Comparison of MDCK Proliferation and Flu Production in Suspension Culture on Various Microcarriers

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1. Abstract

Cell-based influenza vaccine production is quickly growing in the vaccine industry in an attempt to meet the threat of pandemic outbreaks and to eliminate health concerns associated with egg protein allergies. Madin Darby Canine Kidney (MDCK) is a commonly used cell line for production of influenza virus and the cell-based manufacture of inactivated flu vaccines. Expansion of MDCK cells allows for rapid response and quick scale up compared to the traditional egg-based vaccine manufacturing process. This study examines the feasibility of using microcarrier-based cell culture for the growth of MDCK cells in 3D. Several cell culture media were compared for their ability to support MDCK cell growth in planar and suspension cultures as well as their ability to support flu virus production in MDCK cells. The preparation and usage of the various microcarriers were based on the manufacturers’ recommendations and were evaluated for ease of use, ability to support cell proliferation and virus production, and ability to support MDCK expansion without cell disorganization in disposable culture systems. Our results show that ProMDCK™ (2D) and ProMDCK™ (3D) (Lonza) support excellent cell proliferation and virus production in both planar culture and in suspension culture on multiple types of microcarriers. We also demonstrate that MDCK cells can be expanded without cell disassociation by adding new microcarriers to the culture.

2. Methods

2.1 Cell Culture

MDCK (ATCC, CRL-3283) and MDCK (ATCC, CRL-34) were thawed and expanded in serum-containing medium according to instructions. Two media were employed in the adaptation of MDCK cells to Serum-free (SF) medium. For direct adaptation, the MDCK cells were expanded in T-flasks with DMEM-FBS before seeding onto the microcarriers in several commercially available SF media. In the second process, the MDCK cells were seeded 5 passages in T-flasks in the various SF Media before seeding onto the microcarriers in spinner flasks. Cells were seeded at 20,000 cells/cm² of microcarriers and passaged at either Day 5 or by confluence level. 3D cultures were passaged by transfer of confluent microcarriers into new spinner containing fresh SF medium and microcarriers without the use of a detachment enzyme.

2.2 Cell Culture Medium Comparison

Lonza’s serum-free ProMDCK™ Media was compared to four other commercially available serum-free media formulated for MDCK cell proliferation and vaccine production. Those were evaluated for growth performance in 2D and 3D culture formats. Competitor 1 medium contains animal components, while Competitor 2 and Competitor 4 contain secondary animal sourced materials. Ingredients in Lonza’s ProMDCK™ (2D) and (3D) Media are sourced from non-animal origin (NKO) components. Competitor 1, Competitor 2, Competitor 4 and ProMDCK™ Media contain plant material, while Competitor 3 medium is a protein-free defined formulation.

2.3 Microcarrier Evaluation

The microcarriers used in this study included non-porous polystyrene microcarriers, with and without surface treatment; microcarriers composed of dextran; and porous microcarriers made from gelatin or plant material. For consistency in evaluation, all microcarriers were washed with Phosphate Buffered Saline (PBS) without the addition of an attachment factor (serum, gelatin or albumin). The microcarrier density, stir speed and pause/stir cycles were based on the manufactures’ recommendations and were evaluated for ease of use, ability to support cell proliferation and virus production, and ability to support MDCK expansion without cell disorganization in disposable culture systems. Our results show that ProMDCK™ (2D) and ProMDCK™ (3D) (Lonza) support excellent cell proliferation and virus production in both planar culture and in suspension culture on multiple types of microcarriers. We also demonstrate that MDCK cells can be expanded without cell disassociation by adding new microcarriers to the culture.

3. Conclusions

- ProMDCK™ (2D) supports the expansion of MDCK cells in 2D cultures.
- ProMDCK™ (3D) supports the expansion of MDCK cells on a variety of microcarriers in serum-free culture.
- ProMDCK® supports robust influenza virus production.
- Different brands of microcarriers have distinct advantages and disadvantages.
- Microcarrier to microcarrier migration of MDCK cells can be achieved for quick scale up and virus production without the requirement for enzymatic disassociation.