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# SUSPENSION VERO CELL CULTURE TECHNOLOGY FOR HIGH TITER PRODUCTION OF VIRAL VACCINES

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Vero cells are considered as the most widely accepted continuous cell line by the regulatory authorities (such as WHO) for the manufacture of viral vaccines for human use. The continuous Vero cell line has been commercially used, after propagation on microcarriers, for the production of rabies, polio, enterovirus 71, hantaan, more recent COVID19 and other vaccines. Vero cell culture technologies were also explored for productions of many more viral vaccines over the last two decades. The growth of Vero cells is anchorage-dependent, and cells need to be dissociated enzymatically or mechanically for the process of subcultivation. This process is labor intensive and complicated in process scale-up. Adaptation of Vero cells to grow in suspension will significantly simplify scale-up and manufacturing processes. Development of advanced suspension Vero culture technology to improve product titer will further reduce the cost of goods.

We previously reported a successful adaptation of adherent Vero cells originated from ATCC CCL-81 to grow in suspension in serum-free and animal component-free media developed in-house. The suspension adapted cells were found to retain their genetic stability and to be non-tumorigenic. Present work continues the development and optimization of cell culture process and feeding strategy to improve the growth of suspension Vero cell and the production of vesicular stomatitis virus (VSV) and herpes simplex virus-1 (HSV-1). Data from this study showed the suspension adapted Vero cells retained similar VSV productivity to that obtained in adherent culture; volumetric productivity of VSV increased with the increasing cell density at infection in batch culture. However, the maximum cell density in batch culture was about $2.5x10^6$ cells/mL, and was not improved significantly despite tremendous effort dedicated to improve culture conditions such as supplementing various nutrients in batch culture. As a result, perfusion culture was employed as an approach to increase cell density in the culture, which in turn increased the VSV productivity up to one log, at $1.1x10^{10}$ TCID50/mL when the culture infected at $7x10^6$ cells/mL.

High titer production of HSV-1 in the Vero culture is more challenging. The virus productivity is not only limited by the maximum cell density in batch culture, but also reduced by inhibitory metabolites secreted in the culture even at low cell density such as $1x10^6$ cells/mL. Media replacement before virus infection is essential to achieve a high HSV-1 yield. As such, perfusion culture was a preferred mode for high titer production of HSV-1, which improved the HSV-1 titer also by up to one log to $1.8 x10^9$ TCID50/ in a culture infected at $5x10^6$ cells/mL when comparing to a control shake flask culture infected at $1x10^6$ cells/mL. Experimental data also demonstrated that perfusion Vero culture was robust and reproducible.

This study demonstrates that batch or perfusion suspension Vero culture is a much simplified process than current adherent culture technology for manufacturing of viral vaccines, and offers great potentials in reducing the cost of goods. The suspension Vero culture developed in our institute has generated tremendous interests from industry and academia, and are being tested by many different organizations.