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Influenza viruses production: Evaluation of a novel avian cell line DuckCelt®-T17

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ABSTRACT

The influenza vaccine manufacturing industry is looking for production cell lines that are easily scalable, highly permissive to multiple viruses, and more effective in term of viral productivity. One critical characteristic of such cell lines is their ability to grow in suspension, in serum free conditions and at high cell densities. Influenza virus causing severe epidemics both in human and animals is an important threat to world healthcare. The repetitive apparition of influenza pandemic outbreaks in the last 20 years explains that manufacturing sector is still looking for more effective production processes to replace/supplement embryonated egg-based process. Cell-based production strategy, with a focus on avian cell lines, is one of the promising solutions. Three avian cell lines, namely duck EB66®cells (Valneva), duck AGE.CR® cells (Probiogen) and quail QOR/2E11 cells (Baxter), are now competing with traditional mammalian cell platforms (Vero and MDCK cells) used for influenza vaccine productions and are currently at advance stage of commercial development for the manufacture of influenza vaccines.

The DuckCelt®-T17 cell line presented in this work is a novel avian cell line developed by Transgene. This cell line was generated from primary embryo duck cells with the constitutive expression of the duck telomerase reverse transcriptase (dTERT). The DuckCelt®-T17 cells were able to grow in batch suspension cultures and serum-free conditions up to 6.5×10^6 cell/ml and were easily scaled from 10 ml up to 3 l bioreactor. In the present study, DuckCelt®-T17 cell line was tested for its abilities to produce various human, avian and porcine influenza strains. Most of the viral strains were produced at significant infectious titers (>5.8 log TCID50/ml) with optimization of the infection conditions. Human strains H1N1 and H3N2, as well as all the avian strains tested (H5N2, H7N1, H3N8, H11N9, H12N5) were the most efficiently produced with highest titre reached of 9.05 log TCID50/ml for A/Panama/2007/99 influenza H3N2. Porcine strains were also greatly rescued with titres from 4 to 7 log TCID50/ml depending of the subtypes. Interestingly, viral kinetics showed maximal titers reached at 24 h post-infection for most of the strains, allowing early harvest time (Time Of Harvest: TOH). The B strains present specific production kinetics with a delay of 24 h before reaching the maximal viral particle release. Process optimization on H1N1 2009 human pandemic strain allowed identifying best operating conditions for production (MOI, trypsin concentration, cell density at infection) allowing improving the production level by 2 log. Our results suggest that the DuckCelt®-T17 cell line is a very promising platform for industrial production of influenza viruses and particularly for avian viral strains.

1. Introduction

For the last twenty years, and since the outbreaks of avian H5N1 influenza strain (1997) and the pandemic porcine H1N1 influenza strain (2001), World Health Organisation (WHO) and influenza vaccine manufacturers are looking for new production processes

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Table 1
Comparison of cell line production capacity for influenza virus.

Cell lines		Infectious titers, log TCID50/ml	HA titer, log HAU/ml	Ref.
MDCK adherent	Mammalian	10^{8-9} log TCDI50/ml	2.5–3.0 log HAU/100 μl	Lohr, V. et al. [7]
VERO adherent	Mammalian	10^{6-8} log TCDI50/ml	2.3–2.6 log HAU/100 μl	Genzel, Y et al. [25]
MDCK suspension	Mammalian	10^{9-10} log TCDI50/ml	3.0–4.0 log HAU/100 μl	Huang, D et al. [9]
HEK293 suspension	Human	10^{8-9} log TCDI50/ml	2.5–3.0 log HAU/100 μl	Petiot, E. et al [20]
CAP® suspension	Human	10^{8} log TCID50/ml	2.5 log HAU/100 μl	Genzel, Y. et al. [1]
AGE1.CR.pIX® suspension	Avian	10^{7-8} log TCDI50/ml	1.75–2.0 log HAU/100 μl	Lohr, V. et al. [26]
DuckCelt®-T17 suspension	Avian	10^{7-8} log TCDI50/ml	1.5–1.8 log HAU/100 μl	Present work

able to supplement and/or replace historical embryonated egg-based process in order to efficiently react to such threats. Indeed, in the case of a pandemic outbreak issued from animal reservoir, and more specifically in the case of avian influenza viruses, vaccine manufacturing based on ovocultures present several drawbacks to be able to quickly respond and provide in time vaccine doses. The time needed between strain selection and vaccine supply partly due to strain adaptation to eggs, the egg supply and the ineffective growth of some vaccine seeds on eggs, are major drawbacks among the different problematics to overcome to rapidly deliver vaccine doses. In addition, annual vaccine production still face many challenges, as the alteration in virus antigenicity due to adaption of virus to eggs [1].

Consequently, all these challenges necessitated the development of several original approaches to complement egg-production process strategies. As successful examples, two of them should be high lightened, the recombinant vaccine FluBlock® composed of hemagglutinin rosettes and produced from Sf-9 cells and baculovirus vector expression system [2,3], and the Medicago's strategy based on influenza virus-like particles production from tobacco plants [4]. Nevertheless, the most promising strategy, relatively easy to implement to manufacturing scale, is the cell-based production processes. Indeed, it is benefiting from years of experience gained from other cell-based viral vaccine production processes, as rabies or poliovirus [5].

The cell-based strategies for influenza vaccine production get started with the evaluation of the two historical and well characterized adherent cell substrates; Vero and MDCK cells. These cell lines showed acceptable production levels both for infectious particles (2×10^8–10^9 TCID50/ml) and HA antigen activity (2.4–3.3 log HA units/100 μl) as shown in Table 1. They led to different vaccine products already on the market, the Optaflu® (MDCK cells – Novartis), the FluMist® (MDCK cells – Medimmune) and the Influject® (Vero cells – Baxter) [6]. Nonetheless, and for cost and time-consuming reasons, vaccine manufacturers are still looking for new cell lines highly permissive to multiple influenza strains, efficient in terms of viral productivity, but also with culture conditions easily scalable. Indeed, one critical advantage for a producer cell line is its ability to grow in suspension, in serum free conditions, and at high cell densities with fast growth rates (20–30 h doubling times).

One main asset of suspension cell lines over adherent cells is their easier scalability to large manufacturing facilities. Indeed for now, processes based on adherent cells grown on microcarriers are able to deliver only 3 million doses with 6000 L bioreactor [6] while the market demand is of 600 million influenza vaccine doses annually. Moreover, authorities' estimate a need of 13 billion vaccine doses in case of pandemic outbreak. In addition to these aspects, the different strain candidates (H5N1, H1N1, H7N7, H9N2 subtypes), and their host origin (avian or porcine reservoirs) push forward the strategy to enlarge the portfolio of available cell substrates for influenza virus production [6]. Indeed, it is now clear that a sole production cell line will not be able to respond to the demand in the case of a pandemic outbreaks.

To do so, since 2001, several suspension cell lines were constructed and evaluated for influenza virus production (reviewed in [5,6]). So far, suspension cell lines proposed include three MDCK cell lines [7–9], three human cell lines (PER.C6® cells – Crucell [10], HEK293 cells – NRC Montreal [11], CAP® cells– CEVEC Pharmaceuticals [1]) and three avian cell lines (duck EB14® & EB66® cells – Valneva/GSK [12,13]), duck AGE.CR® and AGE.CR.pIX® cells – Probiogen [14] and quail QOR/2E11 cells – Baxter)). Much less information on the production capacities of these cell lines is available, most probably because the production processes are still under development. Indeed, the optimization of the influenza virus propagation process has to focus on the different following critical parameters, (i) the quality of the virus seeds (high infectivity, low MOI), (ii) the trypsin activity, (iii) the fast virus production to reach high titers (>2.5 log10 hemagglutinin HA units/100 μl, $>1 \times 10^8$ 50% tissue culture infectious dose TCID50/ml), (iv) a stable virus titers and (v) a composition of cultivation broths allowing efficient purification (low DNA and protein content) [6]. Such optimization generally include work on cell culture feeding strategy during viral infection, i.e. selection of the best infection medium, implementation of medium exchange prior infection and cell feeding with either batch, fed-batch or perfusion strategies.

Presently, the production capacities of these different suspension cell lines, summarized in Table 1, could be evaluated as comparable to the historical Vero and MDCK adherent cells. Nevertheless, with the ultimate goal to react quickly to pandemic outbreak, having cell lines able to produce viral prototype strains directly isolated from patients in order to shorten the viral seed lot production and the time before vaccine supply would be an important breakthrough. To do so, cell line permissiveness towards various influenza subtypes is an important information. However, it is an aspect which was poorly documented in literature for most of the suspension cell line already evaluated for influenza virus production.

The present work describes the evaluation of the DuckCelt®-T17 cell line (T17), a new avian cell line derived from *Cairina mochata* duck embryo. Our major driver for proposing such a cell line is to complement the egg production strategy with a cell-based production process, and if possible a cell line which would efficiently replicate most of the influenza strains. Consequently, the cell line viral permissiveness as well as its production capacity for influenza virus from various host-origin and subtypes were tested. As described in our manuscript, this alternative cell line supports replication of all subtypes of influenza viruses tested up to now and has demonstrated interesting production capabilities and good scalability. Our main focus was in particular the influenza strains which could put a risk on the current egg supply, namely the avian strains. T17 cell line is an additional option for influenza vaccine production and has the potential to be a great breakthrough for production of animal strains, in particular for avian strains.

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