A new, chemically defined cell-culture medium prototype for direct adaptation of BHK-21 suspension cells to serum-free growth

Ann Lövgren[1], Rena Baktur[2], and Angel Varela-Rohena[2]

[1]Cytiva, Uppsala, Sweden, [2]Cytiva, North Logan, USA

Introduction

Mammalian cells have traditionally been cultured with serum added to the culture medium to support growth. Serum-containing cultures are still common within certain applications such as vaccine manufacturing. However, serum supply is limited and there are several advantages to using serum-free media and suspension culture. Such cultures are easy to scale up and culture medium components are well defined. Therefore, transition to serum-free suspension culture is often desirable. The caveat is that such transition requires that cells are adapted to the new cell culture medium. This is not always easy to achieve and may take a long time. In addition, it is not always possible to obtain the same growth rate and characteristics as with serum-containing culture, and new cell banks may have to be made to support production from the adapted cells.

Baby hamster kidney (BHK-21) cells are currently used for vaccine production (rabies and foot-and-mouth disease), AAV viral vectors, as well as production of recombinant proteins such as Factor VIIa and Factor VIII. We have developed a chemically defined prototype medium (working name, RR18517) for suspension BHK cells previously grown in a serum-containing medium that supports direct adaptation, that is, the BHK cells can be transferred directly into the prototype medium without significant loss in viability and growth rate. Here we show data that suggest that our prototype medium may be a good option for culturing suspension BHK cells under serum-free conditions.

Materials and methods

Cells and cell culture procedures

BHK21C13-2P, suspension-form cells were from ECACC and were purchased from Sigma™-Aldrich Co. (cat. No 84111301). Cells were thawed in DMEM high glucose (HyClone™ SH30285 FS) supplemented with 8 mM L-glutamine (HyClone™ SH30034.01) and 10% characterized US origin fetal bovine serum (HyClone™ SH30071.03), and a research cell bank (RCB) was established in this medium.

Adaptation of suspension BHK cells was performed both by direct and stepwise adaptation. A direct adaptation of cells was performed by transfer of cells directly into the new cell culture medium and continued to culture the cells in the new medium until robust growth was observed. We performed stepwise adaptation by thawing and culturing the cells in the original medium and at the next passage, 50% to 75% of the original medium was replaced with the new medium. When robust growth had been established, we used a larger portion of the new medium. This was repeated until we obtained a complete replacement.

The BHK-21 cells were adapted to and cultured in the following media: DMEM + 10% FBS + 8 mM L-glutamine, GMEM + 10% FBS + 2 mM L-glutamine + 5% Tryptose Phosphate Broth, prototype RR18517, CD BHK-21 (GIBCO™, Thermo Fisher Scientific), and BHK-200 (Sigma™-Aldrich Co.).

We performed cultures in shake flasks or spin tubes in CO_2 incubators with 5% to 7% CO_2. Cells were passaged at 0.3 million cells (Mc)/mL every 2 days (d), or 0.15 Mc/mL every 3 d.

Results and discussion

Adaptation to prototype medium

We adapted suspension BHK-21 to growth in serum-free media using both direct adaptation and stepwise adaptation procedures. An example of results from direct adaptation into prototype medium RR18517 is shown in Figure 1. The growth rate was similar in supplemented DMEM and prototype medium (Fig 2). During this direct adaptation to the prototype medium, cell viability did not drop below 90%, and stable growth was observed from passage 4. When comparing the successfully adapted BHK-21 cells in prototype medium to DMEM supplemented with serum, a similar growth kinetic profile was detected with very high specific growth rates of 1.3 1/d (Fig 2). Sequential adaptation to prototype RR18517 medium gave a similar result (results not shown).

We repeated adaptation to the prototype medium and to other serum-free media — CD BHK-21 (GIBCO™) and BHK 200 (Sigma™-Aldrich Co.) — several times. The only media that allowed direct adaptation to growth without serum were prototype RR18517 and CD BHK-21. However, the cells could readily be adapted to the media tested using the stepwise approach.

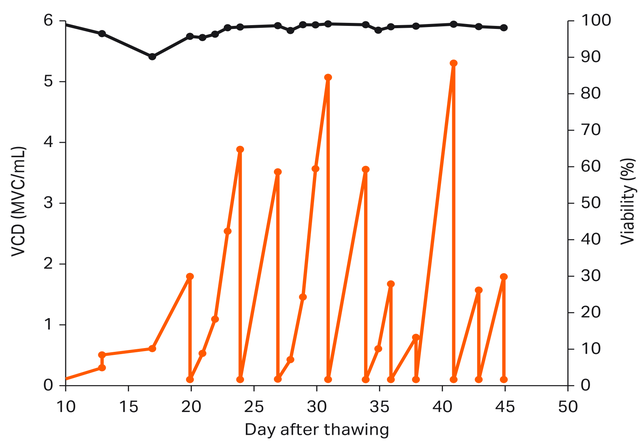

Fig 1. Example of VCD (orange) and viability (black) of suspension BHK cells during direct adaptation into prototype medium. Adaptation was initiated 10 d post-thaw in the original serum-containing medium.

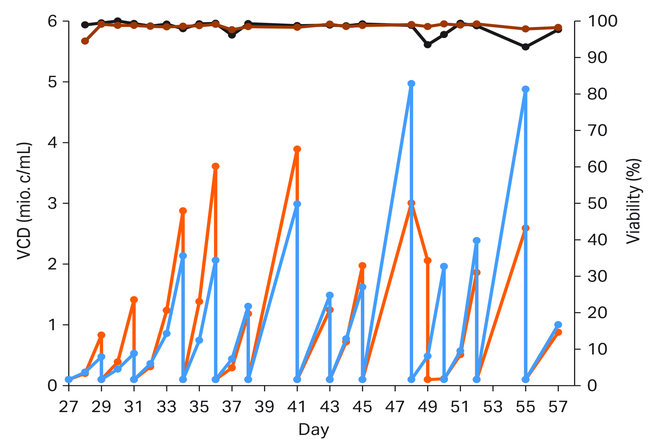

Fig 2. Comparison of growth in supplemented DMEM (orange for VCD, black for viability) and prototype medium (blue for VCD and brown for viability).

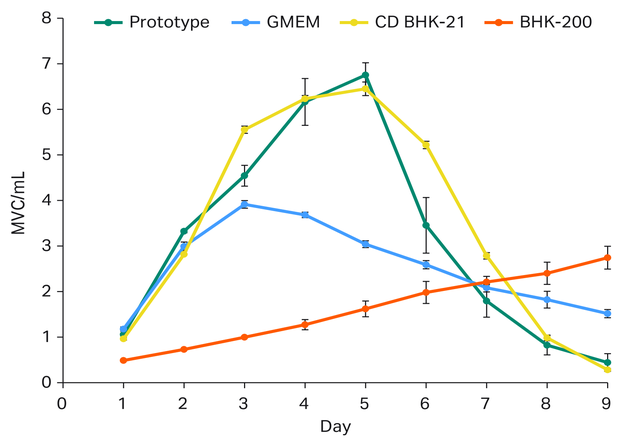

Fig 3. Viable cell density comparing different cell culture media. Cells adapted to respective media were seeded at 0.3 Mc/mL and cultured in shake flasks in triplicate in batch mode. Error bars indicate standard deviation.

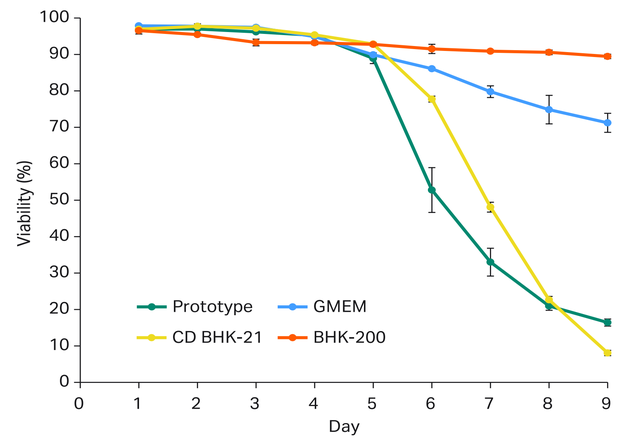

Fig 4. Viability comparing different cell culture media. Cells adapted to respective media were cultured in shake flasks in triplicate in batch mode. Error bars indicate standard deviation.

Cell culture in different media

We compared growth of adapted cells in the different serum-free media and supplemented DMEM or GMEM (Fig 2, 3 and 4). Shake flask cultures were run in triplicate and viable cell density and viability were followed for 9 d. All cultures were started at 0.3 Mc /mL on day 0. Growth in prototype RR18517 and CD BHK-21 was similar with a maximal average VCD of 6.7 and 6.5 Mc/mL, respectively, while growth in supplemented GMEM or BHK-200 was slower with a maximal average VCD of 3.9 and 2.7 Mc/mL, respectively (Fig 3). Consistent with the slower growth rate in GMEM and BHK-200, viability remained higher in these media compared with CD-BHK and prototype RR18517.

The recommended culture medium for BHK21C13-2P, suspension form cells from ECACC is GMEM + 2 mM glutamine + 5% Tryptose Phosphate Broth + 10% fetal bovine serum (FBS). We have, however, also successfully used DMEM + 8 mM L-glutamine (HyClone™ SH30034.01) and 10% characterized US-origin FBS (HyClone™ SH30071.03). In fact, we obtained faster growth and higher maximal VCD using supplemented DMEM.

With prototype RR18517, we have developed a new BHK-21 complete culture medium that is chemically defined and supports growth to high VCD.

References

1. Kallel H, Jouini A, Majoul S, Rourou S. *J. Biotechnol.* 2002;95(3):195–204.
2. Park S, Kim JY, Ryu Kh et al. *Vaccines* 2021;9(5):505.

Conclusions

- The chemically defined serum free medium prototype RR18517 consistently allowed direct adaptation of suspension BHK-21 cells to serum free growth.
- Prototype RR18517 is chemically defined, requires no further supplementation, and does not contain hydrolysates.
- Maximal VCD observed with prototype RR18517 was 6.7 million cells/mL.

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