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Critical phases of viral production processes monitored by capacitance

Emma Petiot[a,b,*], Sven Ansorge[a], Manuel Rosa-Calatrava[b], Amine Kamen[a,c]

[a] NRC, Human Health Therapeutics Portfolio, 6100 Royalmount Ave, Montréal, QC, H4P 2R2, Canada
[b] Virologie et Pathologie Humaine – VirPath Team, International Center for Infectious diseases Research, Inserm U1111, CNRS UMR5308, ENS Lyon, Université Claude Bernard Lyon 1, Faculté de Médecine RTH Laennec, Lyon, France
[c] McGill University, Bioengineering Dpt. 817, Sherbrooke St. W., Montreal, QC, H2 B 2C6, Canada

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ABSTRACT

Over the last decade industrial manufacturing of viral vaccines and viral vectors for prophylactic and therapeutic applications is experiencing a remarkable growth. Currently, the quality attributes of viral derived products are assessed only at the end-point of the production process, essentially because in-process monitoring tools are not available or not implemented at industrial scale. However, to demonstrate process reproducibility and robustness, manufacturers are strongly advised by regulatory agencies to adopt more on-line process monitoring and control. Dielectric spectroscopy has been successfully used as an excellent indicator of the cell culture state in mammalian and yeast cell systems. We previously reported the use of this technique for monitoring influenza and lentiviral productions in HEK293 cell cultures. For both viruses, multi-frequency capacitance measurements allowed not only the on-line monitoring of the production kinetics, but also the identification of the viral release time from the cells. The present study demonstrates that the same approach can be successfully exploited for the on-line monitoring of different enveloped and non-enveloped virus production kinetics in cell culture processes. The on-line monitoring multi-frequency capacitance method was assessed in human HEK293 and Sf9 insect cells expression systems, with viral productions initiated by either infection or transfection. The comparative analyses of all the data acquired indicate that the characteristic capacitance signals were highly correlated with the occurrence of viral replication phases. Furthermore the evolution of the cell dielectric properties (intracellular conductivity and membrane capacitance) were indicative of each main replication steps. In conclusion, multi-frequency capacitance has a great potential for on-line monitoring, supervision and control of viral vector production in cell culture processes.

1. Introduction

In recent years, an increasing number of viral products have been evaluated and approved, either for vaccination or therapeutic approaches, and numerous vectors and viruses are currently evaluated in preclinical and clinical research programs (Brun et al., 2008; Buonagurio et al., 2006; Draper and Heeney, 2010; Haupt and Sings, 2011; Lentz et al., 2011; Van Gessel et al., 2011). Virus production is a broad and highly diverse field, comprising different virus types, production methods and the use of various cellular platforms (Ansorge et al., 2009; Aucoin et al., 2007; Durocher et al., 2007; Ghani et al., 2006; Kamen and Henry, 2004; Knop and Harrell, 2007; Le Ru et al., 2010; Meghrous et al., 2005; Paillet et al., 2009; Pau et al., 2001; Petiot et al., 2012b; Whitford and Fairbank, 2011; Wong et al., 1994). Manufacturing processes require high levels of compliance and quality control. Consequently, guidelines from the regulatory agencies recommend to systematically monitor the "quality" of such productions, not only as an end-point control, but also through implementation of continuous in-process analytical and monitoring technologies. It will allow to document the process and assess its robustness and reproducibility. The goal of this approach is ultimately to i) in-line monitor the accumulation of bioactive viral particles in the cell culture, ii) enable quality-by-design of the process by ensuring in real-time product quality, process consistency and reproducibility (FDA, 2004).

In general, on-line monitoring of the accumulation of the bioactive viral particles in cell culture remains for the moment out of reach due to the fact that non-invasive methods have not been developed to achieve this goal. However, monitoring and super-

* Corresponding author at: Université Claude Bernard Lyon 1, Laboratoire VirPath, CIRI Inserm CNRS U1111, Faculté de médecine RTH Laennec, 7 rue Guillaume Paradin 69008 Lyon, France.
E-mail addresses: emma.petiot@univ-lyon1.fr (E. Petiot), sven.ansorge@cnrc-nrc.gc.ca (S. Ansorge), manuel.rosa-calatrava@univ-lyon1.frm (M. Rosa-Calatrava), amine.kamen@mcgill.ca (A. Kamen).

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vision of key process parameter trends are feasible when using state-of-the-art techniques. To do so, we propose to focus on methods measuring the morphological and physiological alterations of the cell population that occur during each step of the viral replication cycle (cellular entry, replication, genome assembly, budding and/or cell lysis). Many events are associated with the cell infection process and the viral replication cycle. The physiology and morphology of the infected cells are significantly affected. The viral hijacking of cellular machineries and associated remodeling of their ultrastructure and compartments lead to strong modifications of the cellular and the cell populations 'fingerprints' (Josset et al., 2008; Terrier et al., 2012). During the viral production cycles, the intracellular content, the membrane protein composition as well as its integrity are very likely to change. For example, Sf9 cell size increases during baculovirus productions (Palomares et al., 2001), influenza and HIV viral infections induce cell apoptosis (Al-Rubeai and Singh, 1998; Hinshaw et al., 1994) and cytolytic viruses are responsible for modifications of the cell membrane permeability (Costin, 2007). Therefore, the biomass (a function of cell size and number) and the physiological characteristics of the cultivated cells are primary indicators of the progress of viral infection and progeny viral particle production.

Capacitance is probably one of the best and most advanced techniques for the on-line monitoring of cell biomass and physiological state and has been extensively described over the past 20 years (Ansorge et al., 2007; Ansorge et al., 2010b; Elias et al., 2000; Opel et al., 2010; Zeiser et al., 1999) It measures the cell biovolume and has been shown to respond to changes in a number of cellular properties. Its principle is based on the theory of Schwan where cells are considered as small capacitors. The models describing cell capacitance behaviour under polarization at different frequencies link dielectric constants (the permittivity ($\Delta\varepsilon_{max}$) and the characteristic frequency (fc)) to the physiological characteristics of cultivated cells and their biomass (the intracellular conductivity (σi), the membrane capacitance (Cm), the cell biovolume (Bv), and the cell size (r)). This technology has already been assessed and successfully used to monitor growth and death for a number of different cell lines (Ansorge et al., 2010a,b, 2007a,b; Ansorge et al., 2007; Ducommun et al., 2002; Noll and Biselli, 1998; Opel et al., 2010; Párta et al., 2013; Petiot et al., 2012a; Zalai et al., 2015; Zeiser et al., 1999). In addition, the potential to use $\Delta\varepsilon_{max}$ or fc signals to monitor the viral production process evolution has been established (Ansorge et al., 2011; Petiot et al., 2012b).

In parallel, complementary off-line methods, such as electrorotation and dielectrophoresis, have been used to assess correlations between cell dielectric properties (σi, Cm) and specific biological characteristics (Arnold and Zimmermann, 1988). These include cell membrane thickness and folding which both impact the Cm value (Archer et al., 1999; Genet et al., 2000; Zimmermann et al., 2008). Furthermore, the two dielectric parameters, membrane capacitance (Cm) and intracellular conductivity (σ_i), can be inferred from the incremental values of permittivity ($\Delta\varepsilon_{max}$), characteristic frequency (fc), medium conductivity (σ_m) and cell size (r) using Schwan model (see equations 3 & 4). Also, Cm and σi have been used to study the impact of cell death on macroscopic signals (Opel et al., 2010; Tibayrenc et al., 2011), and intracellular conductivity was recently identified as an indicator of the cell physiological state in cell culture process (Ansorge et al., 2010b).

In the present study, the on-line capacitance data from different viral production systems (virus type, cell-lines, production mode) were extensively analyzed. Three enveloped viruses (lentivirus, influenza virus, baculovirus) and one non-enveloped virus (reovirus) were produced on two cellular platforms, HEK293 cells and Sf9 cells. All the viruses were produced by direct infection with the exception of lentivirus that was generated from a co-transfection of four plasmids. The selected viruses were different in many aspects, including the localization of viral replication (nucleus vs cytoplasm) and assembly (cytoplasm vs cell membrane), the mode of viral release (budding vs lysis) and the viral l morphology (spherical vs rod-shape) which broadened the scope of the study and allowed to propose general conclusions. Moreover, two different multiplicities of infection (MOI) were used in baculovirus productions to evaluate the impact of viral production kinetics on the capacitance monitoring signals. Signal trends were correlated with biological events occurring during the viral replication process. These biological events were directly correlated with cell dielectric parameters profiles (r, Cm and σi) focusing on three key phases for any viral production i) intracellular accumulation of viral components and viral assembly (**Phase I**), ii) viral release (**Phase II**) and iii) cell death (**Phase III**). Overall the results of this work demonstrate that dielectric spectroscopy is a convenient and generic on-line method to detect phases and progression of viral productions.

2. Material & methods

2.1. Viral productions

Different viruses and modes of production were selected for their variable effects on cellular morphology and cell dielectric properties during the course of viral replication. Typical virus production runs were monitored using an on-line capacitance probe and one non-enveloped and three enveloped virus productions were analyzed in detail. To do so, we focused on the analysis of infectious viral particles which we are considered as having completed their viral life cycle.

On-line measurements from the capacitance probe acquired the dielectric parameters of the cultures as described in the M&M Section 3. The descriptions of the bioreactor set-up and feeding strategy have been already presented in detail in the associated published work for lentivirus (Ansorge et al., 2011), influenza virus (Petiot et al., 2011) and baculovirus (Bernal et al., 2009). The cultures presented herein for influenza and lentivirus productions were also part of these articles, describing either viral production process development and/or production kinetics. Both Reovirus and Baculovirus cultures are original work that was not previously published, but for baculovirus viral production kinetics (cell density, cell viability, viral titers) were comparable with previous work published by the group on baculovirus production processes (Elias et al., 2000). Critical information necessary for the understanding of the present work is provided in the following section and Table 1.

2.1.1. Non-enveloped virus production: reovirus production

Reovirus production in HEK 293 cells was selected to represent a non-enveloped viral production process. Productions were performed in batch mode including a dilution of the culture prior to infection. Cultures were inoculated at cell density of 0.4×10^6 cell/mL in I.A. 65719 medium (SAFC) supplemented with 4 mM glutamine. The dilution-step was performed immediately before infection after 2 days of culture once a cell density of $1.8 - 2.4 \times 10^6$ cell/mL was reached. The bioreactor system used was a Chemap 3.5 L (working volume 2.8–3 L), previously described in Le Ru et al. (2010), but with minor modifications.

2.1.2. Enveloped virus productions

2.1.2.1. Influenza virus production. Influenza virus was produced in a 3.5-L Chemap bioreactor with a 2.9 L working volume. The bioreactor set-up has been already well described elsewhere (Le Ru et al., 2010). Cells were seeded at 0.25×10^6 cell/mL and were infected when the culture reached 6×10^6 cell/m. Infection of culture is performed at a MOI of 0.01 with the preliminary addition of TPCK-Trypsin (Sigma) at a final concentration of 1 μg/mL. Progeny