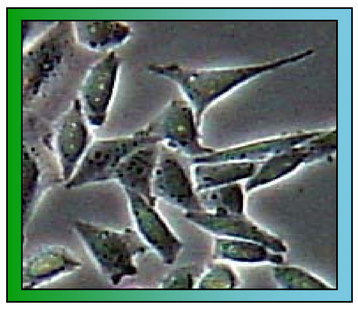

Sheff-Vax supplements significantly reduce the amount of FBS required for culture of Vero, MDCK, MRC-5 and BHK-21 cells

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Introduction:

The presence of Fetal Bovine Serum (FBS) in the cell culture medium is necessary for the growth of some adherent and suspension mammalian cell lines; however, it has the inherent disadvantages of high cost, variability, ethical issues along with the risk of introducing adventitious contaminating agents. The Sheff-Vax systems have been developed to help reduce or eliminate the FBS or other types of animal sera from the medium of industrially relevant cell lines such as Vero, MRC-5, MDCK and BHK-21. The Sheff-Vax systems provide an additional advantage of simple supplementation ; directly to the basal medium and an easy weaning method to help reduce or eliminate the FBS being used in the medium. The purpose of this study was to demonstrate the efficacy of the Sheff-Vax systems in reducing or eliminating the FBS in the medium of a variety of industrially relevant cell lines.

Materials and methods:

In this study, the four different Sheff-Vax system supplements (Sheff-Vax ACF, Sheff-Vax Plus ACF, Sheff-Vax PF ACF and Sheff-Vax Plus PF ACF) were evaluated to determine their efficacy in reducing the FBS in the growth medium of industrially relevant cell lines.

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS was used as a basal medium for the experiments performed with Vero and MRC-5. DMEM supplemented with 5% FBS was used for MDCK cells. The cell lines were maintained in T-75 flasks incubated at 37°C, 5% CO_2 and routinely passaged every three to four days once the cells were found to have reached a confluency of 95% when observed under the microscope. It was necessary to apply a range of concentrations of all four of Sheff-Vax supplements initially to each of the adherent cell lines to determine the optimum concentration. All four Sheff-Vax supplements were made at 100g/L stock concentration in DMEM. Sheff-Vax was added to each cell line at final concentration of 2, 4, 6 and 8 g/L. The cells were then weaned off FBS by slowly decreasing it's concentration each passage by 25%. The optimum Sheff-Vax concentration for each cell line was then selected based on the cell growth and viability.

The experiments for Vero cells were performed in triplicate in T-25 flasks with a working volume of 5 mL and a seeding density of 0.05 x 10^6 cells/ml. For the experiments with the MRC-5 and MDCK cells, the cells were seeded into T-25 flasks at a seeding density of 20,000 cells/cm^2 with a working volume of 10 ml. Routine protocols were used for detachment of the cells from the T-flasks and seeding of the cells into new T-25 flasks to begin an experiment. A commercially available recombinant trypsin was used to detach the cells from the surface of the flasks. Once the serum levels were below 1.5% a commercially available animal component free trypsin inhibitor was used to reduce FBS carryover. Growth and viability were measured on the Nova Bioprofile Flex automated cell counter. The flasks were then incubated for 3-4 days, morphological characteristics were captured, and routine detachment/counting of the cells was performed.. Sheffield Insulin CC was included in the medium once the FBS levels dropped below 1.5% to maintain high growth rate and viability

The four Sheff-Vax systems were initially added to BHK-21 at much higher concentrations of 8, 10 and 12 g/L. The BHK cell lines were then weaned off FBS by gradually decreasing the concentration by 25%. The optimum Sheff-Vax concentration was then selected based on the cell growth and viability. Glasgow Minimal Essential Medium (GMEM) was used as the basal medium for the experiments performed with BHK-21 suspension cells. Pluronic F-68 was used at 0.5% in the medium to maintain the cells in suspension. The BHK-21 suspension cells were maintained in 250 mL Erlenmeyer flasks with a 100 mL working volume. This study was performed in triplicate 125 mL Erlenmeyer flasks with a working volume of 35 mL each and a seeding density of 0.3 x 10^6 cells/mL . The flasks with the BHK-21 cells were incubated at 37°C, 5% CO_2 and constant mixing at 135 rpm. The cells were passaged routinely by centrifuging appropriate volume of the cell suspension at 200g for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in the fresh medium in the corresponding flasks.

Summary:

Using all four of the Sheff-Vax systems, it was possible to adapt and sustain Vero cells at 0% FBS for multiple passages. The optimum Sheff-Vax concentration was found to be 2 g/L. The cells at 0% FBS were reaching cell densities about half that of the 10% FBS control and similar cell viability in the same amount of time (Figure 2). It was observed that the cells growing at 0% FBS reached peak densities similar to the control when allowed to grow for a longer time as compared to the control. The cells did not show any morphological changes as compared to the control (Figure 1). The growth of the Vero cells at 0% FBS was confirmed by passaging the cells in the serum-free medium for a minimum of eight passages.

Sheff-Vax Plus ACF proved to be the best supplement for FBS reduction in the MDCK cell line. Supplementation with Sheff-Vax Plus ACF resulted in reduced usage of FBS from 10% to 0.1%. The cell growth at reduced FBS levels was not affected as seen in Figure 4. When observed under the microscope the cells did not show any morphological changes as compared to the control (Figure 3).

It was observed with the MRC-5 cell line that using Sheff-Vax ACF can reduce the FBS in the medium from 10% to 2.5% with minimal effect on the cell growth (Figure 6). The morphology of the cells was also not affected as observed in Figure 5. Although, further reduction of the FBS in the medium is possible, it will lead to some reduction in the cell proliferation rate as well as morphological changes.

For the BHK-21 cells, all four of the Sheff-Vax systems were successful in reducing the FBS in the medium to 0.5%. The growth of the cells in reduced FBS levels with Sheff-Vax supplements was comparable to the control with 10% FBS (Figure 7,8).

The study shows the high efficiency of the Sheff-Vax systems in reducing the FBS in the growth medium in a variety of industrially relevant adherent as well as suspension cell lines. The advantage of using the Sheff-Vax systems is evident from the simple weaning method involved in adapting the cells to lower FBS levels and in some cases completely eliminating the FBS from the medium.

Morphology of Vero cells at 10% FBS and Sheff-Vax supplemented 0% FBS

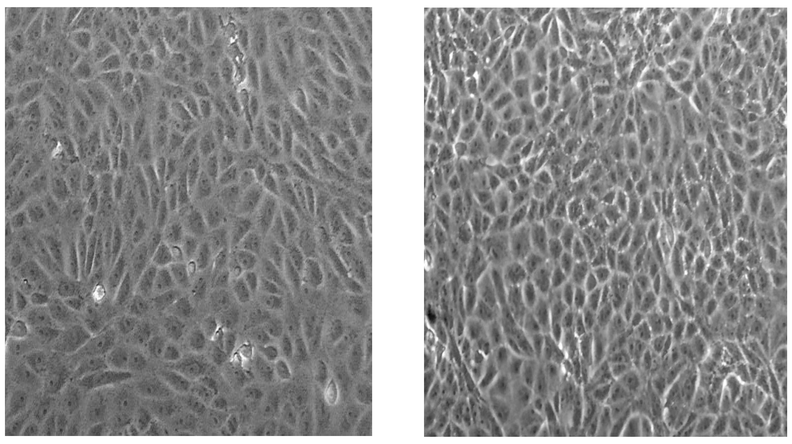

Figure 1

Images comparing the morphology of Vero cells growing at **(A)** *10% FBS control and* **(B)** *Sheff-Vax supplemented cells at 0% FBS supplemented with 2g/L Sheff-Vax ACF + 10mg/L Sheffield Insulin CC. There was no observable morphological difference between the control cells and the cells growing at 0% FBS. All the images were taken using a Leica inverted light microscope at 100x.*

Growth profile for Vero cells in 10% FBS compared to cells grown in Sheff-Vax ACF with DMEM at 0% FBS

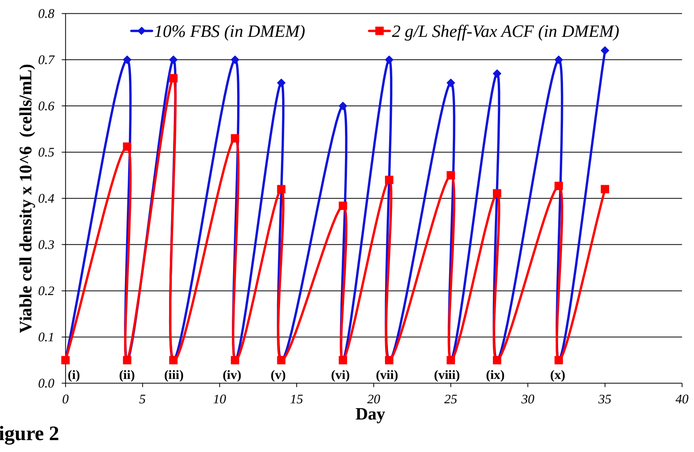

Figure 2

Growth profile for Vero cells in 10% FBS control v/s sequential weaning of FBS at each subsequent passage for the Sheff-Vax supplemented cells. DMEM was used as the basal medium. (i) 10% FBS, (ii) 7.5% FBS, (iii) 5% FBS,(iv) 2.5% FBS, (v) 1.5% FBS, (vi) 1% FBS, (vii) 0.5% FBS, (viii) 0.25% FBS, (ix) 0.1% FBS, (x) 0% FBS. Using the sequential weaning method, it was possible to grow Vero cells at 0% FBS with Sheff-Vax supplementation. The supplemented cells grew to half the peak densities that of control in same incubation time, however higher peak densities can be obtained by using longer incubation periods.

The effect of Sheff-Vax Plus ACF on the morphology of MDCK cells

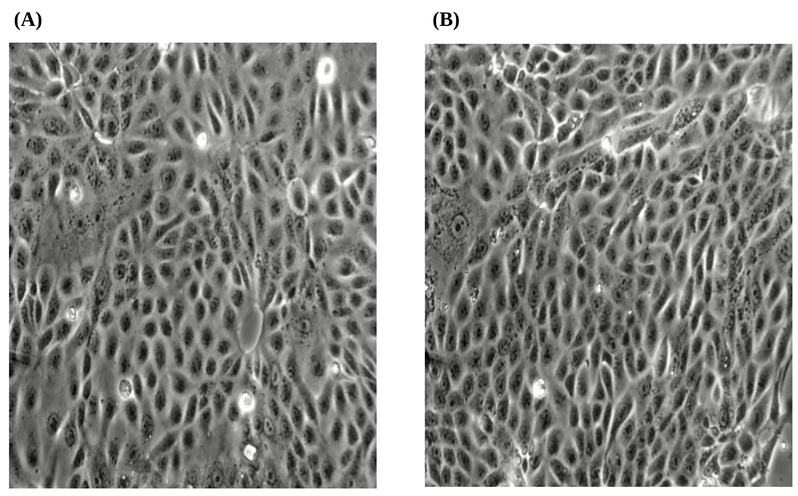

Figure 3

The images were obtained from the MDCK cells cultured in DMEM with **(A)** *5% FBS and* **(B)** *0.1% FBS supplemented with 2 g/L of Sheff-Vax Plus ACF + 10 mg/L Sheffield Insulin CC, respectively. It was shown that no distinguishable morphological changes were observed when Sheff Vax Plus ACF was used.*

Growth profile for MDCK cells grown in 5% FBS compared to cells grown in 0.1% FBS supplemented DMEM with 2 g/L Sheff-Vax Plus ACF

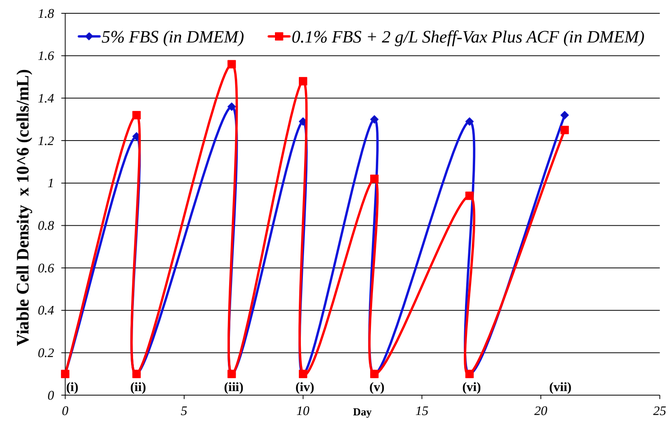

Figure 4

Growth profile for MDCK cells in 5% FBS control versus sequential weaning of FBS at each subsequent passage for 2 g/L Sheff Vax Plus ACF supplemented cells. DMEM was used as the basal medium. (i) 5% FBS, (ii) 2.5% FBS, (iii) 1% FBS, (iv) 0.5% FBS, (v) 0.25% FBS, (vi) 0.1% FBS. Using the sequential weaning method, it was easy to grow MDCK cells at 0.1% FBS with 2 g/L Sheff-Vax Pus ACF supplementation at the same rate as 5%FBS control although the cell density was a little bit lower. However, the normal density could be recovered by using 10mg/L Sheffield Insulin CC (vii).

The effect of Sheff-Vax Plus ACF on the morphology of MRC-5

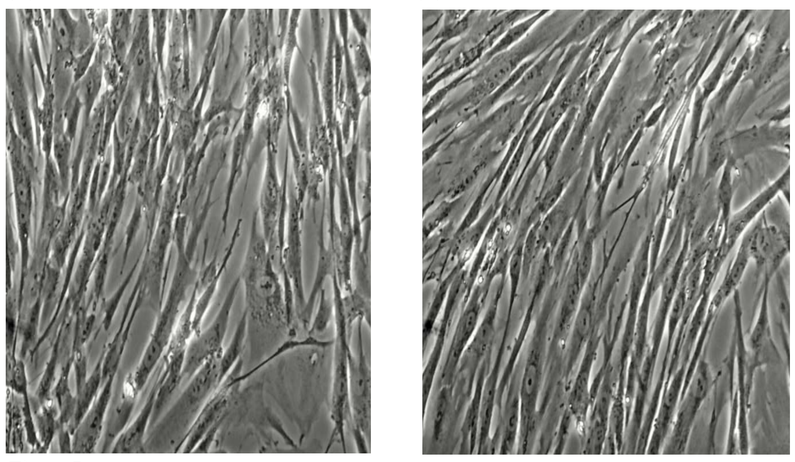

Figure 5

The images were obtained from the MRC-5 cells cultured in DMEM with **(A)***10%FBS and* **(B)** *2.5% FBS supplemented with 2.5 g/L of Sheff-Vax Plus ACF, respectively. It was shown that no distinguishable morphological changes were observed when Sheff-Vax Plus ACF was used.*

MRC-5 cell growth profile in reduced FBS medium with 2.5 g/L Sheff-Vax Plus ACF

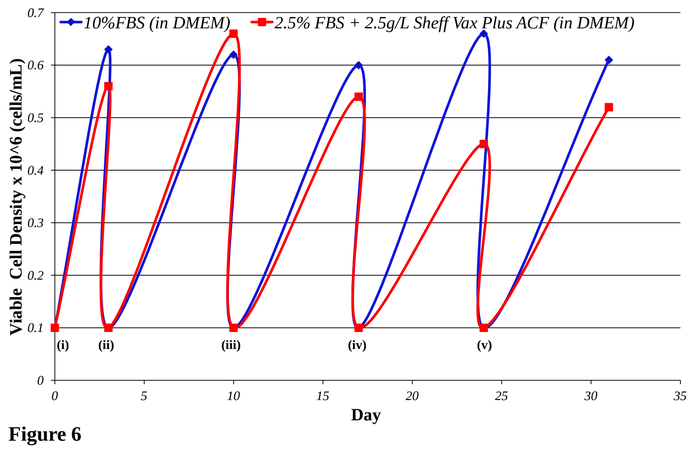

Figure 6

Growth profile for MRC-5 cells in 10% FBS control versus sequential weaning of FBS at each subsequent passage for 2.5g/L Sheff-Vax Plus ACF supplemented cells. DMEM was used as the basal medium. (i) 10% FBS, (ii) 7.5% FBS, (iii) 5% FBS, (iv) 3.5% FBS, (v) 2.5% FBS. Using the sequential weaning method, MRC-5 cells almost could grow at 2.5% FBS with 2.5g/L Sheff Vax Plus ACF supplementation at the same rate as at 10% FBS.

BHK21 cell growth profile in reduced FBS medium with 12 g/L Sheff-Vax ACF

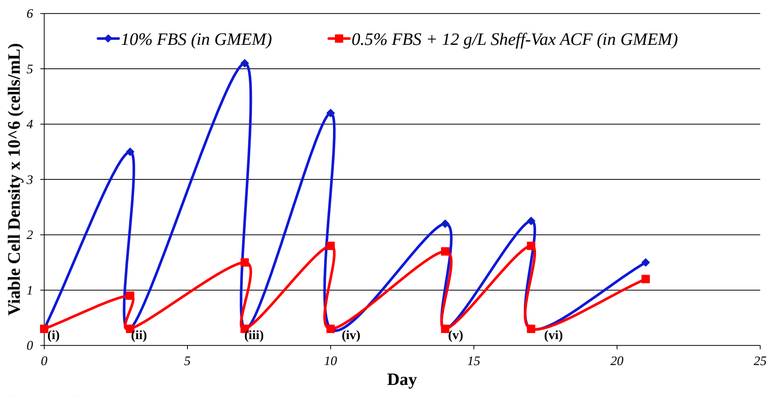

Figure 7

Growth profile for BHK21 cells in 10% FBS control versus sequential weaning of FBS at each subsequent passage with 12 g/L Sheff-Vax ACF, 0.5% Pluronic-F68 and 10 mg/L Sheffield Insulin CC. GMEM was used as the basal medium. (i) 10% FBS, (ii) 7.5% FBS, (iii) 5% FBS, (iv) 2.5% FBS, (v) 1% FBS, (vi) 0.5% FBS. The sequential weaning method for the reduction of FBS was successful for the BHK21 cells with the cells in reduced FBS medium with Sheff-Vax ACF reaching similar peak densities as the control medium in the same time period.

BHK21 cell growth profile in reduced FBS medium with 12 g/L Sheff-Vax PF ACF

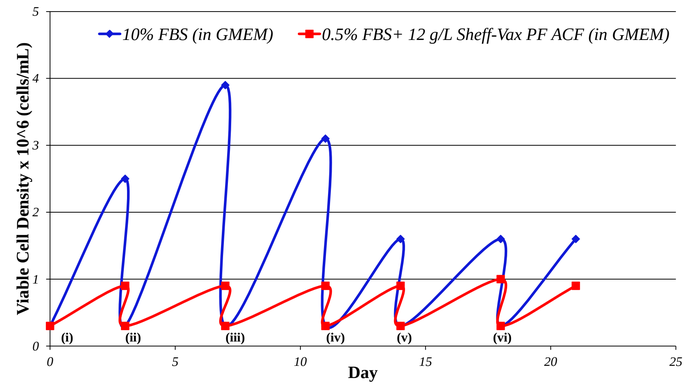

Figure 8

Growth profile for BHK21 cells in 10% FBS control versus sequential weaning of FBS at each subsequent passage with 12 g/L Sheff-Vax PF ACF, 0.5% Pluronic-F68 and 10 mg/L Sheffield Insulin CC. GMEM was used as the basal medium. (i) 10% FBS, (ii) 7.5% FBS, (iii) 5% FBS, (iv) 2.5% FBS, (v) 1% FBS, (vi) 0.5% FBS. Using the Sheff-Vax PF ACF in the weaning method, it was possible to reduce the FBS to 0.5% and the cells reached similar peak densities as compared to the control with 10% FBS.