Evaluation of animal-origin free peptones to enhance viral vector vaccine production

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ABSTRACT

Vaccines have proven to be an effective tool in controlling numerous emerging and re-emerging infectious diseases. To improve reliability, consistency, and safety and optimize production, global vaccine manufacturers and regulatory agencies are gradually shifting away from using animal serum in their processes. However, completely chemically-defined (CD) media and feeds often compromise on titer goals, driving up the manufacturing costs. The upstream production process can be improved by supplementing cell culture media with peptones. Peptones (or protein hydrolysates) consist of peptide fragments and various other nutritional components created from the hydrolysis of plants, yeast, or animal tissues. Adenovirus (AdV) and Vesicular Stomatitis Virus (VSV) are common viral vectors used for vaccine development, including for SARS-CoV-2 vaccine and Ebola, respectively. Previously, we demonstrated that suspension HEK293 cell growth and wildtype AdV5 production in a CD medium (Dynamis) achieved high cell density and virus titer. In the present study, we evaluated the effect of various animal-origin free (AOF) peptones—soy, yeast, cotton, wheat, and malt—on AdV and VSV productions. Viral titers were quantified by Focus Forming Assay (FFA). In suspension HEK process, our results show that cell density and cell viability decreased for all AdV5 infected cultures from 3 to 6 days post infection (dpi). Addition of 2g/L or 6g/L peptones on 0 and 2dpi (with temperature downshift on 3dpi), resulted in peak AdV titers on 4 or 5 dpi for most conditions. While peak viral titers in several peptone conditions (soy, yeast, and cotton) matched peptone-free media with feed, peptone blend conditions showed greater titer increase.

Addition of peptones (4g/L; with 2% reduced serum) in adherent Vero process showed comparable cell growth to 10% FBS demonstrating potential cost savings for vaccine manufacturers looking to scale up their process. Furthermore, post-infection, VSV production titers were much higher in peptone and peptone blend supplemented reduced serum (2%) conditions compared to 2% FBS conditions.

Overall, these results demonstrate that various AOF peptones can effectively support suspension and adherent cell growth and enhance viral vector production. AOF peptone supplementation can be an economical and efficient option for viral vector-based vaccine manufacturing in the fed-batch or serum reduction process.

INTRODUCTION

Viral vector-based vaccines have been a research focus for decades leading up to the recent (2019) approval of the vesicular stomatitis virus vector-based and human adenovirus-based Ebola vaccines. The SARS-CoV-2 pandemic further highlighted the continued need for vaccines against emerging pathogens. Effective AdV vector-based SARS-CoV-2 vaccines have been developed by several vaccine manufacturers. However, manufacturing cost can be a barrier to accessibility to vaccine. Reduction in cost can be achieved by improvement in the total yield of the viral-vector vaccines and optimization of the processes with peptones in serum-free or serum reduction media. Furthermore, use of AOF peptones can alleviate concerns for stakeholders that desire to move away from animal-derived components. Therefore, vaccine manufacturers continuously require optimization of cell culture parameters.

Requirements for media/supplements used in viral vector-based vaccines:

- Ease of scalability (Advanced Granulation Technology (AGT™) media)
- Free of animal-derived components
- Manufactured under cGMP conditions
- Regulatory support (DMF) for media
- Comparable performance to current media

Goal: Provide process recommendations for AOF peptone usage in viral vector vaccine productions, media, feed/supplement to maximize peak viral vector titers.

MATERIALS AND METHODS

For the growth performance study, suspension HEK293 cells (Gibco Viral Production Cells 2.0 (VPC 2.0), Cat. No. A51218) were evaluated in simple fed-batch conditions in Dynamis Medium (Thermo Fisher Scientific, Cat. No. A2617504). Cells were seeded in triplicate at 1×10^6 cells/mL and cultured in shake flasks (Corning™ flask) at 37°C and 8% CO_2. EfficientFeed™ C+ (2X) (EFC+ (2X)) (Thermo Fisher Scientific, Cat. No. A3937601) was fed at 5% on day 0 and day 2. Viable cell density (VCD) was monitored daily for up to 10 days. For the adenovirus production study, suspension HEK293 cells were adapted to Dynamis Medium for a minimum of 3 passages. Cultures were incubated in triplicate at 37°C and 8% CO_2 and infected with wild-type human adenovirus type 5 (AdV5) (ATCC, VR-1516™) at MOI 0.3 at 1×10^6 cells/mL. EFC+ (2X) was fed at 5%, and Feed A (alternative commercially available feed) at 5% on day 0 (1 hour post infection) and 2 days post infection (dpi) in an ambr15 micro-bioreactor (Sartorius). Glucose was fed to 6g/L when levels dropped below 3g/L. Temperature was downshifted to 33°C on 3 dpi. Samples were collected daily from 3-6dpi. After three freeze-thaw cycles, viral titers were measured with and focus-forming assay. Vero cells were grown in DMEM/F12 (Gibco™ DMEM/F-12, HEPES, Cat. No. 11330032) supplemented with 10% FBS. To evaluate the effect of peptones on cell growth, Vero cells were further adapted in DMEM/F12 neat medium containing 4g/L peptone with 2% FBS for four passages. DMEM/F12 with 10% FBS was used as the control. The VCD and cumulative population doublings were calculated. For VSV production evaluation, Vero cells adapted in various conditions were seeded in T75 flasks. Once the confluency reached to 100%, the cells were washed with DPBS with Ca^{2+} and Mg^{2+} and then infected with VSV at MOI 0.0001 prepared in 2mL of neat DMEM medium. After 1 hour of virus adsorption, 18mL of peptone-containing reduced serum media were added back to each designated flasks. The infected flasks were incubated at 37°C, 5% CO^2. The flasks were harvested on 2dpi. The titer was determined by plaque assay.

RESULTS

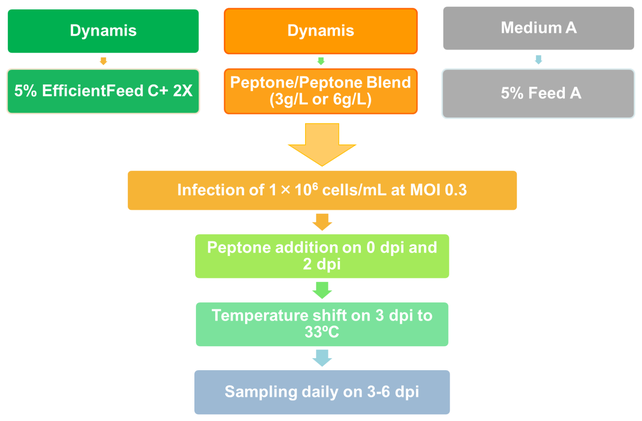


Figure 1. Experimental design for evaluation of adenovirus production in Dynamis Medium with peptones. HEK293 suspension cells were adapted to media and infected with AdV5 in shake flasks. Peptone or peptone blends (2 or 6g/L), 5% EFC+ (2X) and Feed A were fed on 0 dpi and 2 dpi. Temperature shift was performed on 3 dpi to 33°C. Titer was determined daily on 3-6 dpi with focus-forming assay.

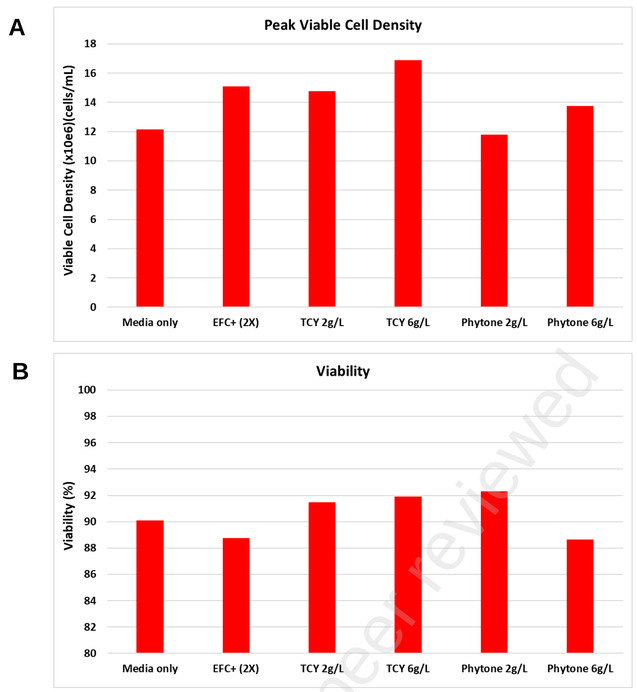


Figure 2. Peak VCD and viability for media with peptones. VPC 2.0 cells were seeded in shake flasks at 1×10^6 cells/mL. Dynamis medium without and with 5% EFC+ (2X) by volume were included as controls. The peptones, TCY and Phytone (2g/L or 6g/L) were supplemented with the media. **(A)** Peak VCD were observed between days 5 and 8 post-seeding. (**B)** Viability at the time of peak VCD.

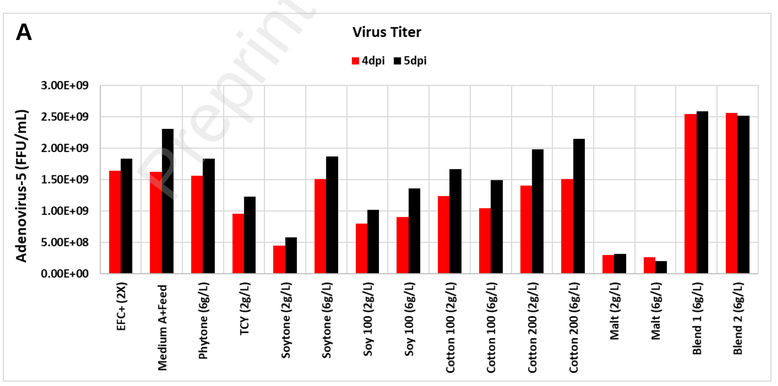
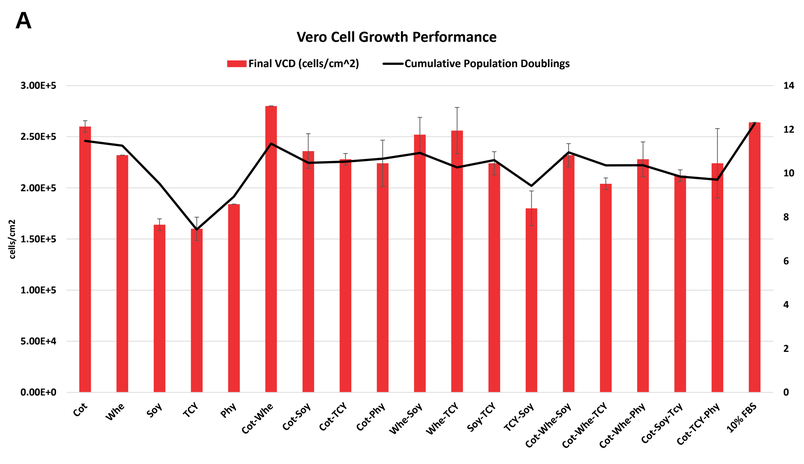


Figure 3. AdV5 production in peptone supplemented suspension HEK293 cells. VPC 2.0 cells were seeded in ambr15 micro-bioreactors (Sartorius) at 1×10^6 cells/mL and infected at MOI 0.3. Infectious virus titers were determined with Focus Forming Assay. Peptones or feed were added on day 0 post-infection and 2dpi. **(A)** Virus titers on peak titer days, 4 (blue bars) and 5 (orange) dpi. **(B)** Change (%) in virus titer compared to EFC+ (2X) control.
Blend 1: Phytone (75%) + TCY (25%); Blend 2: Phytone (50%) + TCY (50%)



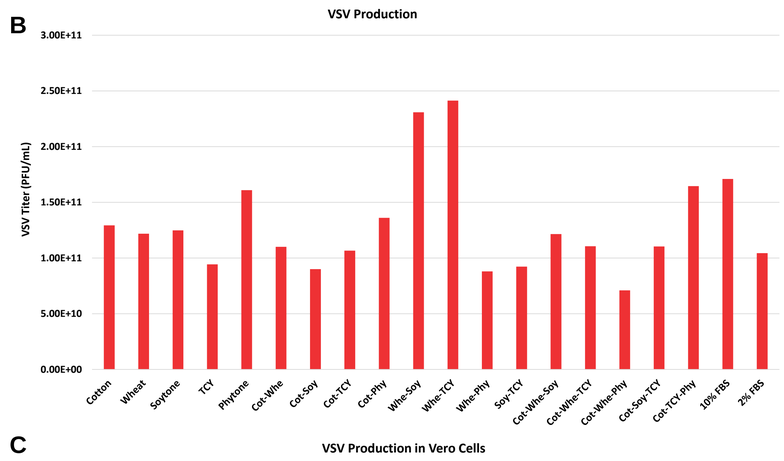


Figure 4. AOF peptones support adherent Vero cell growth in reduced serum conditions and enhance VSV Production. (A) Vero cell growth performance in DMEM F12 + 2% FBS supplemented with 4g/L AOF peptones. Vero cells were passaged in various AOF peptone-containing DMEM F12 Medium with 2% FBS. The VCD from the 4th passages and cumulative population doublings were shown here. **(B)** VSV production evaluation in Vero cells adapted in AOF peptone-containing and reduced serum media. Vero cells were seeded in T75 flasks. While cell density reached to 90 to 100% confluency, the flasks were infected with VSV at MOI 0.0001. After 3 days post infection, most flasks were observed for CPE and then harvested. After one freeze-thaw, the virus samples were aliquot for plaque assay. **(C)** The fold changes of VSV titers in peptone-containing reduced serum media compared to control conditions with 2% or 10% FBS.

Table 1. Gibco® peptone recommendations for viral vector vaccine production.

Peptone	Cat. No.
Phytone	**211906**
TC Yeastolate (TCY)	**292804**
Soytone	**212488**
Soy 100	**215383**
Cotton 100	**215381**
Cotton 200	**215382**
Malt	**218630**
Wheat 100	**670140**

CONCLUSIONS

In this study, we evaluated (1) HEK293 cell growth for AdV5 production in AOF peptone supplemented suspension cell culture process, and (2) adherent Vero cell growth in reduced-serum with AOF peptone for VSV production.

For the suspension HEK293 cell culture process, we observed an improvement in cell growth performance with TCY and Phytone. Wheat peptone is not recommended for suspension HEK293 cells since it causes significant amount of cell clumping (data not shown). For AdV5 production, several peptones had comparable titers to 5% Gibco EfficientFeed C+ (2X) or competitor medium with competitor feed. However, using two peptone blends (TCY and Phytone) demonstrated near 40% increase in AdV5 titers. Ongoing studies are looking into the effects of other two (or more) blends on cell growth and virus production.

For adherent cell culture process, several peptone and peptone blends matched Vero growth performance in reduced-serum conditions compared to 10% FBS. Cotton 200 by itself and as blend with Wheat 100 showed the best results in reduced-serum (2%) condition on par with 10% FBS data. The VSV production data showed peptone blends (4g/L), wheat-soy and wheat-TCY, outperforming serum-only conditions.

Overall, our results demonstrate that Gibco® catalog peptones can offer great value to vaccine manufacturers by improving cell growth performance as well as viral vaccine production. More importantly, peptones can be a great alternative to reduce or remove serum from cell culture process. In addition to the potential cost savings with peptones, AOF peptones as demonstrated here can reduce reliance on animal-derived components.

REFERENCES

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TRADEMARKS

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