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Influenza is considered a major threat to human health. The current influenza vaccines production system in embryonated eggs does not satisfy the market needs in cases of pandemic or high seasonal demand. Furthermore, even though the existent seasonal vaccines are effective to induce protective immunity in healthy adults, poor immunogenicity in the elderly and early childhood is elicited (Wu et al., 2010). Therefore, there is a need to develop new generation influenza vaccines produced in robust and flexible production platforms capable of providing complete immune protection and support the vaccines demand. Virus-like particles (VLP) constitute a promising alternative to safely elicit a potent immune response against influenza. These particles do not contain viral genome and their structure mimic influenza virus (Thompson et al., 2015). In this study, a HEK-293SF inducible stable cell line expressing hemagglutinin (HA) and neuraminidase (NA) of the strain A/PR/8/34 (H1N1) has been developed. Once the stable cell line, named 293HA/NA, was established, the production of VLPs was mediated by transient transfection with the plasmid pAdCMV5-GAGGFP that encodes the HIV GAG structural protein fused with GFP. The transient expression of GAG was efficient to abundantly release the particles in the culture medium. The VLPs production in 293HA/NA cells using GAGGFP protein as scaffold was studied in 250 mL shake flasks by following the increase of fluorescence in the supernatant (Spectrophotometry) and the accumulation of HA (Dot-Blot) (Figure 1). The process was successfully operated in a 3L-Bioreactor and at 72h post-transfection the cell supernatant was ultracentrifuged on a 25 % sucrose cushion. High levels of well-structured influenza GAG-VLPs were obtained by this production process and their concentration was estimated by electron microscopy at $5.9*10^9$ VLPs/mL. The influenza GAG-VLPs were purified by Tangential Flow Filtration and the concentration of HA was estimated by three different techniques: Single Radial Immunodiffusion, Hemagglutination assay and Dot-Blot. With further optimization, the production system proposed in this work could be a promising alternative to support the influenza vaccines manufacturing in the future.

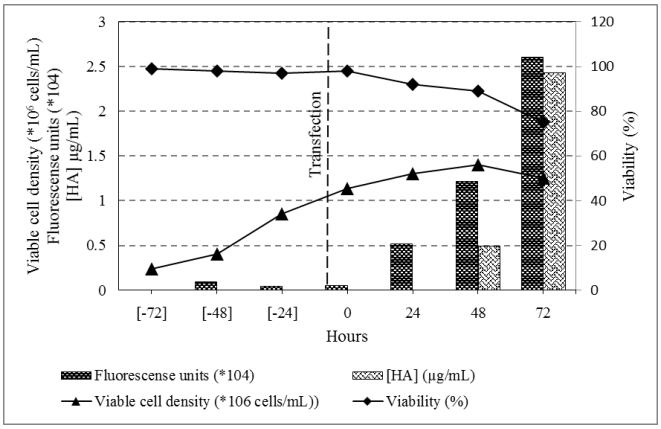

Figure 1 – Kinetic of expression of GFP and HA during the production of influenza GAG-VLPs in 293HA/NA stable cell line.

References

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