

PERFUSION PROCESS FOR THE PRODUCTION OF A NEW, VLP-BASED YELLOW FEVER VACCINE CANDIDATE

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Yellow fever (YF) is an acute viral hemorrhagic disease endemic in tropical areas of Africa, Central and South America, which is transmitted by the bite of infected mosquitoes. It is a “historically devastating disease” (Paules and Fauci, 2017) that killed during outbreaks in past centuries, before the introduction of the current vaccine, approximately 10% of the population of cities like Philadelphia (USA) and Barcelona (Spain). According to Garske et al. (2014), YF caused in 2013 78,000 deaths worldwide, which is a disease burden comparable to influenza. In the past few years, outbreaks in Angola (2016) and in Brazil (2017-2018) led to the depletion of the WHO vaccine stockpile and to the introduction of the emergency use of a fractional dose (1/5). Furthermore, the Angola outbreak in 2016 caused the first cases of YF ever to occur in Asia (11 imported cases to China), rising the concern about approximately 2 billion immunologically naïve people who would be at high risk in Asia in case local transmission of the virus starts to occur (Wilder-Smith et al., 2019).

The urgent need for a new YF vaccine becomes evident from two major issues concerning the current vaccine, which consists of a live-attenuated virus propagated in chicken embryos: (i) vaccine shortage due to limitations in the manufacturing technology; (ii) rare, but fatal adverse effects. Therefore, this work focuses on the development of a safe, non-replicating YF vaccine, produced by a high-productivity perfusion process.

Stable recombinant HEK293 cell lines constitutively expressing the structural proteins prM (pre-membrane) and E (envelope) of YFV were generated, enabling long-term production and secretion of recombinant virus-like particles (VLPs). FACS (fluorescence activated cell sorting) was used to sort the transfected population for high producer cells and allowed obtaining an enriched cell pool producing significantly higher amounts of VLPs. Small scale kinetic studies under intermittent perfusion (pseudoperfusion) were performed in order to investigate possible feeding strategies and to evaluate the use of short-chain fatty acids as productivity enhancers. Subsequently, perfusion runs were carried out in stirred-tank bioreactors in order to investigate optimal conditions for VLP production, as well as to evaluate different cell retention devices (e.g. inclined lamella settler and ATF-2). Partial retention of the VLPs in the perfusion bioreactor system occurred when the ATF-2 was used. VLPs produced by perfusion were purified by a two-step chromatographic process, and transmission electron microscopy (TEM) images confirmed the expected size and morphology of the VLPs, enabling their use in mouse immunogenicity studies.

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