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InVitria

Mitigating Risk Beyond Xeno-Free: A Critical Component for Cell-Based Vaccines

The human serum albumin protein is known to perform a multitude of beneficial biological functions. From nutrient delivery to hungry cells, toxin and waste sequestration, and neutralization of free radicals, this unique ~66.5 kD protein does it all (Theodore Peters, Jr.'s All About Albumin). It has become widely accepted that most mammalian cell types require the presence of albumin in their growth environment in order to facilitate adequate in vitro expansion and predictable cellular function. Cells that have been utilized extensively for vaccine production, including VERO, Wi38, and MRC-5, are no exception. This two-part series will capture the usefulness of recombinant human serum albumin and its potential in all areas of the vaccine industry. First, we will show recombinant albumin's ability to promote the expansion of cell lines used for the production of virus. Part two of this series will establish the utility of recombinant human serum albumin in virus particle stabilization.

Traditionally, vaccines produced by mammalian cells have involved cell culture protocols that require the cells be expanded in a fetal bovine serum-containing medium. While bovine serum does contain adequate amounts of albumin as well as a plethora of other helpful components that cells require for sufficient expansion and subsequent virus production, it also comes with the possibility of contamination, skyrocketing prices, and reduced availability. These caveats, as well as the recent advancements in serum free media formulations and increasing pressure from regulatory agencies, have helped transition the vaccine industry away from the use of bovine serum or bovine serum-derived components in all cell culture processes for novel emerging vaccines.

In order to create a xeno-free cell expansion medium, bovine serum and its intrinsic albumin source must be removed and replaced by human serum-derived albumin to maintain adequate cell expansion and proliferation prior to virus production. While this substitution is beneficial for cell growth and viability, the transition from bovine-sourced to human-sourced materials does not reduce the risk of potential viral contamination. As an industry, we are reminded from time to time of the reality of potential blood supply contamination as most recently demonstrated by Zika Virus. Confirming the absence of adventitious agents can be costly, and risk cannot be totally mitigated due to the failure to detect new or emerging virus types, which can become a real fiscal and scientific nightmare. A recombinant human serum albumin that exhibits equivalence to animal or human derived components would minimize viral contamination risks of vaccine products and at the same time maintain or improve vaccine productivity.

The recombinant albumin Cellastim has been shown to be capable of supporting a multitude of different cell types, including the vaccine workhorse VERO and the well characterized MRC-5 fibroblast line. The critical functionality of Cellastim is demonstrated in Figure 1 (below). Cellastim was supplemented into 13 different in-house animal component free (ACF) VERO media formulation candidates (i.e. media formulations completely void of any human or animal serum-derived components) and was shown to significantly boost VERO cell growth in all 13 formulations. The average performance of the 13 formulations with and without Cellastim supplementation are shown in Figure 1. A single candidate formulation was selected to be further optimized, including further optimization of the Cellastim supplementation level, resulting in a serum free, animal component free, defined VERO medium able to propagate VERO cells for 5 passages comparable to FBS in T flasks (Figure 2). Scaling these cultures up to microcarrier culture on 150 mL spinner flasks (Figure 3), the Cellastim-containing ACF media resulted in significantly greater performance versus 10% FBS.

Figure 1.

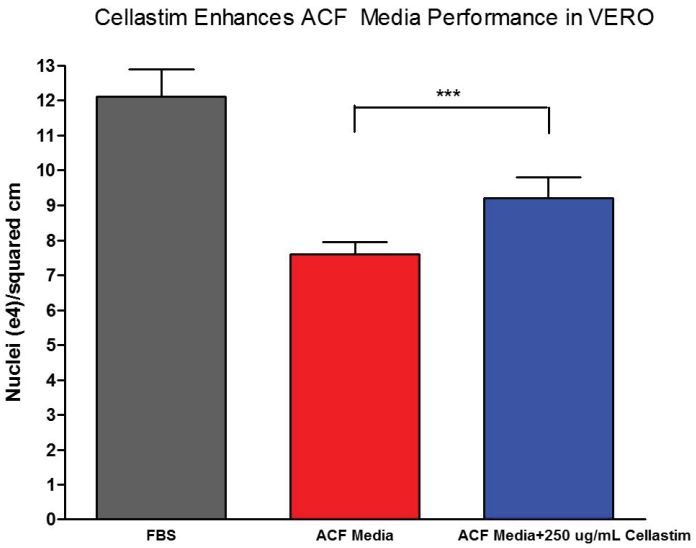

Figure 1. Cellastim Enhances ACF Media Performance in VERO. VERO cells were maintained in EMEM + 2 mM Glutamine + 10% FBS. Cells were washed, trypsinized, and incubated until cell detachment was observed. Trypsin was neutralized with 0.25% soybean trypsin inhibitor and cells were collected in basal EMEM. Cells were pelleted and resuspended in enough basal EMEM to seed VERO cells at an initial seed density of 5,000 cells/cm2 in triplicate in 48-well plates containing 13 different serum free candidate media with or without 250 ug/mL Cellastim. Cells were incubated for 96 hours at standard growth conditions. At the end of the subculture time, medium was removed from the wells and cells were lysed using 1% Triton + 10 mM Citrate in water. Nuclei were stained and counted via a pregated flow cytometer. Data presented is normalized nuclei counts/squared centimeter. Significance of mean differences were determined via T Test between non Cellastim and Cellastim-treated formulations, respectively. Removal of Cellastim demonstrates a significant reduction in VERO cell proliferation. *** $p > 0.001$.

Figure 2.

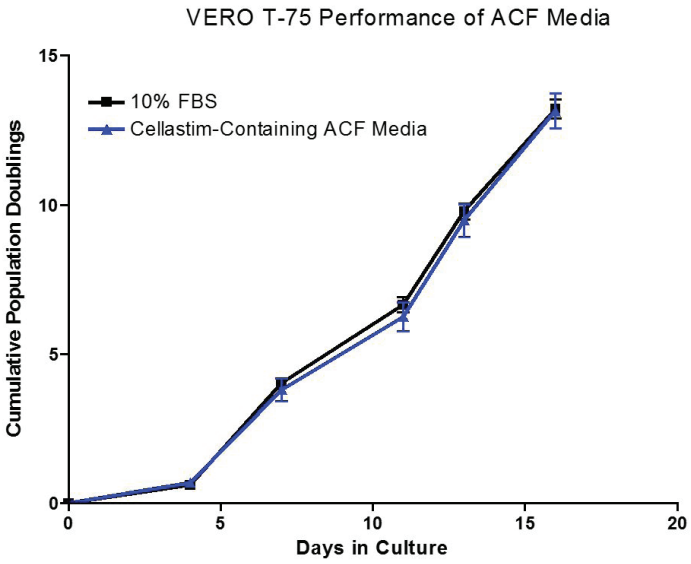

Figure 2. Cellastim supports the growth of VERO cells in an animal component free media in T flasks. Once optimization of the AOF media formulation was complete (figure 1), VERO cell proliferation in T Flasks was compared in EMEM + 10% FBS versus the Cellastim-containing AOF media. At the day of experiment initiation, VERO cells previously grown in serum were washed 3x with DPBS to remove all traces of serum, trypsinized, and harvested after trypsin neutralization. Cells were pelleted and resuspended in basal EMEM and seeded either into T-75 flasks containing 12 mL of EMEM with 2 mM Glutamine + 10% FBS or the Cellastim-containing AOF media at an initial cell density of 10,000 cells/cm2. Cells were subcultured for 96 hrs and were passaged into new flasks using the protocol described in (figure 1). Cells were subcultured for a total of 5 subcultures and population doublings were determined at each passage. The Cellastim-containing AOF media exhibits equivalent doubling time and population doublings as 10% FBS. *** $p > 0.001$.

Figure 3.

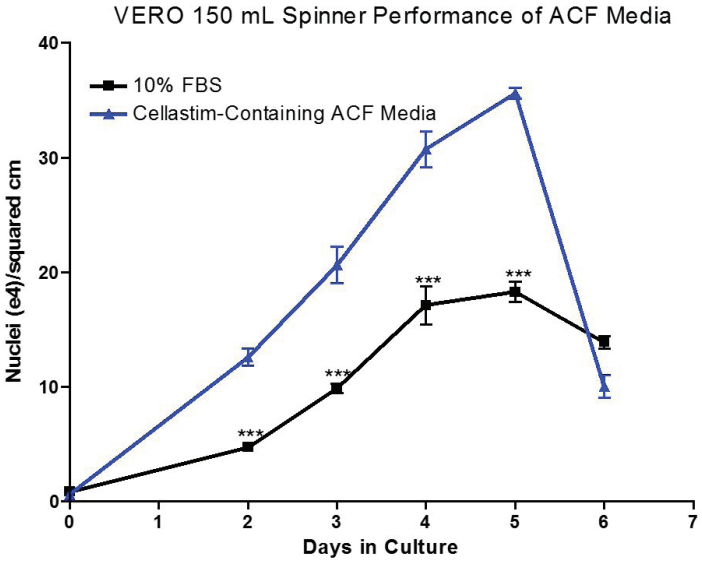

Figure 3. Cellastim supports the growth of VERO cells in an animal component free media in 150mL spinner flasks. VERO cells were thawed directly into either EMEM + 2 mM Glutamine supplemented with 10% FBS or the Cellastim-containing AOF media. Cells were subsequently expanded from a single T-75 to 1 T-150 followed by 3 T-150s in the time span of 14 days. Cells were harvested and seeded into 150 mL spinner flasks containing 10 cm2 /mL plastic microcarriers (SoloHill). Starting at day 2, cultures were sampled every day in order to monitor glucose and pH. Glucose levels were maintained at 1.5 g/L by adding a 45% glucose feed while pH was kept at 6.7 using a 7.5% bicarbonate solution. Cell density was determined by harvesting 500 µL of culture and lysing the cells with 10 mM Citrate 1% Triton solution and determined counting nuclei on a pregated flow cytometer. Data is normalized to nuclei/cm2. Data presented is the average run of 2 independent experiments. The Cellastim-containing AOF media demonstrates robust cell growth in 150 mL spinner flasks.

Similar results were obtained using another animal component free media for MRC-5 cells in 6-well plates (Figure 4):

Figure 4.

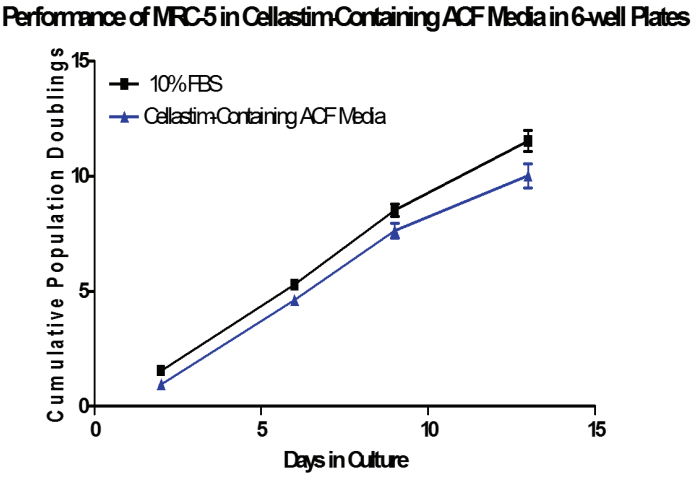

Figure 4. Cellastim Supports the growth of MRC-5 cells in an animal component free media in 6-well plates. MRC-5 cells were maintained in EMEM + 2 mM Glutamine + 10% FBS. Cells were washed, trypsinized, and incubated until cell detachment was observed. Trypsin was neutralized with 0.25% soybean trypsin inhibitor and cells were collected in basal EMEM. Cells were pelleted and resuspended in enough basal EMEM to seed MRC-5 cells at an initial seed density of 2,000 cells/cm2 in an optimized serum free animal component free medium containing Cellastim. Cells were incubated for 96 hours at standard growth conditions. Cells were subcultured as described above every 4 days for a total of 4 subcultures. Total population doublings were quantified at every passage and totaled for the data presented. Significance of difference of mean total population doublings between the two media was determined by T test. Performance was determined to be statistically equivalent.

The combination of these results shows that Cellastim is an extremely useful component in the expansion of cells used to produce virus. Further, inclusion of recombinant animal-free human serum albumin provides risk mitigation of viral contamination in cell culture processes that won't break the bank.

It is a common misconception that the cost of recombinant versions of critical proteins like human serum albumin is too high to be incorporated into large scale cell culture for vaccine production and other applications. With InVitria's animal component free, recombinant HSA (Cellastim), the incorporation of a recombinant human albumin can be affordable and eliminate key disadvantages of working with an unpredictable product like human blood derived serum. Cellastim has already proven its unique ability to enhance performance with its recent incorporation into the production processes of several novel vaccines. While eliminating serum from a cell culture protocol is not always as simple as replacing the albumin source, InVitria offers a full product line of high performing, recombinant, animal-free proteins and supplements that are used to eliminate serum in cell culture from bench scale to large scale vaccine manufacturing. In addition, InVitria offers custom cell culture media development services that will ensure your animal component free media meets your requirements and will allow you less hassle, investment, and regulatory difficulty down the road.

Product Ordering Information

InVitria Cellastim Recombinant Human Serum Albumin-10G (10847-718), 100G (10847-716), 1KG (10847-792)

0716 Lit. No. 091689W