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OPTIMIZATION OF rAd5 VECTORED NEWCASTLE VACCINE PRODUCTION IN HEK293 AT HIGH CELL DENSITIES

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Newcastle disease (ND) is a highly contagious and often severe global spread syndrome that affects birds including domestic poultry. It is caused by a virus belonging to the *paramyxoviridae* family.
Indeed, an outbreak of ND can be quite severe, and is considered as a constant threat to the industry and food security worldwide. The disease can be controlled through the administration of effective vaccines.
Immunizations with inactivated or live vaccines, although protective, have some eminent disadvantages.
The aims of this work is the development of a vectored vaccine using a non-replicative human adenovirus vector, expressing the F antigen from Newcastle Disease Virus (rAd-F-ND) in bioreactor. The recombinant vaccine is produced using the HEK293 cell line.

HEK293 cultures were carried out in suspension, first in shake flasks and then in stirred bioreactor at 37°C, 5% CO2 and 150 rpm in chemically defined media. The virus titers were determined by qPCR.

To improve rAd-F-ND virus productions in HEK-293 cells, we studied the effects of the following parameters in shake flask cultures: culture media (Hycell Trans FX-H and Xell-GM), cell density, multiplicity of infection (MOI) and feed (Xell-FS , Xell-GM and cell Boost 5).

HEK293 cells were infected at $2x10^6$ cells/mL with rAd-F. Virus replication optimization was pursued at different MOIs (0.1, 1, 3 and 5). The highest virus titers ($5.25x10^{11}$VG/mL) were obtained with an MOI of. The specific productivity obtained in Hycell Trans FX-H medium was very low compared to that obtained in Xell-GM medium. When feeding was started before infection, the virus productivity improved when HEK293 cells were infected at $6x10^6$ cells/mL. The maximum virus titer reached $1.65x10^{11}$VP/mL with feeding with Xell-FS at 3%. The specific productivity was more pronounced under this condition. It was 2-fold higher compared to the same culture without feed (virus titers were 2.17 10^{10} Vg/mL versus 6.6 10^8 Vg/mL).

In the current work, we also studied the rAd-F-ND virus production in HEK-293 cell line in a 7L bioreactor. Batch and fed-batch culture modes were investigated. The cells were infected at a cell density of $5.5x10^6$cells/mL and the maximum virus titer was about $4x10^{10}$Vg/mL. The osmolarity level during feeding phase was maintained under the limiting value to maximize virus productivity.