SAFC Biosciences™
Accelerate Success™

Research Report

Evaluation of MDCK Cell Growth and Virus Production in EX-CELL™ MDCK

Manisha Sahni, Shelley Wilcox, Pamela Mettner, Cynthia Reiss, Sarah L. Gilliland, Douglas R. Purtle, Karen J. Etchberger

Abstract

EX-CELL™ MDCK is a serum-free, animal-protein free medium designed and optimized to support high-density culture of Madin Darby Canine Kidney (MDCK) cells. The MDCK cell line can be easily adapted to EX-CELL™ MDCK using a direct adaptation method or a sequential adaptation method. EX-CELL™ MDCK supports MDCK cell density up to 5 x 10^5 cells/cm^2 with doubling time as short as 30 hours. MDCK cells in EX-CELL™ MDCK were also infected with Canine Adenovirus (CAV) and produced $10^{7.5}$ $TCID_{50}$/mL at a multiplicity of infection (MOI) of 0.1 and $10^{7.4}$ $TCID_{50}$/mL at a MOI of 0.01. It was concluded that EX-CELL™ MDCK supports rapid MDCK cell growth and produces high adenovirus production.

Introduction

The MDCK cell line is used in applications such as development of viral vaccines, anticancer agents and the production of recombinant adenoviral vectors.

Traditionally, MDCK cells are grown in serum-supplemented basal medium such as Minimum Essential Medium (MEM). EX-CELL™ MDCK is a serum-free, animal-protein free medium specially formulated to support large-scale, high-density MDCK culture and adenovirus production. The medium contains very low levels of recombinant protein (approximately 1.1 mg/L), facilitating downstream processing of expressed products and eliminating regulatory concerns associated with serum and animal proteins. Additionally, the liquid formulation of EX-CELL™ MDCK is formulated without L-glutamine, which avoids problems associated with L-glutamine degradation, therefore improving product shelf-life. Our experiments show that EX-CELL™ MDCK supports high-density, serum-free MDCK cell growth and the production of high yields of adenovirus.

Materials

Cells

- Madin Darby Canine Kidney cell line, American Type Culture Collection, Catalog No. CCL-34

Virus

- Canine Adenovirus (CAV) Strain: Toronto A 26/61, American Type Culture Collection, Catalog No. VR-800, Lot Number 217526

Serum-Free Media

- EX-CELL™ MDCK, SAFC Biosciences, Catalog No. 14580

Other Media and Supplements

- Minimum Essential Medium, Alpha Modification (α-MEM), SAFC Biosciences, Catalog No. 51451
- Fetal Bovine Serum (FBS) Gamma Irradiated, SAFC Biosciences, Catalog No. 12107
- L-glutamine 200mM, SAFC Biosciences, Catalog No. 59202
- Trypsin 0.25%, 0.1% EDTA, Gamma Irradiated, SAFC Biosciences, Catalog No. 59429
- Dulbecco's Phosphate Buffered Saline (DPBS), without Calcium and Magnesium, SAFC Biosciences, Catalog No. 59321
- Soybean Trypsin Inhibitor (STI), Sigma-Aldrich, Catalog No. T-6522
- Trypan Blue 0.4%, Sigma-Aldrich, Catalog No. T-8154
- Dimethyl sulfoxide (DMSO), Sigma-Aldrich, Catalog No. D-2650

Methods

Media and Supplement Preparation and Storage

EX-CELL™ MDCK was prepared by adding L-glutamine at a final concentration of 6 mM at time of use. Unless otherwise

United States
SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe
SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific
SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com

noted, all EX-CELL™ MDCK was supplemented before use. 10% FBS was added to α-MEM at time of use (referred to as α–MEM-10%). Soybean Trypsin Inhibitor (STI) was prepared as a concentrated (10 mg/mL) solution in DPBS and filter sterilized (0.2 µm). Working stock solutions of STI were diluted to 1 mg/mL with sterile DPBS as needed.

All media were stored at 2 to 8 C protected from light. Other supplements were stored at their recommended temperatures.

Basic Culture Techniques

Cells were routinely subcultured every 3 days (72 hours ± 6 hours) at a seeding density of 1 x 10^5 cells/cm^2 (in EX-CELL™ MDCK) or 5 x 10^4 cells/cm^2 (in α-MEM-10%) in 75 cm^2 vent-cap T-flasks (Corning). The total volume of media was 20 mL per flask. Cultures were maintained at 37 C ± 1 C in a humidified incubator with 5% CO_2. All experiments were performed in triplicate flasks, except where noted. Cell counts were determined by standard counting technique using a hemocytometer (i.e. typically a 1:10 dilution in 0.04% trypan blue, with 5 squares of the hemocytometer counted). All cultures were maintained using aseptic technique and without the use of antibiotics or fungicides.

Trypsinization

MDCK cultures in α-MEM-10%:

Spent medium was aspirated and the flasks rinsed with 5 mL DPBS. The DPBS was aspirated and 3 mL trypsin was added to the flasks. The flasks were incubated at 37 C for 13 - 15 minutes until the cells dissociated. 10 mL of α-MEM-10% was then added to the flasks, the cells gently resuspended and a sample taken for counting. Cells were subsequently diluted in the appropriate quantity of medium and incubated as above.

MDCK cultures in EX-CELL™ MDCK:

Spent medium was aspirated and the flasks rinsed with 5 mL DPBS. The DPBS was aspirated and 3 mL trypsin was added to the flasks. The flasks were incubated at 37 C for 13 - 15 minutes until the cells dissociated. 7 mL of STI was added and the cells gently resuspended and transferred to sterile 15 mL conical tubes. The tubes were centrifuged at 1000 rpm (228 *g*) for 5 minutes to pellet the cells. The supernatant was removed, discarded and the pellet resuspended in 10 mL of prepared medium. A small aliquot was taken for counting and the cells were subsequently diluted in the appropriate quantity of medium and incubated as above.

Notes:

1. MDCK cells in EX-CELL™ MDCK medium appeared sensitive to mechanical force. It was found that rapping the flask to dislodge the cells resulted in decreased viability.
2. Over-trypsinizing the cells may occur quickly. It is important to inactivate the trypsin within 15 minutes (less time in smaller flasks). Over-trypsinized cells do not recover.
3. The use of STI is not absolutely required. After trypsinization, cells may be resuspended in a small quantity of EX-CELL™ MDCK and then centrifuged to remove the trypsin. The use of STI may, however, ease the adaptation to EX-CELL™ MDCK.

Direct Adaptation

MDCK cells were started from frozen cells in α-MEM-10% in 25 cm^2 T-flasks, expanded and maintained in 75 cm^2 T-flasks. MDCK cells were subcultured directly into EX-CELL™ MDCK at a seeding density 1 x 10^5 cells/cm^2, the cells reached 100% confluency within 3 days and displayed normal doubling times.

Direct Adaptation of MDCK Cells to EX-CELL™ MDCK

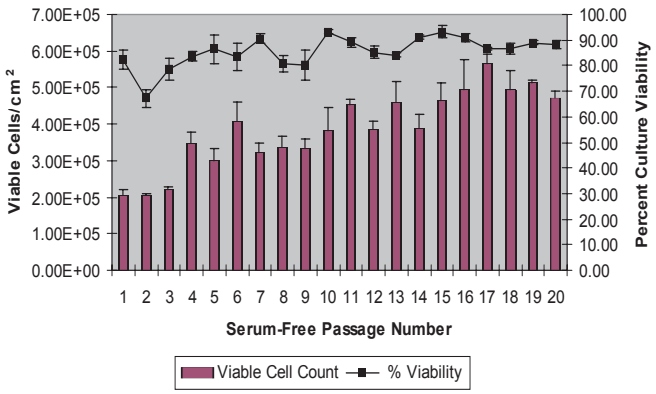

Figure 1: MDCK cells were maintained in EX-CELL™ MDCK for 20 passes after direct adaptation. Cell counts during the first 3 - 4 passes in EX-CELL™ MDCK mimicked those seen in α-MEM-10%, then cell counts gradually increased during the course of the study.

Doubling Times (Hours) of MDCK Cells in EX-CELL™ MDCK

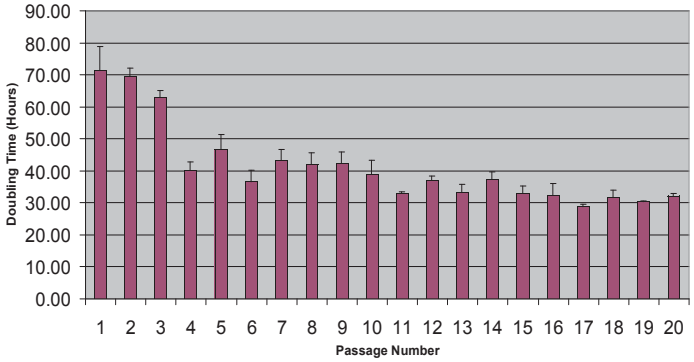

Figure 2: As MDCK cell densities increased, the cell doubling times decreased. In addition, as the cell counts increased, it was noted the morphology of the cells appeared more normal. Average MDCK cell density in EX-CELL™ MDCK was 3.9 x 10^5 cells/cm^2 and average doubling time was 30 - 40 hours. In comparison, the average cell density in α-MEM-10% was approximately 2 x 10^5 cells/cm^2 with doubling times of about 60 hours.

Gradual (Sequential) Adaptation

MDCK cells were adapted over 4 passages whereby the concentration of serum-containing medium was reduced by 25% at each pass. Cell densities and doubling times were similar to those seen during the direct adaptation.

Sequential Adaptation of MDCK Cells to EX-CELL™ MDCK

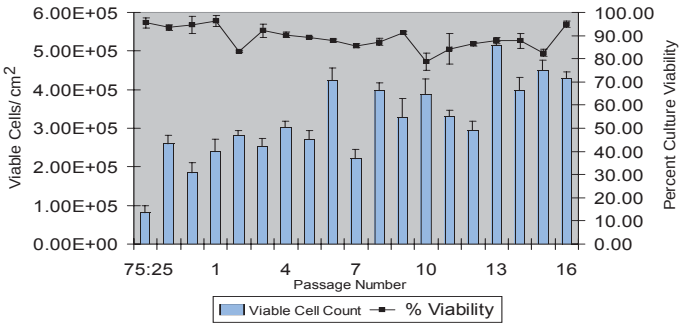

Figure 3: MDCK cells were adapted to EX-CELL™ MDCK in a sequential manner with decreasing concentration of serum-containing medium.

Doubling Times (Hours) During Sequential Adaptation to EX-CELL™ MDCK

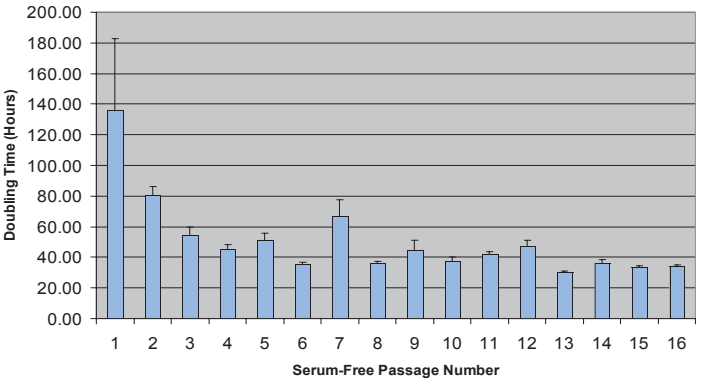

Figure 4: As MDCK cell densities increased, the cell doubling times decreased (Figure 2). In addition, as the cell counts increased, it was noted the morphology of the cells appeared more normal.

Cryopreservation

MDCK cells were frozen using a cryopreservation medium consisting of 90% fresh EX-CELL™ MDCK with 10% DMSO (referred to as "90:10"). Three 175 cm^2 T-flasks were seeded with 1 x 10^5 cells/cm^2 in EX-CELL™ MDCK. After 3 days, the cells were harvested and resuspended in the cryopreservation media as follows: flasks were rinsed with 10 mL DPBS and trypsinized with 6 mL of trypsin per flask (37 C for 15 minutes). 10 mL of STI was added to each flask and the cells were gently resuspended and pooled together in one flask. A sample was then taken to assess viability (>90%). The cells were then transferred to conical tubes and centrifuged at 1000 rpm (228 *g*) for 5 minutes. The cells were resuspended in cold "90:10" medium to a final concentration of approximately 1 x 10^7 cells/mL. The cell mixture was dispensed in 1 mL aliquots into sterile cryovials. The tubes were placed in a Styrofoam container and placed at -20 C for 4 hours, then transferred to -70 C overnight. The next day, the tubes were placed in liquid nitrogen vapor.

MDCK cells were recovered from liquid nitrogen after 3 days. Cells were quickly thawed and resuspended in 10 mL fresh medium in 15 mL conical tubes. A sample was taken to determine viability and then to remove residual DMSO, the cells were centrifuged at 1000 rpm (228 *g*) for 5 minutes. The medium was discarded and the cells resuspended in 20 mL fresh medium and plated into 75 cm^2 T-flasks.

MDCK Cells Frozen in 90% Fresh EX-CELL™ MDCK with 10% DMSO

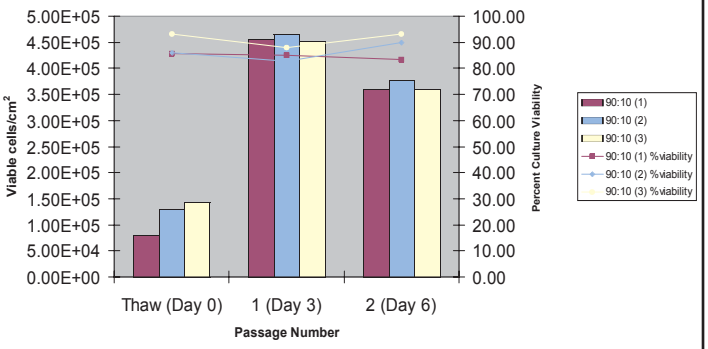

Figure 5: MDCK cells recovered from cryopreservation with initial thaw viabilities > 85%. Cells grew to confluency within 3 days and displayed no apparent lag in growth. Cells were monitored for 2 additional passes and grew to normal densities in both passages.

Canine Adenovirus Production

To determine the capability of EX-CELL™ MDCK to support the production of virus, MDCK cells were infected with CAV. Prior to testing, the CAV was amplified twice on MDCK cells in α-MEM-10% to a final titer of 2.54 x 10^6 $TCID_{50}$/mL. CAV production was assessed at two different MOI's, 0.01 and 0.1, over 7 days in EX-CELL™ MDCK.

MDCK cells were seeded in EX-CELL™ MDCK in 25 cm^2 T-flasks at 1 x 10^5 cells/cm^2 in a total volume of 5 mL medium per flask. Flasks were incubated overnight at 37 C with 5% CO_2. The next day, one flask was sacrificed and the total cell count was determined. The cells were then infected with the appropriate amount of virus to yield a MOI of 0.01 or 0.1. Flasks were incubated as above and a single flask from each trial was removed daily over the next 7 days and frozen at -70 C. During the infections the cells were monitored and displayed cytopathetic effect (CPE). The cells became rounded and many, although not all, of the cells detached from the bottom of the flask.

Prior to titration, each flask was subjected to 3 rounds of thawing (37 C) and freezing (-70 C) to lyse the cells. Any remaining attached cells were sloughed off the flask by rapping the flasks against the palm of the hand and by pipetting the lysate forcefully against the bottom of the flask. The cell lysates were transferred to 15 mL conical tubes and centrifuged at 3000 rpm (~2000 *g*) for 10 minutes to pellet the cell debris. The supernatants were transferred to new tubes and stored at -70 C until titration.

Canine Adenovirus Production in EX-CELL MDCK

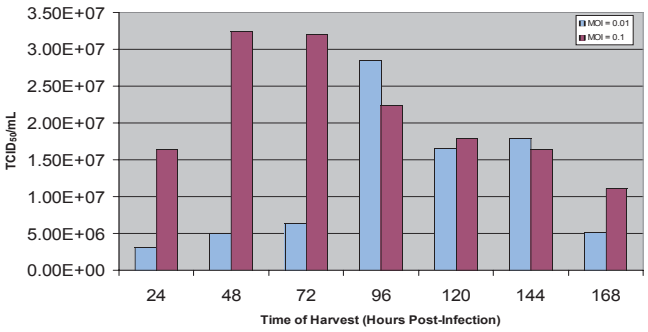

Figure 6: EX-CELL MDCK supported the production of CAV in MDCK cells adapted to the medium. Cells infected at a MOI of 0.1 produced slightly better yields than those infected at a MOI of 0.01. Peak production ($TCID_{50}$/mL=$10^{7.5}$) occurred between days 2 - 3 at a MOI of 0.1, and a day later at the MOI of 0.01 ($TCID_{50}$/mL=$10^{7.4}$). At both MOI's production declined after day 4, presumably due to unfavorable conditions for CAV such as cell lysis, pH shift and accumulation of toxins.

$TCID_{50}$/mL Titrations

CAV production in EX-CELL™ MDCK was determined by $TCID_{50}$/mL. The titration procedure was devised by modifying an existing procedure from Qbiogene (AdenoVator™ Adenoviral Vector System, Applications Manual, Version 1.1).

MDCK cells growing in α-MEM-10% were harvested by trypsinization, counted and diluted to a final concentration of 1 x 10^5 cells/mL in α-MEM supplemented with 5% FBS (α-MEM-5%). Using a 12-channel pipettor, 100 mL of cells were dispensed into 96-well microtiter plates (1 x 10^4 cells/well) and allowed to attach at 37 C.

Duplicate serial dilutions of each lysate were made in α–MEM-0% in sterile snap-cap disposable tubes (1:10 dilutions, 10^{-3} to 10^{-10} plated). ***Note:*** α-MEM used for dilutions was not supplemented with FBS, therefore, the final concentration of FBS in the wells was 2.5%. The dilutions were poured into sterile reservoirs and 100 mL of each dilution was dispensed in wells 1 - 10 (wells 11 and 12 in all rows served as controls). The rows were dispensed with the highest (10^{-10}) dilution in the bottom row, the lowest dilution (10^{-3}) in the top row. The plates were incubated at 37 C, 5% CO_2 for 7 days and then observed on an inverted microscope for CPE. Wells were considered positive even if only a small area of the well showed CPE. The titer (T) was determined as follows:

$$T = 10^{1+d(S-0.5)}$$

Where d = Log_{10} of the dilution and S = Sum of the ratios

Conclusions

MDCK cells were adapted from α-MEM-10% to EX-CELL™ MDCK in static cultures. During adaptation a seeding density of 1 x 10^5 cells/cm^2 was used to obtain cultures that reached confluency within 3 days. MDCK cells took 3 - 4 passages to adapt to EX-CELL™ MDCK. However, the cells appeared to be continually adapting to the medium, with increasing cell densities and shorter doubling times as the culture time in EX-CELL™ MDCK increased. Cell densities in EX-CELL™ MDCK were approximately twice those seen in α-MEM-10%.

Cryopreservation and recovery of adapted cells in EX-CELL™ MDCK was easily accomplished with only the addition of 10% DMSO. It was not found to be necessary to add conditioned medium or supplements (e.g. Bovine Serum Albumin).

EX-CELL™ MDCK also supported CAV production in MDCK cells. The cells exhibited classic CPE in culture and produced CAV titers in the range of $10^{7.5}$ $TCID_{50}$/mL. This range is comparable with those seen in serum-supplemented cultures.

Warranty, Limitation of Remedies

SAFC Biosciences warrants to the purchaser for a period of one year from date of delivery that this product conforms to its specifications. Other terms and conditions of this warranty are contained in SAFC Biosciences' written warranty, a copy of which is available upon request. ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING THE IMPLIED WARRANTY OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, ARE EXCLUDED. In no case will SAFC Biosciences be liable for any special, incidental, or consequential damages arising out of this product or the use of this product by the customer or any third party based upon breach of warranty, breach of contract, negligence, strict tort, or any other legal theory. SAFC Biosciences expressly disclaims any warranty against claims by any third party by way of infringement or the like. THIS PRODUCT IS INTENDED FOR PURPOSES DESCRIBED ONLY AND IS NOT INTENDED FOR ANY HUMAN OR THERAPEUTIC USE.

Additional Terms and Conditions are contained in the product Catalog, a copy of which is available upon request.

EX-CELL™ is a trademark of SAFC Biosciences, Inc.
AdenoVator™ is a trademark of Qbiogene, Inc.

© 2006 SAFC Biosciences, Inc.

Issued February 2006 R024
0103

www.safcbiosciences.com

United States
SAFC Biosciences, Inc.
13804 W. 107th Street
Lenexa, Kansas 66215
USA
Phone +1 913-469-5580
Toll free-USA 1 800-255-6032
Fax +1 913-469-5584
E-mail info-na@sial.com

Europe
SAFC Biosciences Ltd.
Smeaton Road, West Portway
Andover, Hampshire SP10 3LF
UNITED KINGDOM
Phone +44 (0)1264-333311
Fax +44 (0)1264-332412
E-mail info-eu@sial.com

Asia Pacific
SAFC Biosciences Pty. Ltd.
18-20 Export Drive
Brooklyn, Victoria 3025
AUSTRALIA
Phone +61 (0)3-9362-4500
Toll free-AUS 1 800-200-404
Fax +61 (0)3-9315-1656
E-mail info-ap@sial.com