an IAD apoptosis rate for infected cells, i.e. a delay in apoptosis induction. Therefore, we tested a logistic function, the Hill equation, and a Gombertz function. After comparing the approaches we decided on using a logistic function

$$k_{\mathrm{I}}^{\mathrm{Apo}}(\tau) = \frac{K_{\mathrm{I}}}{1+\exp(-\nu_{\mathrm{LS}}(\tau-\tau_{\mathrm{LS}}))} \tag{1}$$

as it resulted in the lowest sum of squared residuals. Furthermore, the introduced parameters are connected to various biological processes, i.e. τ_{LS} as the average infected cell survival time, and ν_{LS} as its inverse distribution. Another modification of the model of Heldt et al. was the introduction of a new state variable to describe the total virus particle concentration, which is important in manufacturing of inactivated influenza vaccines as it correlates directly with harvest yields. In particular, we take into account that immediately after infection, a higher percentage of the released virus particles is infectious than at later stages. Therefore, we implemented the fraction of infectious particles released as a first order degradation

$$\frac{dF_{\mathrm{Par}}}{dt} = -k_{\mathrm{Red}}^{\mathrm{Rel}} F_{\mathrm{Par}} \tag{2}$$

where F_{Par} is the ratio of infectious to total particle release and $k_{\mathrm{Red}}^{\mathrm{Rel}}$ denotes the decrease of this ratio. The adjusted model containing the additional IAD functions was then calibrated

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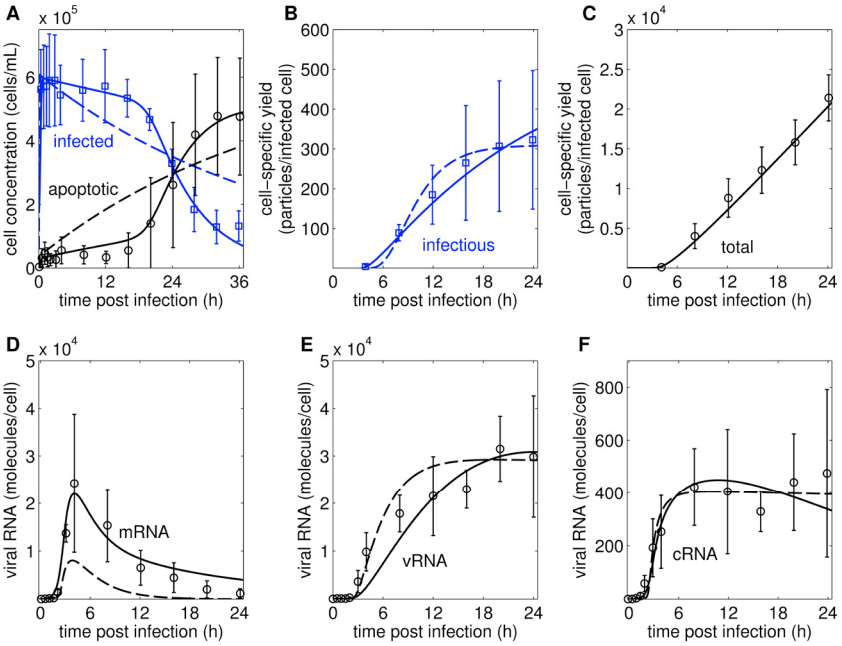

Fig. 1. The extended model of influenza virus replication captures intra- and extracellular infection dynamics: Curves depict the model fit to (A) cell population, (B) infectious and (C) total cell-specific yield, and (D-F) viral RNA measurements from adherent MDCK cell cultures infected with influenza A/PR/8/34 (H1N1) at 10 PFU per cell (Frensing et al., 2016). Dashed lines indicate the model fit of the original model (Heldt et al., 2013), solid lines represent the model fit of the extended model.

to experimental data obtained for MOI=10 (Frensing et al., 2016), which results in the synchronous infection of all cells. Besides, virus dynamics, molecular dynamics (viral RNA) and population dynamics (infected and apoptotic cells) were investigated in the same experimental setup. Our results show that the extended model describes both intra- and extracellular dynamics of this high MOI experiment well (Fig. 1). In particular, the dynamics of infected cell apoptosis and the distinct viral mRNA peak can be captured closely.

To test the applicability of this model for low MOI conditions, we checked its ability to describe experimental data that were used for the validation of the original model (Heldt et al., 2013). Following re-calibration, the extended model successfully reproduced viral replication dynamics for low MOI conditions and provided a better fit than the original model (data not shown).

3. DISCUSSION

We implemented IAD kinetics in a multiscale model of influenza A virus infection to describe infection dynamics for MOIs differing significantly from each other.

By distinguishing between infectious and total virus particles, the model can describe $TCID_{50}$ and HA assay results, two quantification methods relevant in cell culture-based influenza vaccine production. Taking into account explicitly the change in the fraction of released infectious particles, i.e. a decline over time resulting in a mostly non-infectious particle release at late infection stages, corresponds to experimental data obtained. The biological mechanisms involved are not fully understood but a decline of cellular resources and effects related to virus-induced apoptosis and genome packaging play a crucial role. In case the timing of these events could be influenced by cell line engineering (i.e. a delay in cell death) or cultivation conditions, higher yields in influenza vaccine production would be feasible.

In summary, we developed a mathematical model that reproduces the dynamics in both low and high MOI infection conditions. Results obtained suggest that this is another step towards a predictive model for influenza A virus infection in animal cell culture. Follow-up studies are planned to confirm the predictive power of this model. In addition, we want to investigate whether the model allows a description of different infection conditions without re-calibration. Therefore, further experiments utilizing the same experimental setup as for the high MOI infections (Frensing et al., 2016) need to be performed.

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