

Engineering Conferences International

ECI Digital Archives

Integrated Continuous Biomanufacturing IV

Proceedings

10-6-2019

Production of Zika virus-like particles (VLPs) by perfusion processes

Renata G. F. Alvim

Leda R. Castilho

Follow this and additional works at: https://dc.engconfintl.org/biomanufact_iv



Part of the [Engineering Commons](#)

Production of Zika virus-like particles (VLPs) by perfusion processes

Renata G. F. Alvim, Leda R. Castilho

Federal University of Rio de Janeiro (UFRJ), PEQ-COPPE, Cell Culture Engineering Lab., Brazil
rga@peq.coppe.ufrj.br

AIM

Production of Zika virus-like particles (VLPs) in perfusion mode, enabled by the development of cell pools showing stable and constitutive expression, was investigated with the aim of developing a more cost-effective technology for vaccine production than traditional batch production of whole viruses.

BACKGROUND

- **Zika virus (ZIKV): mosquito-borne flavivirus**
 - Since 2015: transmission detected in more than 80 countries
 - Zika congenital syndrome (ZCS): microcephaly and several other fetal malformations (from ~ 42% of infected mothers, Brasil et al., 2016)
 - Also linked to autoimmune neurological syndrome in adults (Guillain-Barré Syndrome)
- **Need for Zika vaccine: to avoid future periodic outbreaks in endemic areas or future spread to currently non-endemic regions**
- **Virus-like particles: 3D structures that mimic the virus, made of recombinant structural proteins of the virus, but lacking the viral genome**
 - Epitopes displayed in repeated regular pattern: effective immunogens for vaccines
- **Perfusion processes: higher volumetric productivities, reduced bioreactor sizes, less plant footprint and lower investment costs when compared to batch processes**

METHODOLOGY

- **Cell line development**
 - Transfection to produce structural pre-membrane (prM) and envelope (E) ZIKV proteins (GenBank # KU365779)
 - HEK293SF-3F6 cell line (NRC, Canada) cultivated in HEK TF medium (Xell AG, Germany)
 - Enrichment of stable cell pools producing Zika VLPs by FACS (Fluorescence Activated Cell Sorting)
- **Process development**
 - Kinetic studies in shake flasks: batch and pseudoperfusion evaluation (intermittent medium exchange): definition of optimal feeding strategy in shaken tubes
 - Perfusion cultivation in stirred-tank bioreactor (ez-Control, Applikon Biotechnology, The Netherlands) coupled to XCell™ ATF-2 (Repligen, USA) as cell retention device. Working volume of 1 L, at 37 °C, pH 7.1, and 30% of air saturation.

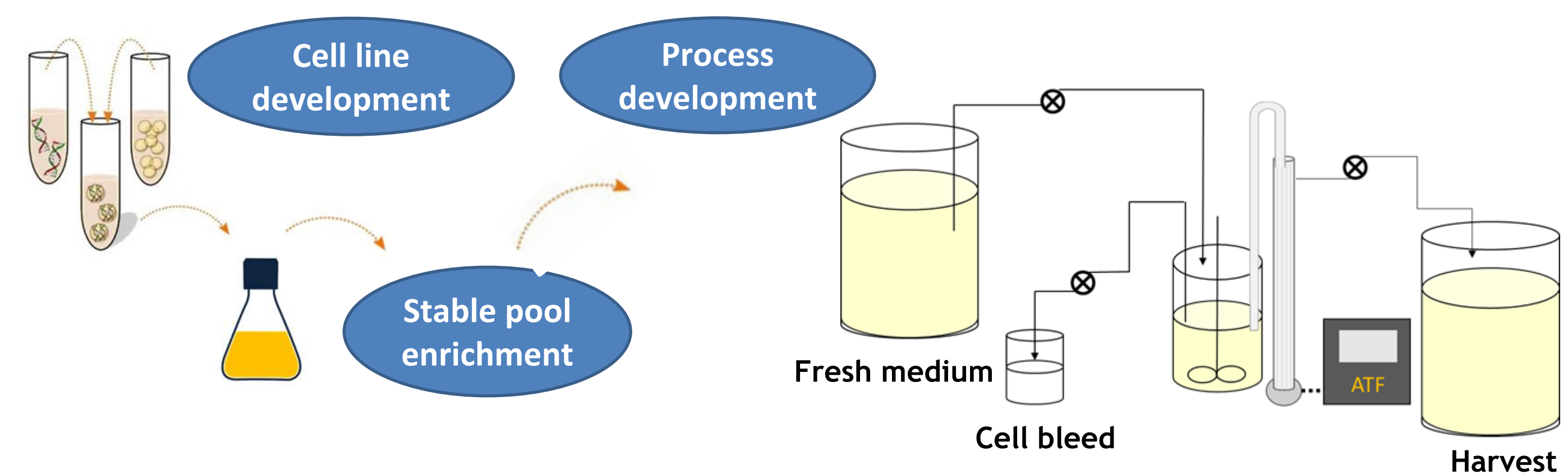


Fig. 1: Development of a HEK293 stable cell pool producing Zika VLPs and its cultivation in perfusion mode. All steps since host cell line maintenance and transfection were carried out in animal component free medium.

RESULTS

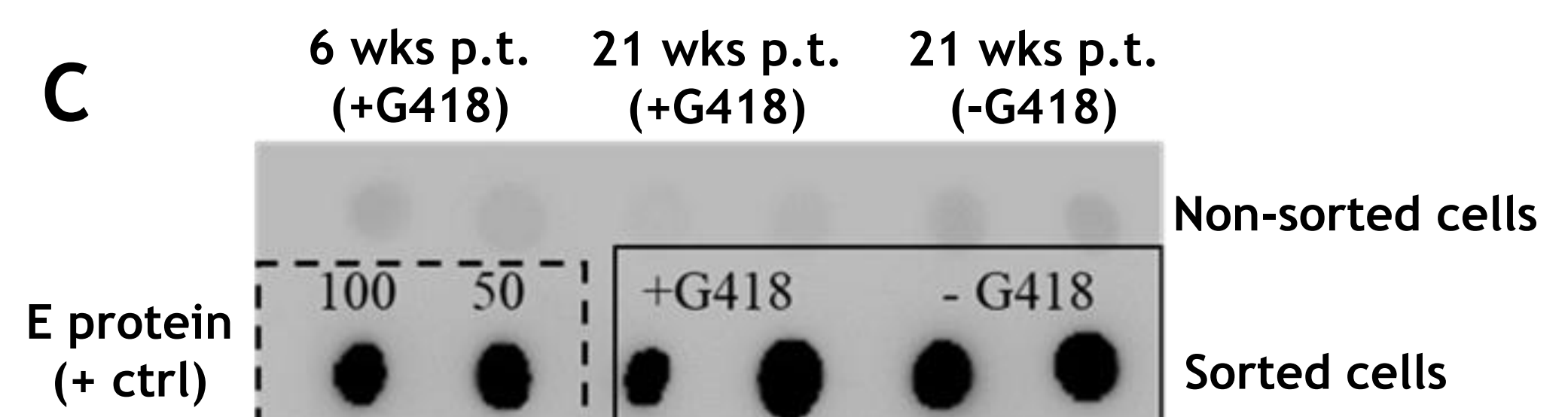
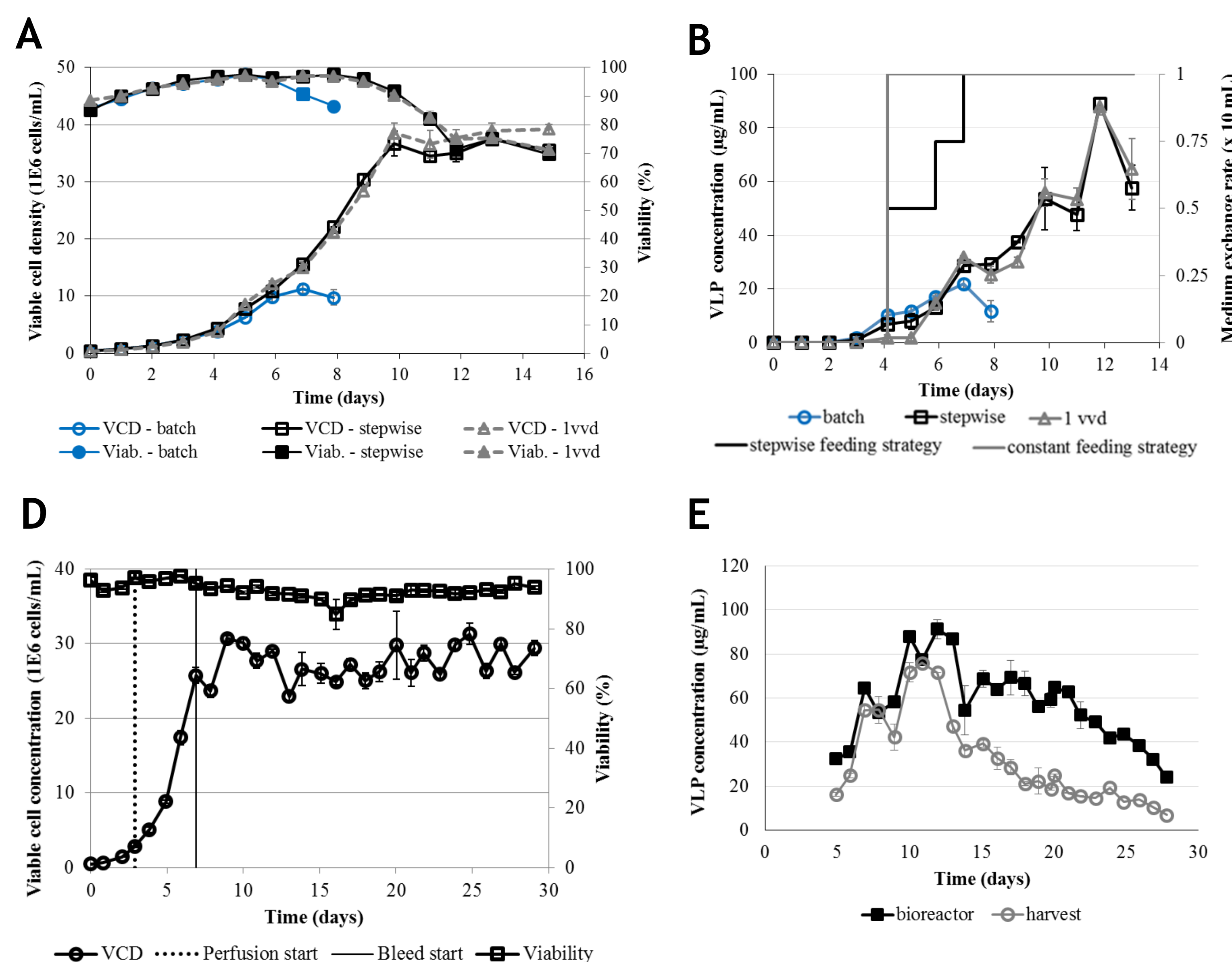


Fig. 2: Production of Zika VLPs under different modes of operation. Viable cell density (VCD) and viability (A) and VLP concentration and medium exchange rate shown for cells cultivated in shaken tubes, under batch and pseudoperfusion (PP) modes of operation. (C) Confirmation of the increase in VLP production after cell sorting and stability production over time. (D) and (E) show results for cells cultivated in perfusion mode in stirred-tank bioreactor.

Table 1: Maximum VCD, product titer and volumetric productivity of HEK293-L3 cells cultivated under different modes of operation.

Parameter	Batch	PP	Perfusion
VCD_{max} ($\times 10^6$ cells/mL)	11.3 \pm 0.8	37.4 \pm 0.2	30.7 \pm 0.4
P_{max} (μ g/mL)	21.9 \pm 1.8	89.1 \pm 1.9	91.3 \pm 11.2
P_V (mg/L/d)	3.4	22.4	25.0

CONCLUSIONS

- **Stable cell lines producing VLPs: enables the development of a continuous production platform that could potentially lead to an affordable vaccine to be manufactured in low biosafety level facilities;**
- **Intermittent (PP) or continuous perfusion medium exchange led to a 3-fold increase in maximum VCD and 4-fold increase in maximum product titer;**
- **Perspectives for the near future: use of a CHO-K1 cell line constitutively producing ZIKV VLPs - robustness and lower media costs.**