HIGH TITER PRODUCTION OF HIV-1 VIRUS-LIKE PARTICLES BY CAP-T CELLS

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Novel vaccine approaches are moving towards recombinant technology. Upon these, virus-like particles are promising candidates because they have been demonstrated to efficiently elicit both humoral and cellular immune responses. Moreover, they are non-infectious as they are formed basically by the structural viral proteins, mimicking the native virus but without containing the viral genome. CAP-T is a novel human cell line that has been previously demonstrated to be superior to other common cell lines in the production of recombinant proteins and viruses. They grow in suspension in serum-free, chemical defined media and they are easily transfectable with PEI, so they were evaluated for the production of HIV-1 virus-like particles by PEI-mediated transient transfection. Upon transfection of the HIV-1 Gag-GFP protein using the standard conditions, spherical particles with a size consistent with immature HIV virions (130 nm) were observed by TEM and NTA and supernatants containing 3×1010 VLPs/mL were harvested in batch culture 72 hours post-transfection. Several key steps of the production protocol were studied to establish the best transfection conditions both in terms of VLP yield and protocol simplicity. It was determined that for optimal production cells need to be growing at mid-exponential phase and can be transfected by independent addition of DNA and PEI with no prior complexation. Noticeably, a medium exchange step from PEM to FreeStyle is required before transfection since the former is not compatible with PEI-transfection while the latter is not compatible with high-density cell growth. A Box-Behnken experimental design was used to optimize cell density at time of transfection and DNA/cell and PEI/cell ratios. For optimal production, cells were transfected at a density of 3.3E+06 cells/mL with 0.5 pg of DNA/cell and 3 pg of PEI/cell. Using the optimized protocol titers of 6×1010 VLP/mL were achieved, 20-fold higher than optimized production with HEK293 cells, making CAP-T a suitable and promising cell line for the production of HIV-1 VLPs and potentially other complex viral-based biotherapeutics. Scale-up of the optimized transfection protocol to 1L bioreactor was evaluated. The medium exchange required before transfection is trivial at Erlenmeyer scale, but becomes cumbersome when working with a bioreactor. For this very reason, transfection media development as well as optimizing transfection protocol are in progress.