

SARS-COV-2 PRODUCTION IN A SINGLE-USE SCALABLE HIGH CELL DENSITY BIOREACTOR

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic and the fast development of first-generation vaccines have demonstrated the value of applying a variety of vaccine technology platforms (1). Inactivated vaccines represent a well-known approach, and their manufacturing depends on high-yield virus production in appropriate biosafety level facilities. This study (2) aimed to establish efficient Vero (WHO) cell-based and animal component-free SARS-CoV-2 production in the CelCradle bioreactor (Esco Aster Pte Ltd.). The single-use culture vessels pre-packed with 0.1 L BioNOCII carriers are highly useful for small scale cultivation of adherent cell lines. Cultures were seeded with 1.5×10^8 cells and total cell numbers peaked at 9 days post cell seeding (dpcs) with $2.7\text{--}2.8 \times 10^9$ cells/vessel in non-infected cultures. To produce virus, cultures were infected at 7 dpcs at total cell numbers of $2.2\text{--}2.5 \times 10^9$ cells/vessel at a multiplicity of infection of 0.006. Harvest of virus-containing supernatant twice instead of once per day improved the virus yield by 2–7 fold. Introducing a temperature shift from 37°C to 33°C upon the time of infection improved virus yield by 2–9 fold with a considerable decline of infectious titer only after 72 h post infection (hpi). Infectious titers peaked at 7.3 \log_{10} 50% tissue culture infectious dose (TCID₅₀)/mL at 72 hpi, and a total of 10.5 \log_{10} TCID₅₀ were produced in ~5 L (11 harvests). While trypsin has been reported to enhance SARS-CoV-2 spread in cell culture, addition of 0.5% recombinant trypsin from the time of infection did not affect virus yield. Overall, animal component-free production of SARS-CoV-2 in Vero (WHO) cells was successfully established in a single-use packed-bed bioreactor. β -propiolactone inactivated SARS-CoV-2 from this study was immunogenic and induced neutralizing antibodies in mice with mean 50% neutralization titers of 1/150 or 1/580 after three immunizations with doses containing 0.1 μ g or 0.5 μ g S1 protein, respectively. The CelCradle represents a scalable technology and is a small version of the TideXCell system applying single-use culture vessels with packed-bed volumes of up to 100 L. The potential rapid response to outbreaks with inactivated vaccines has been demonstrated in the SARS-CoV-2 pandemic (1). Vero cells are susceptible to a wide range of viral pathogens (3), and these scalable single-use bioreactors provide a high level of flexibility and potentially decreased response time for production of future emerging viruses for vaccine purposes.

References: (1) Poland 2020 Lancet [PMID: 33065034], (2) Offersgaard 2021 Vaccines [PMID: 34209694], (3) Barret 2009 Expert Rev. Vaccines [PMID: 19397417]