Adaptation of Adherent HEK293T Cells to Suspension Cell Culture in Chemically Defined Culture Medium

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BACKGROUND AND NOVELTY

HEK293 cells are immortalized human embryonic kidney cells and the daughter cell line HEK293T is among the most commonly used cell lines from research to commercial gene therapy and bioprocessing applications, including large-scale protein and virus production. While both adherent and suspension culture systems are utilized, adherent HEK293T cells are limited by scaling up in production and a narrow selection of chemically defined (CD) media.

In this study, we describe a process of adapting adherent HEK293T cells into a suspension culture utilizing a CD media and show comparable growth to Expi293F, a commercially available suspension-adapted 293 cell line. Both adherent HEK293T cell culture and adapted suspension HEK293T cell culture were verified similar to 293[HEK-293] cells by ATCC Human Cell STR Profiling, indicating genomic stability during the adaptation process. Our in-house adapted suspension HEK293T cells also maintain the function of producing adeno-associated virus serotype 2 (AAV2). The viral DNA titer was measured side-by-side using qPCR and digital PCR assay. The results from both assays are comparable, indicating reliable assays established for both instruments for quantification of AAV2 production by suspension HEK293T cell cultures.

MATERIALS AND METHODS

- Dulbecco's Modified Eagle's Medium (**DMEM**), **BalanCD HEK293**, **L-Glutamine**: FUJIFILM Irvine Scientific
- GibcoTM **TrypLE**™ Express Enzyme (1X) (no phenol red), **Pluronic**™ **F-68** non-ionic surfactant : Thermo Fisher Scientific
- **Fetal Bovine Serum (FBS)**: Millipore-Sigma
- Cell Stock: **Adherent HEK293T** (ATCC); **Expi293F** (Thermo Fisher Scientific)
- Culture vessels: **T75** Corning cell culture flasks; **125-mL** Corning Erlenmeyer flask with vent cap
- **PEIpro**: Polysciences
- **Cell counting method**: 500uL of cell culture or harvested cell resuspension for measurements of viable cell density (VCD) and viability using Vi-Cell (Beckman Coulter)
- **Static culture condition**: 37°C, 5.0% CO2, 80% humidity
- **Shake flask culture condition**: 37°C, 5.0% CO2, 120rpm, 80% humidity
- **AAV2 harvest and preparation for viral DNA quantification**: AAVpro Titration kit (Takara Bio)
- **qPCR**: BioRad
- **Digital PCR**: Qiagen QIAcuity

RESULTS

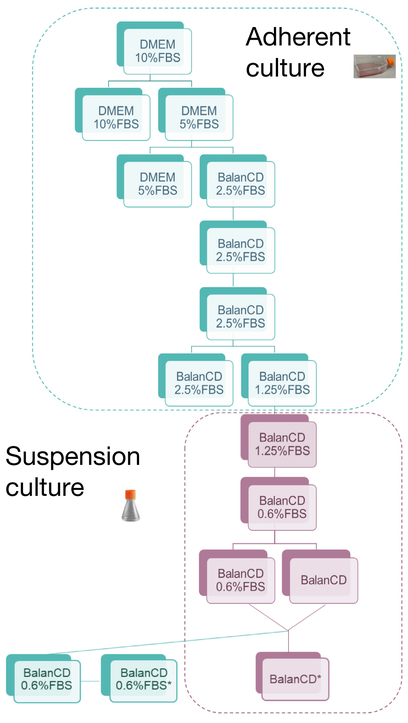
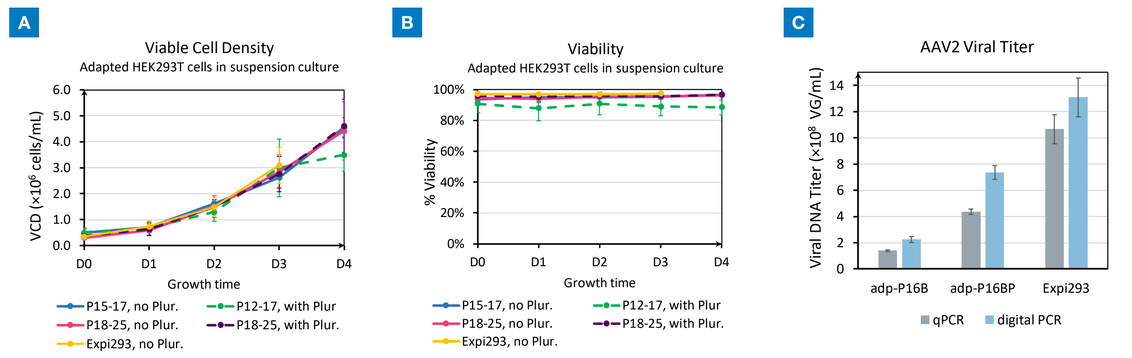


Figure 1. Subculture HEK293T cells from adherent cell culture (top) to suspension cell culture (bottom) with reduced amount of fetal bovine serum (FBS), passage number: 1-10. Adherent HEK293T cells were initially cultured in DMEM with 10% FBS as a static culture in T75 flask, and the amount of FBS was reduced from 10% to 1.25% through sequential adaptation. If the cells grew at the targeted rate, from 0.5×10^6 cells/mL to 1.5×10^6 cells/mL in 2-3 days, they would be sub-cultured in 50% reduction of FBS in next passage. BalanCD HEK293 medium replaced DMEM when the concentration of FBS was reduced to 2.5%. Upon reduction to 1.25% FBS, cells were transferred to 125 Erlenmeyer flask for suspension culture, until complete transition to chemically defined media conditions was achieved. 0.6%FBS can be kept as a static culture in T75 flask and/or cryopreserved as a backup if adaptation in suspension cultures fail.



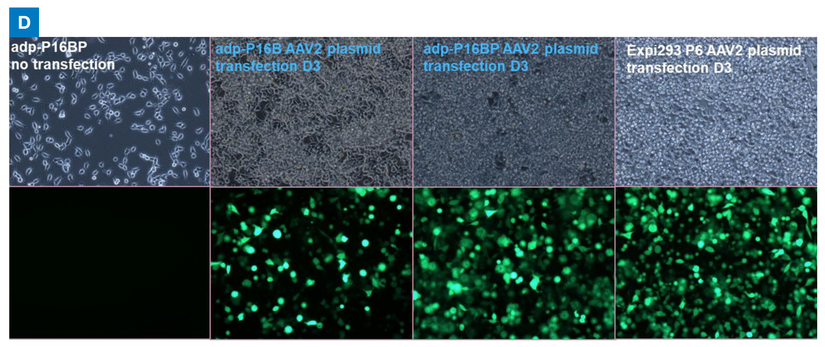


Figure 4. Adapted suspension HEK293T cell growth in BalanCD HEK293 and AAV2 viral titer by the adapted suspension cell culture. Suspension-adapted HEK293T cells (or control Expi293F cells) were passaged in BalanCD HEK293 without FBS, and with or without Pluronic F68. (**A**) VCD of adapted cell culture passage number: P12-25, dash lines are cultures with 0.01% Pluronic F68. (**B**) Viability of cell culture passage number: P12-25. (**C**) AAV2 viral DNA titer from the cell culture lysate assayed by qPCR and digital PCR. Cells were lysed by three cycles of freezing-thawing, cellular DNAs were removed by DNase I digestion, AAV2 particles were lysed by lysis buffer from TakaraBio followed by dilution of the vial lysate for PCR quantification. (**D**) Transgene ZsGreen expression of adapted suspension cell cultures with ("BP") or without ("B") Pluoronic F-68 after PEI-assisted transfection with AAV2 plasmid vectors. Expi293F cells served as a control suspension culture for all experiments shown.

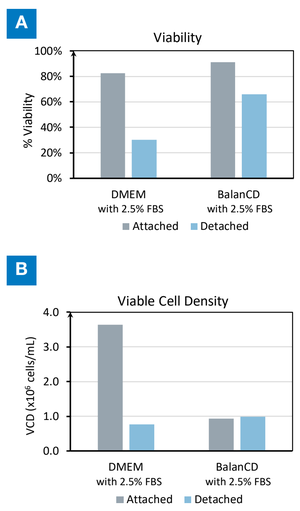


Figure 2. Viability (A) and viable cell density (VCD) (B) of attached and detached HEK293T cells cultured in DMEM or BalanCD HEK293 with 2.5% FBS at P5, Day 7. T75 culture flask were gently shaken before cell harvesting. For unattached cells, the culture medium was collected from each flask separately, and cells were centrifuged and re-suspended in equal volume of media for cell counting using ViCell. For attached cells, the cells were disassociated from the flask using TrypLE, collected and re-suspended in equal volume of media for cell counting using ViCell. Detached cells grown in BalanCD HEK293 show higher viability than in DMEM, suggesting that further adaptation in suspension culture may be supported in shake flask culture format.

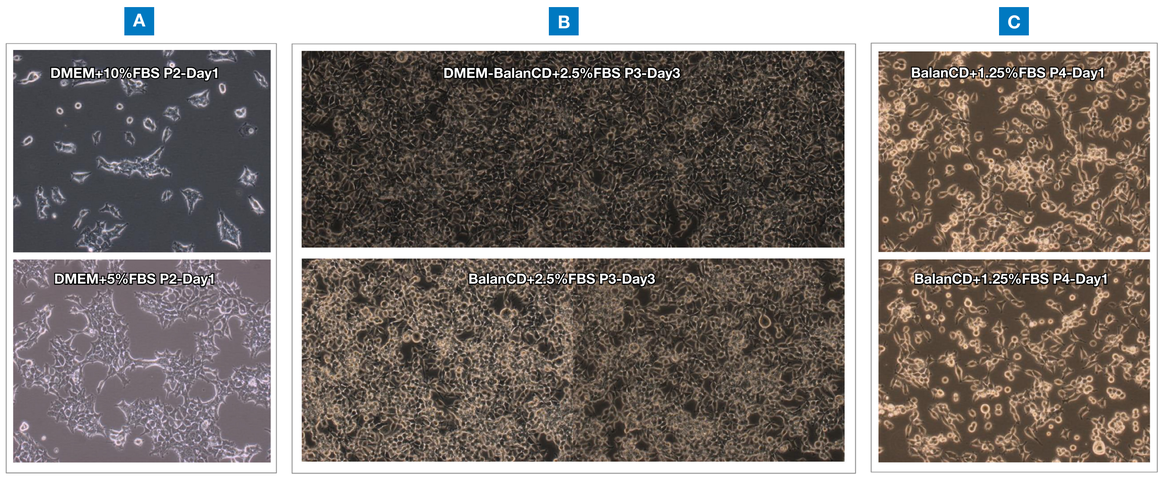


Figure 3. Cell morphology of HEK293T cells cultured in DMEM or BalanCD HEK293 medium with reduced amounts of FBS. (**A**) HEK293T cells grown in DMEM with 10% and 5% FBS showed similar morphology of monolayer. (**B**) With 2.5% FBS, cells appear less attached to the culture surface with replacement of DMEM with BalanCD HEK293. (**C**) With further reduction of FBS (1.25%) in BalanCD HEK293 medium, cells tend to grow scattered instead of forming in monolayer patches.

SUMMARY

- Successfully adapted adherent HEK293T cells to suspension cells cultured in serum-free, chemically-defined conditions
  - Gradual replacement of serum-containing media (DMEM+10% FBS) with BalanCD HEK293 with reduced amount of FBS
  - 0.01% Pluronic F68 may be needed for adapting to suspension cultures
- BalanCD HEK293 encourages detachment of cells growing in static cultures so that the cells are better prepared for successful adaptation in suspension cultures in shake flasks
- Adapted suspension HEK293T cell cultures can be transfected with AAV2 plasmid vectors and produce AAV2
- Short Tandem Repeat (STR) profiling for both adherent HEK293T and adapted suspension HEK293T cells verified that the cells are similar to 293[HEK293] cell line (CRL-1573) from ATCC