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OPTIMIZATION, PRODUCTION, PURIFICATION AND CHARACTERIZATION OF HIV-1 GAG VLPs FUNCTIONALIZED WITH SARS-COV-2 SPIKE GLYCOPROTEIN

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Virus-like particles (VLPs) constitute a promising approach for recombinant vaccine development. They are robust, safe, versatile and high immunogenic supra-molecular structures that closely mimic the native conformation of the viruses without carrying its genetic material. HIV-1 Gag VLPs share similar characteristics with wild type Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus, making them a suitable platform to express the Spike membrane protein to generate a potential vaccine candidate for COVID-19. This work proposes a methodology for the generation of SARS-CoV-2 VLPs by its co-expression with HIV-1 Gag protein by transient transfection of HEK 293 cultures. We first evaluated the cellular co-expression of SARS-CoV-2 Spike glycoprotein with HIV-1 Gag: confocal microscopy analysis showed that after its expression, native envelope Spike glycoprotein travels to the plasmatic membrane of the HEK 293 producer cells, where it co-localizes with Gag::eGFP (Figure 1.A,B) and Spike-functionalized VLPs (S-VLP) generation occurs. Electron microscopy observations of the S-VLPs led us to conclude that they had no significant structural differences with Gag-based non functionalized VLPs (G-VLPs). Spike protein presence was confirmed by western blot (Figure 1.C) and by immunogold labeling at the surface of S-VLPs (Figure 1.D), a key feature to present immunogenic SARS-CoV-2 epitopes to patient's immune system when used as vaccine. Further, we optimized the production bioprocess using design of experiments to increase S-VLP productivity. Using this approach, we identified the transfection conditions maximizing the cellular population co-expressing simultaneously Gag and S proteins. The model predicted a double positive population of 57.5±2.3% for the optimal transfection condition, which was validated and then implemented to transfect a 1L stirred tank bioreactor. The bioreactor production was carried out satisfactorily achieving similar behavior to the parallel Erlenmeyer shake flasks in terms of cellular growth, Spike concentration, VLP concentration and purity of the produced S-VLPs. The production was then followed by a downstream purification process consisting in two clarifications, an ion exchange and a size exclusion chromatography. Overall, the DSP process had a low yield in terms of VLP recovery but highly succeeded in concentrating and purifying the desired S-VLPs while generating a final product with low undesired contaminants. The approach proposed could be expanded to produce additional Gag-based VLPs against different diseases or COVID-19 variants.

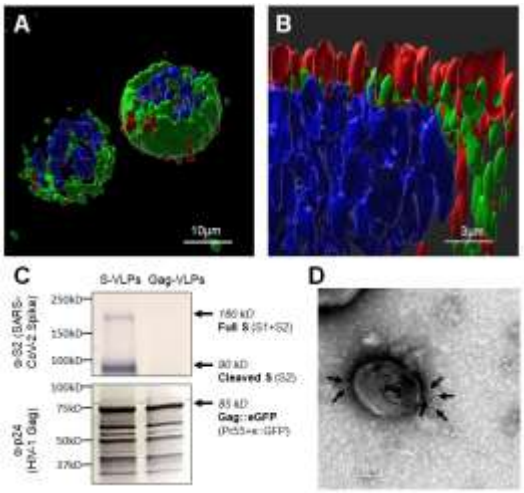

Figure 1 – Spike protein cellular localization and characterization of the produced S-VLPs. (A,B): Co-localization of Spike (red) and Gag (green) can be observed at (A): cells external boundary and (B): Cross-section of the plasmatic membrane. Cell nuclei is shown as blue. (C): Western Blot of purified S-VLPs and G-VLPs. Top: anti SARS-CoV-2 S2 Spike. Bottom: anti HIV-1 p24. Both S-VLPs and G-VLPs showed HIV-1 Gag bands, while only S-VLPs showed Spike bands. (D): Electron microscopy images of the Gold-immunolabeled S-VLPs showing S protein localization (arrows) on the surface of the chimeric VLPs.