A New Chemically Defined Medium Improves Viral Vector Production from HEK293 Cells

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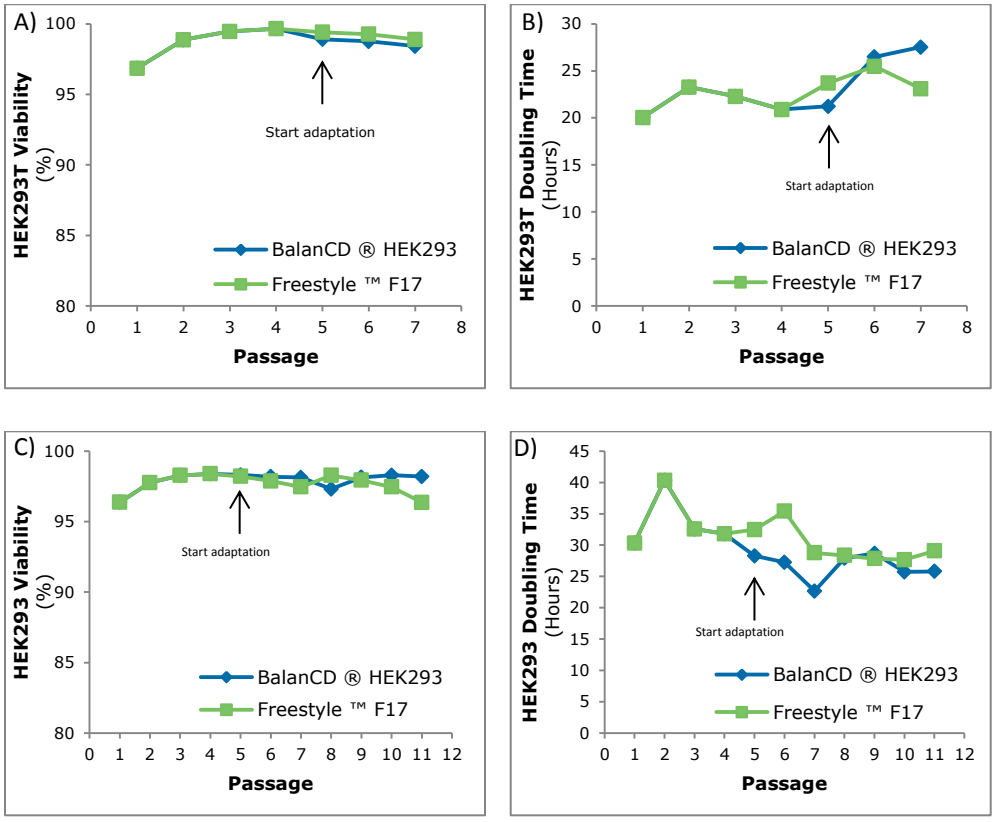
The human embryonic kidney cell line, HEK293, is widely used in the production of therapeutic biologicals, including viral vectors for human gene therapy. At Genethon, we are pursuing a variety of projects (including two that have progressed to Phase I/II clinical trials) using adeno-associated virus (AAV) and lentivirus (LV) to deliver genes that express therapeutic proteins in human tissues. In an effort to improve our yields of AAV from HEK293 cells, and LV from HEK293T cells, we compared our current cell culture medium, FreeStyle™ F17(Thermo Fisher Scientific), with a new chemically-defined medium from Irvine Scientific, known as BalanCD® HEK293. The evaluations we report here were performed in a 2L benchtop bioreactor. Future studies will determine if the advantages of BalanCD® HEK293 shown here will carry over during scale-up to a 200L stirred-tank bioreactor.

Since our cell lines were originally adapted to grow in FreeStyle™ F17 medium, the first thing we wanted to determine was whether they would require any adaptation to the BalanCD® HEK293 medium. This was tested by thawing the cells into F17, measuring cell viability and doubling time for 5 passages (3-4 days/passage), then switching to BalanCD® HEK293 and continuing to monitor both parameters for several more generations. As illustrated in Figure 1, both types of cells exhibited virtually identical viability and doubling times before and after transfer to BalanCD® HEK293, indicating that essentially no adaptation is required.

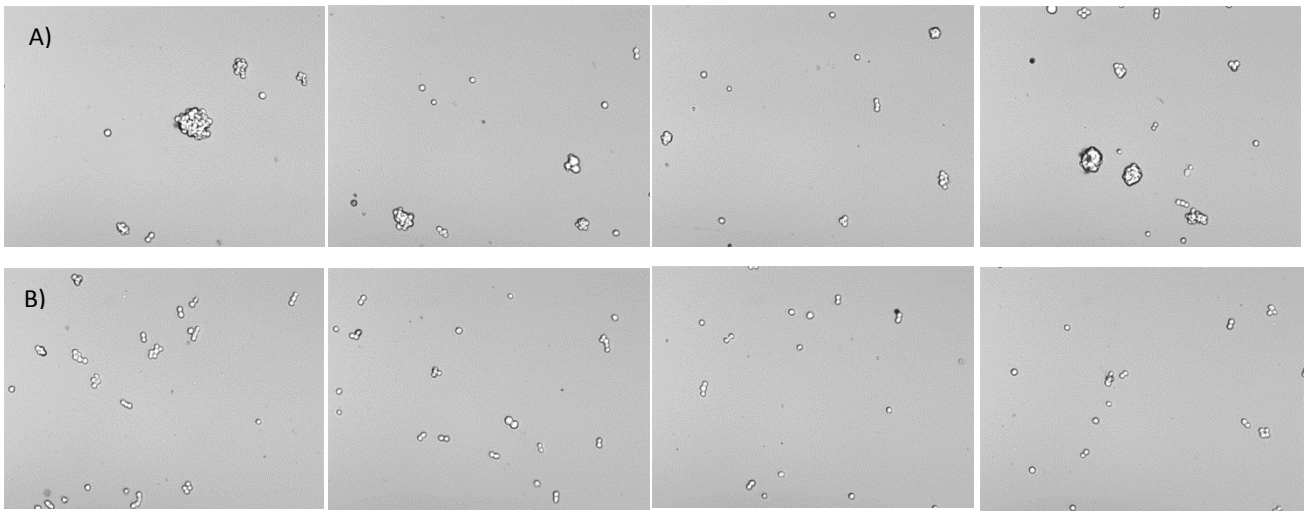
Figure 1. Adaptation of HEK293T and HEK293 cells



Two suspension cell lines, HEK293T and HEK293, banked in Freestyle™ F17 Expression Medium (Thermo Fisher Scientific), were thawed into 250mL shake flasks. Passages were performed every 3-4 days. After 5 passages, cells were transferred into BalanCD® HEK293 (Irvine Scientific). Viability (A, HEK293T; C, HEK293) and doubling time (B, HEK293T; D, HEK293) were measured for subsequent passages.

Whenever mammalian cell lines are grown in culture, one must be aware of their tendency to form clumps of cells. Clumping can result in many cells being inaccessible to transfection with the plasmids that drive vector production, while cells in the middle of large clumps will be starved for nutrients. Therefore, we compared the tendency of HEK293 cells to form clumps in each type of medium during a transfection experiment. In Figure 2, we see that the cells grown in F17 exhibited a much greater tendency to form clumps, and when cells did form clumps in BalanCD® HEK293, they tended to be much smaller.

Figure 2. Reduced cell aggregation in BalanCD® HEK293 compared to Freestyle™ F17

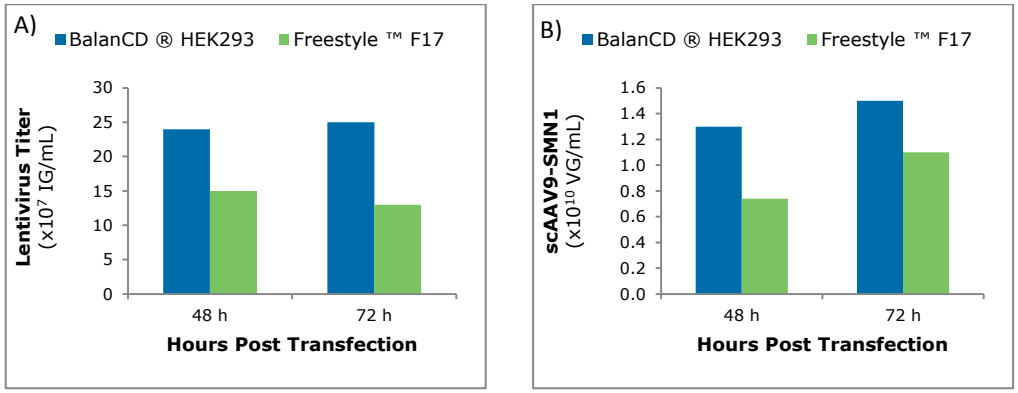


HEK293 cells were cultured in Freestyle™ F17 Expression Medium and BalanCD® HEK293 in 2L bioreactors. At the time of transfection, samples were removed from the bioreactors and images were taken to observe cell aggregation. Cells grown in Freestyle™ F17 Expression Medium (A) showed higher cell aggregation overall compared to BalanCD® HEK293 (B).

Our final set of experiments in the 2L bioreactor was designed to quantify the production of AAV or LV after transfection of HEK293 or HEK293T, respectively, with the appropriate plasmids. At 48 and 72 hours post-transfection, we measured the number of viral genomes produced using qPCR. For reactions transfected with AAV, we quantified the number of viral genomes in cell lysates. For LV transfections, the supernatants were first serially diluted and inoculated onto monolayers of HCT116 cells. One day later, the HTC116 cells were lysed for DNA extraction, followed by quantification of integrated viral genomes via qPCR. At each time point, and for both kinds of virus vectors, the yield was consistently about 40-50% higher in BalanCD® HEK293 medium, compared to F17 medium.

These results were particularly encouraging in the case of the AAV vectors, since we evaluated a self-complementary genome construct (scAAV9-SMN1) which typically exhibits lower yields than common single stranded AAV vectors.

Figure 3. Enhanced lentivirus and AAV production in a 2L benchtop bioreactor



HEK293T (lentivirus) and HEK293 (AAV) cells were thawed directly into each medium and passaged every 3 to 4 days before going into a 2L benchtop bioreactor run. Cells were seeded and cultured for 3 days before being transfected by PEIpro (Polyplus). For transfection, four plasmids were used for lentivirus and three plasmids were used for AAV. Lentiviral (A) and scAAV9-SMN1(B) titer were measured 48 and 72 hours post transfection. For lentivirus titer, the culture supernatants were collected and treated with DNase and serially diluted before inoculation on HCT116 cell monolayers. One day later, the cells were trypsinized and lysed for DNA extraction. The integrated vector genomes (IG) were quantified by qPCR. For AAV, the cultures were lysed using triton and centrifuged. The supernatants were collected and treated with DNase before DNA extraction and the vector genome (VG) copy number was quantified by qPCR.

We are currently testing various culture parameters with BalanCD® HEK293 medium to optimize the current process, which had been initially designed for FreeStyle™ F17 medium. Our goal is to consolidate and possibly improve the gain of productivity that we observed in this preliminary study. Then, the process will be scaled up to a 200L stirred-tank bioreactor. We will again compare growth characteristics of cells in both kinds of media during this process, in addition to viral vector production. Assuming similar increases in productivity can be achieved at the 200L level by using BalanCD® HEK293 medium, this chemically-defined serum-free medium should prove to be an advantageous and reliable component of the viral vector production process.