BioResearch

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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 culture 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 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 dissociation 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 dissociation by adding new microcarriers to the culture.

2. Methods

Cell Culture

MDCK (ATCC, CRL-34) were thawed and expanded in serum-containing medium according to instructions. Two methods 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-10% FBS before seeding onto the microcarriers in several commercially available SF media. In the second process, the MDCK cells were carried 5 passages in T-flasks in the various SF Media before seeding onto the microcarriers in spinner flasks. Cells were seeded at 20,000c/cm^2 of microcarriers and passaged at either Day 5 or by confluence level. 3D cultures were passaged by transfer of confluent microcarriers into new spinners containing fresh SF medium and microcarriers without the use of a detachment enzyme.

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. These 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 (NAO) components. Competitor 1, Competitor 2, Competitor 4 and ProMDCK™ Media contain plant material, while Competitor 3 medium is a protein-free defined formulation.

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 manufacturers' recommended procedures.

Cell Proliferation

Cell yield and viability were measured for the 2D and 3D culture systems using an automated cell counter (ViCell). Trypsin/EDTA was used to dissociate cells from the T-flasks for planar culture passages and for performing cell counts from 3D cultures.

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 dissociation.

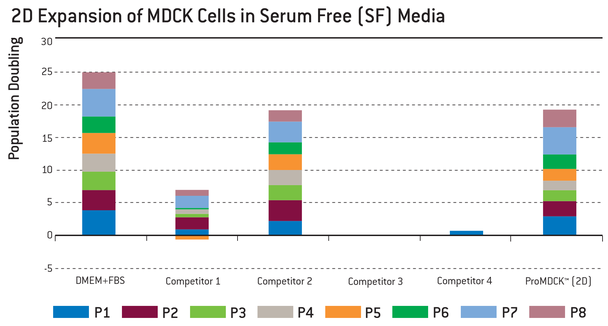

Figure 1. Population doubling of MDCK cells in 2D (planar) cultures in a variety of SF media directly adapted from DMEM-10% FBS. Cells in Competitor media 3 and 4 were unable to adapt directly from serum-containing medium. Competitor 1 medium experienced a significant lag before cells were able to proliferate and never supported robust cell growth. Cells successfully expanded directly from serum containing medium in Competitor 2 medium and in ProMDCK™ (2D).

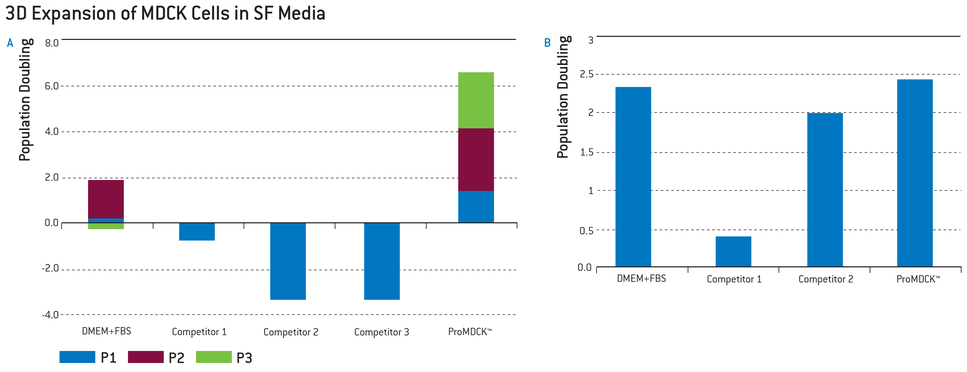

Figure 2. Population doubling of MDCK in 3D culture using Corning untreated microcarriers. Direct adaptation from DMEM-10% FBS 2D culture (Fig. 2A). Cells carried for 5 passages in each test medium in 2D culture before seeding onto microcarriers (Fig. 2B). For all media tested, except ProMDCK™ Media, cells required adaptation to the medium in 2D culture before successful attachment and expansion in 3D culture was possible.

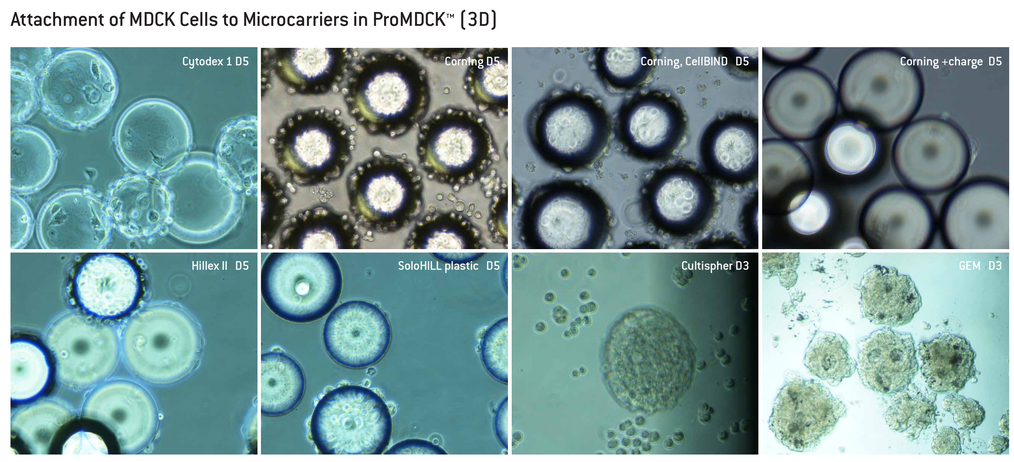

Figure 3. MDCK cells attach to different microcarriers in ProMDCK™ (3D) Medium. Porous microcarriers, GEM and Cultispher, were visualized on Day 3. Cytodex, Corning and SoloHILL–based cultures were visualized on Day 5 (Images 100X) to yield images at maximal cell density.

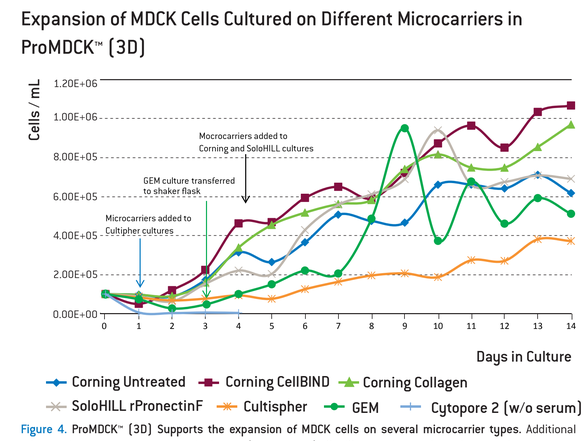

Figure 4. ProMDCK™ (3D) Supports the expansion of MDCK cells on several microcarrier types. Additional microcarriers were added where indicated (black arrow). CultiSpher microcarriers dissolved in culture by Day 1; new microcarriers were added (blue arrow). The metallic component of the GEM microcarriers caused them to attach to the magnetic impeller so culture was transferred to shaker flask culture (green arrow).

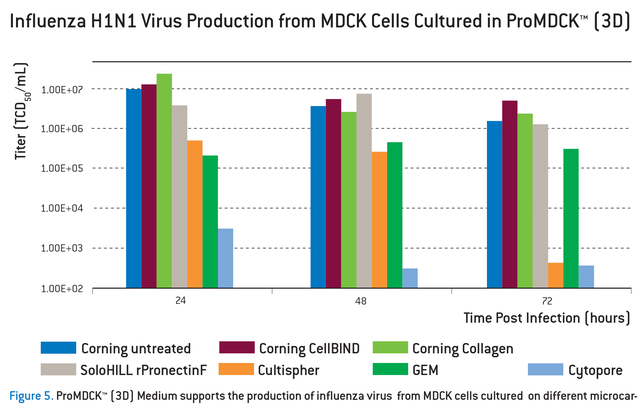

Figure 5. ProMDCK™ (3D) Medium supports the production of influenza virus from MDCK cells cultured on different microcarrier types. MDCK cells were infected with 0.01 MOI of H1N1 (strain A/PR/8/34) at Day 4 (80-90% confluence) of the 3D culture. Supernatants were harvested and titered at different days post-infection as indicated.

Manufacturer	Item	Animal Component	Material	Porous	Features
GE Healthcare Pall	Cytodex 1	No	Dextran	Microporous	– Can be used in rocking bioreactors, siliconized glass or polished stainless steel vessels – Cytodex's transparent material allows for microscopic inspection of attached cells
	Cytodex 3	Yes (Porcine Gelatin)	Dextran	Microporous	– Can be used in rocking bioreactors, siliconized glass or polished stainless steel vessels – Cytodex's transparent material allows for microscopic inspection of attached cells – Collagen surface is enzyme degradable for cell harvesting
	Cytopore	No	Cellulose	Macroporous structure with Micro matrix	– Designed for use in stirred suspension cultures – Hygroscopic – Non-toxic and biodegradable material
SoloHILL	Hillex II	No	Plastic	Microporous	– Can be autoclaved – Solid structure made of polystyrene material
	Pronectin F	No	Plastic	No	
Corning	+ Charge	No	Plastic	No	– SAL 10-6 sterilization level – Class VI material – Solid structure made of polystyrene material
	Untreated	No	Plastic	No	
	Enhanced Attachment	No	Plastic CellBIND Surface	No	
	Collagen coated	Yes (Porcine Collagen)	Plastic	No	
PerCell Biologics	CultiSpher-G, standard porosity	Yes (Porcine Gelatin)	Gelatin	Yes	– Can be autoclaved – Enzyme degradable for cell harvesting
Global Cell Solutions	Global Eukaryotic Microcarrier (GEM)	Yes (various coatings)	Alginate core with silicated iron particles Gelatin layer Also available with other coatings	Yes	– Sterile – Magnetic particles for easy media exchange – Enzyme degradable for cell harvesting

* Cost per gram in USD: Low ≤ $12 per gram; Medium between $12 and $17 per gram; High > $17 per gram

Figure 6. Features of common microcarriers on the market used with SFM ProMDCK™

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