Measles virus replication in Vero cells grown in the chemically defined Hektor and InVitrus VP-6 media

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1 Purpose

The investigation reports on the proliferation of Vero cells and the propagation of the measles virus in the chemically defined Hektor and InVitrus VP-6 culture media.

2 Background

2.1 Vero cells and measles virus

Vero cells are immortalized kidney epithelia cells from the African green monkey. The Vero cell line is widely applied for the production of different viruses and vaccines including the measles virus - a negative single-stranded RNA-virus of the family *Paramyxoviridae*, which causes the Measles disease. In its attenuated form, the measles virus (Edmonston strain) has been used as a vaccine against measles for several decades. Measles virus' oncolytic activity was discovered in the late 70's, based on the observation that cancer regressed with an ongoing Measles infection (Bluming und Ziegler 1971). More recently, clinical studies proved that the amount of infectious measles viruses for cancer treatment must be 6 log values higher compared to vaccination (Russell et al. 2014).

2.2 Chemically defined, protein- and peptide-free cell culture media

Typically, commercially available serum-free culture media for Vero cells contain chemically undefined complex additives like plant-derived hydrolysates. As hydrolysates are characterized by lot-to-lot variability, the vaccine industry tends develop next-generation bioprocesses based on the use of true chemically defined media devoid of serum, extracts and/or other chemically undefined complex additives. Obviously, such chemically defined media must support cell growth in the same way as serum-containing media.

3 Material and Methods

3.1 Cell line, culture media and measles virus

Vero cells (CCL-81, ATCC) were used for the study of different cell culture media for the purpose of cell propagation. The MV strain MVvac2 GFP (P) was kindly provided by Dr. Michael Mühlebach (Paul-Ehrlich-Institute, Langen, Germany). Hektor and InVitrus VP-6 media were supplemented with 4 mM L-Glutamine and used to support Vero cells growth under static culture conditions.

3.2 Growth kinetics

Cells were directly adapted from a serum-free, high hydrolysate-containing culture medium to chemically defined Hektor and InVitrus VP-6 media in 8 passages. The newly adapted Vero cells were in passage 183. Vero cells were seeded at a density of 15,000 cells per cm² in T-25 flask (Sarstedt, Germany) and cultivated for 7 days to study their growth kinetics. The tissue culture flasks were incubated at 37°C and 5% CO_2 and cells counted every day.

3.3 Determination of cell density

As cells must be detached before counting, the spent medium was discarded and the cells washed with 150 µl cm-2 PBS. Cells were then detached with 20 µl cm^{-2} Trypsin/EDTA incubation for 6 minutes at 37°C. Detachment was stopped by adding 20 µl cm^{-2} Trypsin-Inhibitor. The cells were resuspended with 100 µl cm^{-2} PBS. 50 µl cell suspension was transferred into a 1.5 mL reaction vessel, mixed with 50 µl 0.5% (v/v) trypan blue and counted with a hemocytometer (Neubauer counting chamber).

The maximum growth rate μ_{max} was determined during the log phase of cell growth using the following equations (X = cell concentration, t= time):

$$\mu \left[\frac{1}{\mathrm{h}}\right] = \frac{1}{X} \times \frac{d(X)}{dt} = \frac{\ln(\mathrm{X_2}) - \ln(\mathrm{X_1})}{\mathrm{t_2} - \mathrm{t_1}}$$

3.4 Determination of glucose and lactate concentration

Glucose and lactate concentrations in cell culture supernatants were analyzed with a EKF analyser (EKF Diagnostics).

3.5 Virus production

Vero cells adapted to the chemically defined media were seeded at a density of 5,000 cells per cm². Measles viruses were first produced in serum-containing medium prior to cell infection. The cells were infected with the virus after 4 h cultivation (Passage 3) at a MOI of 10. Samples from the supernatant were stored at – 80°C for the determination of the virus titer.

3.6 Measurement of infectious virus titer

The titer of infectious viruses was determined by the $TCID_{50}$ method (Reed und Muench 1938; Kärber 1931).

4 Results and Discussion

Vero cells were first adapted to Hektor and InVitrus VP-6 culture media and passaged 8 times before undergoing determination of the growth kinetics. Both chemically defined media were tested for the purpose of Vero cell proliferation and virus production against a commercially available serum-free high hydrolysate containing medium (HCM).

4.1 Growth kinetics

In a first step, the growth behavior of Vero cells was analyzed in chemically defined media and compared to proliferation in HCM, the latter serving as a reference (Figure 1).
Vero cells grew to a confluent monolayer in all media. The morphology of Vero cells in Hektor medium was slightly different compared to the reference medium as cells grew in a clustered net-like structure.

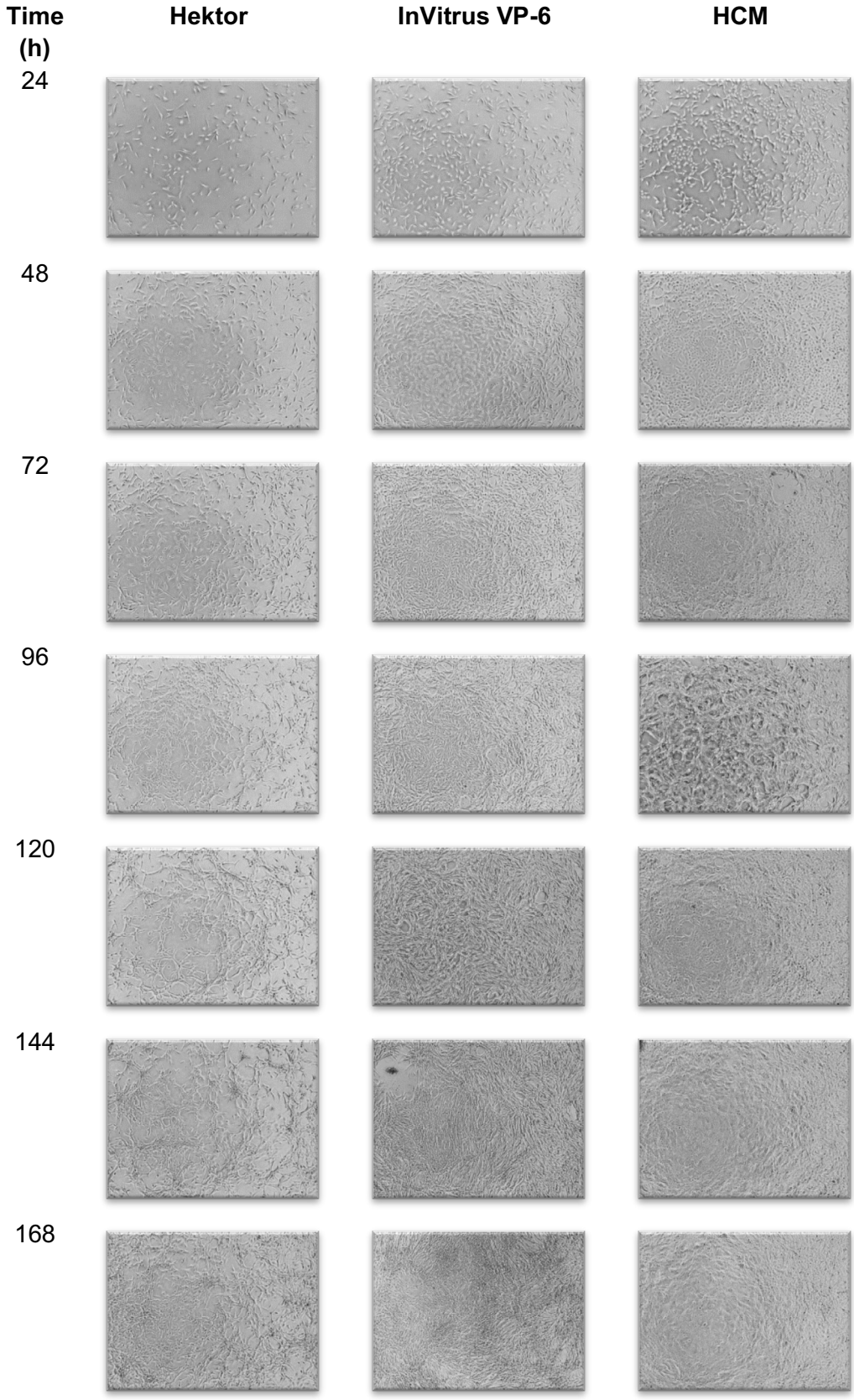

Figure 1: Microscopic images of cell growth in the chemically defined Hektor and InVitrus VP-6 culture media compared to a commercially available hydrolysate containing medium (HCM). Images were taken by a 5-fold magnitude.

Concerning growth kinetics (Fig. 2a), Vero cells showed similar results in InVitrus VP-6 medium and in HCM. A longer lag-phase and a lower maximum cell density were detected in Hektor medium. The cells showed similar maximum growth rates in the chemically defined media, ie 0.036 h^{-1} in InVitrus VP-6 medium and 0.033 ± 0.001 h^{-1} in Hektor medium, respectively (Fig. 2b). The maximum growth rate in HCM was 0.040 ± 0.004 h^{-1}.

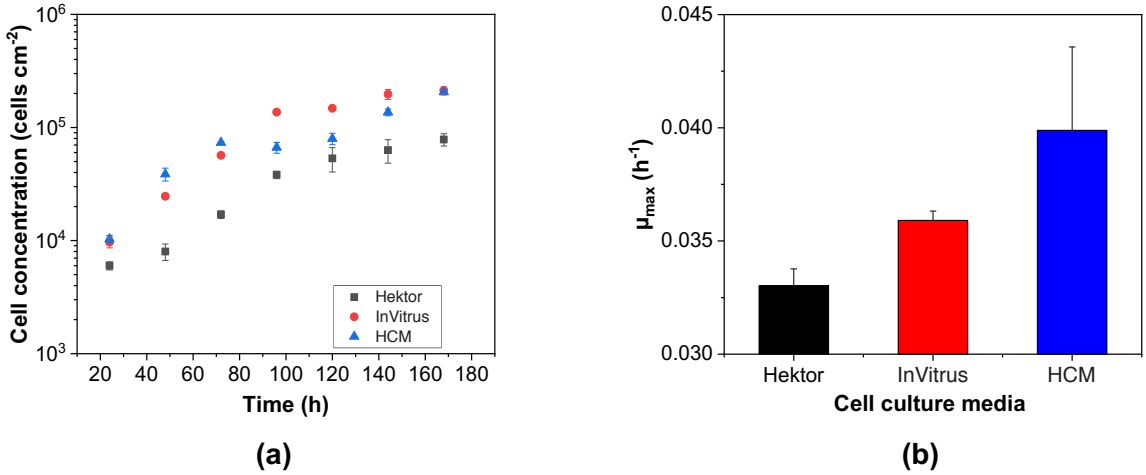

Figure 2: Comparison of the cell growth (a) and maximum cell growth rate (b) for Vero cells in Hektor, InVitrus VP-6 and hydrolysate containing medium (HCM).

The glucose concentration decreased in all media during cultivation, whereas the lactate concentration increased in a similar but inverse proportional behavior (Fig. 3). The growth behavior and the basic metabolic activities of the cells looked similar in InVitrus VP-6 medium and in HCM. Cells cultivated in Hektor medium showed a somewhat different morphology and slightly lower growth rate and maximum cell density.

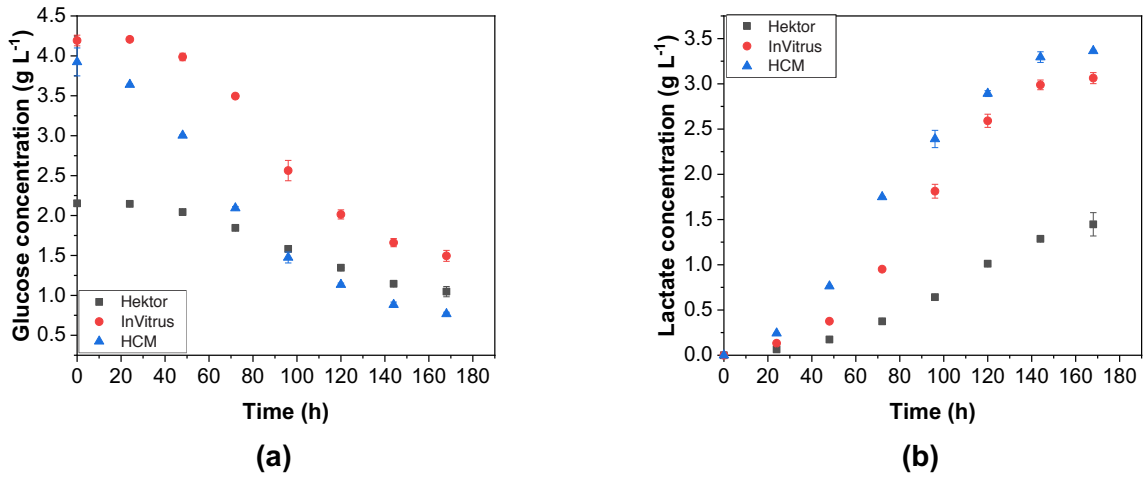

Figure 3: Glucose (a) and lactate (b) concentrations during the cultivation of Vero cells in the chemically defined Hektor (black symbols) and InVitrus (red symbols) media, and hydrolysate containing medium (HCM, blue symbols).

4.2 Measles virus production

For the determination of the measles virus (MV) production, Vero cells were seeded at 5,000 cells cm^{-2} and infected with a MOI of ~10. A similar kinetic of MV production was measured for all media (Fig. 4a). First, the MV titer in the supernatant decreased up to 48 h post-infection (p.i.), because of the MV adsorbed by the cells. With increasing time p.i., the MV titer increased

similarly for all cell culture media to approx. 6 log ($TCID^{50}$ mL^{-1}). At the time of harvest (144 h p.i.), the MV titer was slightly higher for the reference medium with 6.27 ± 0.33 log($TCID_{50}$ mL^{-1}). The chemical defined media reached a MV titer of 5.65 ± 0.22 log($TCID_{50}$ mL^{-1}) in InVitrus VP-6 and 5.73 ± 0.26 log($TCID_{50}$ mL^{-1}) in Hektor media. As a result, both chemically defined media proved to be suitable for the production of MV regardless of their effect on Vero cell growth. While Hektor and InVitrus VP-6 media supported Vero cell growth in different ways, we observed no differences between the two nutrient mixtures regarding MV production.

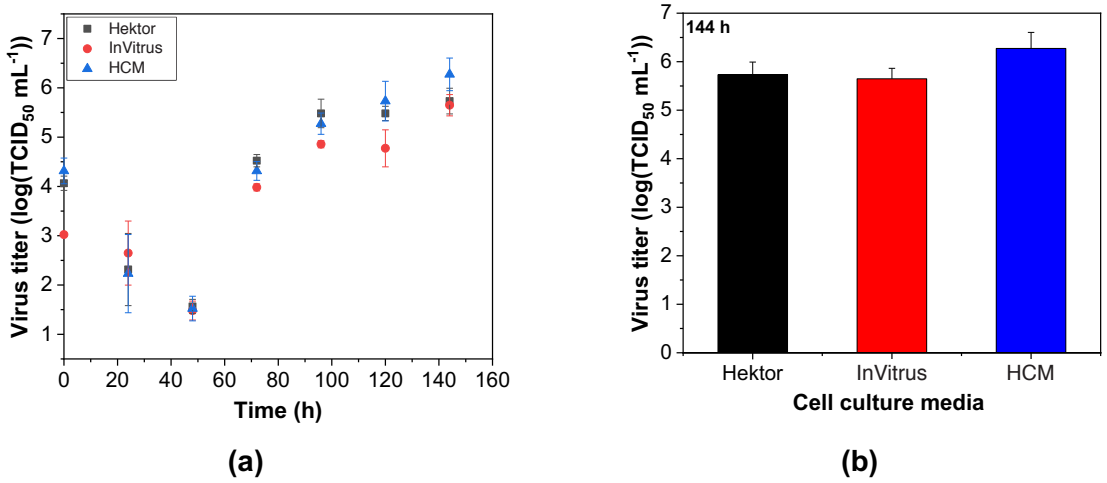

Figure 4: Virus titer during the cultivation of Vero cells in the chemically defined Hektor (black symbols) and InVitrus (red symbols) media, and hydrolysate containing medium (HCM, blue symbols). Fig. (b) shows the final titers at t=144 h p.i.

5 Outlook

The bioprocess presented here will be transferred to a dynamic system and tested for its suitability under controlled culture conditions. The aim is the generation of competitive virus titers at large-scale. Furthermore, Hektor and InVitrus VP-6 media will be investigated to confirm the expected benefits on downstream operations, as both media consist exclusively of small molecules of highest purity.

References

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