

feature article

MegaCell™: The Next Generation of Classic Cell Culture Media

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Introduction

Historically, the majority of cell culture has been performed using classical media that were developed in the golden age of cell culture (1950's and 60's) such as Dulbecco's Modified Eagle Medium (DME), Minimum Essential Medium Eagle (MEM), and RPMI-1640. Depending on the exact medium formulation and cell type, 5-20% fetal bovine serum (FBS) is added to the medium to ensure proper cell growth. Today the trend is to use more defined media that have been developed to be less dependent on serum supplementation, but there are still numerous applications that use classical media supplemented with FBS.

Due to increasing cost and decreasing availability of FBS, a new product line has been developed for those investigators interested in reducing their dependence on serum. The MegaCell product line is a set of five media based on classical media formulations that have been optimized to support cell growth with 3% serum supplementation comparable to growth observed with classical media containing 10% serum. Our understanding of cellular biochemistry and nutrition has increased greatly during the almost half century since these classical formulations were first reported in the literature. Utilizing this knowledge, very specific components have been added to each individual medium to enrich them such that the required level of serum could be lowered. Various supplements, including lipids, buffers, amino acids, trace elements, and alternate energy sources, were added to each classical medium to create the MegaCell media product line.

Sigma-Aldrich now offers five fortified MegaCell media products: MegaCell MEM (Product Code [M 4067](#)), MegaCell DME (Product Code [M 3942](#)), MegaCell MEM:F12 (Product Code [M 4317](#)), MegaCell DME:F12 (Product Code [M 4192](#)), and MegaCell RPMI-1640 (Product Code [M 3817](#)). Customers who utilize the MegaCell media will be able to save money by reducing the levels of FBS needed and save time because the cells can be directly transferred into the MegaCell media without weaning.

Materials and Methods

All materials were supplied by Sigma-Aldrich Corporation (St. Louis, MO) unless otherwise stated.

Media Preparation

All five of the MegaCell media were supplemented with 3% FBS and 4 mM L-glutamine and warmed to 37 °C before use. The competitor media tested were GIBCO's Advanced D-MEM (12491015), Advanced MEM (12492013), and Opti-MEM® I (31985070). All of these media are supplied by Life Technologies (Grand Island, NY) and were used according to the manufacturer's directions (supplemented with 3% FBS).

Suspension Cell Culture

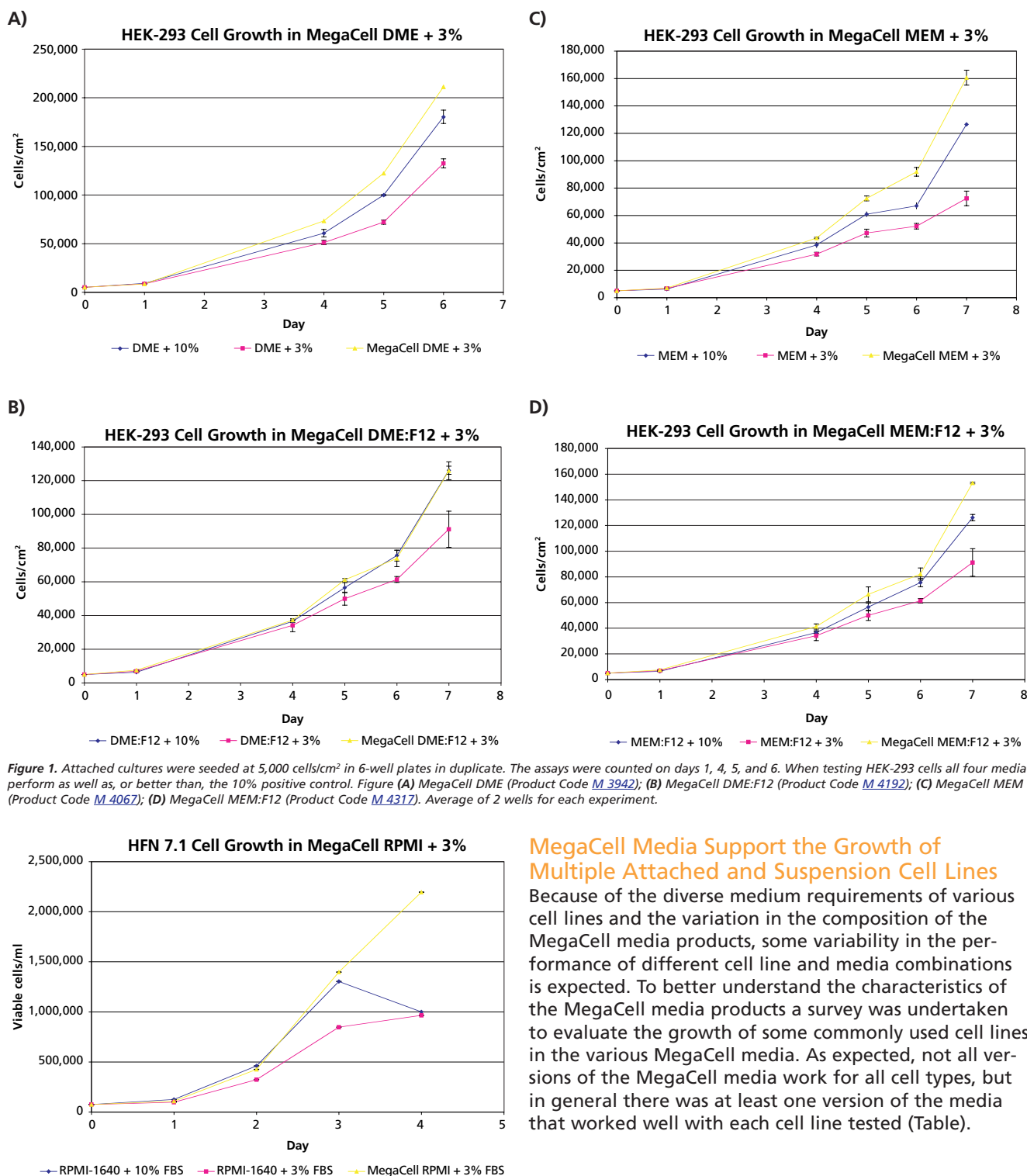
A hybridoma cell line, HFN 7.1, was used to evaluate the performance of MegaCell RPMI-1640 (Product Code [M 3817](#)). The cell stocks were cultured in classical RPMI-1640 + 10% FBS in static T-flasks. The MegaCell RPMI-1640 development assays were performed in duplicate T-25 static flasks. The flasks were inoculated at 75,000 cells/ml with a final volume of 5 ml/flask. The assays were counted using a hemacytometer and CASY® Counter (Scharfe Systems, Reutlingen, Germany) on days 1-4 and subcultured on day 3. The subculture was counted on days 1 and 4.

Attached Cell Culture

A human embryonic kidney cell line, HEK-293, was used to evaluate the performance of MegaCell DME (Product Code [M 3942](#)), MegaCell MEM (Product Code [M 4067](#)), MegaCell DME:F12 (Product Code [M 4192](#)), and MegaCell MEM:F12 (Product Code [M 4317](#)). The cell stocks were cultured attached in T-225 flasks in classical DME + 10% FBS. For each development assay the cells were seeded in duplicate at 5,000 cells/cm² in 6-well plates containing 3 ml of media in each well. The wells were counted on days 1, 4-6 and subcultured on day 5. The subculture was counted on days 1 and 6.

Results and Discussion

MegaCell media supplemented with 3% FBS are able to perform as well as, or superior to, the classical version of the same medium supplemented with 10% FBS. This is illustrated in Figures 1 and 2. Figure 1 (A-D) shows the results of the attached cultures and Figure 2 shows the results of the suspension cultures.



MegaCell Media Support the Growth of Multiple Attached and Suspension Cell Lines

Because of the diverse medium requirements of various cell lines and the variation in the composition of the MegaCell media products, some variability in the performance of different cell line and media combinations is expected. To better understand the characteristics of the MegaCell media products a survey was undertaken to evaluate the growth of some commonly used cell lines in the various MegaCell media. As expected, not all versions of the MegaCell media work for all cell types, but in general there was at least one version of the media that worked well with each cell line tested (Table).

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	Cell Line	Seeding Density	DME M 3942	DME:F12 M 4192	MEM M 4067	MEM:F12 M 4317	RPMI M 3817
Attached	VERO	5000/cm ²	-	+	+++	++	ND
Attached	MDBK	5000/cm ²	++++	+	+	+++	ND
Attached	MRC-5	5000/cm ²	++	+++	++	+++	ND
Attached	WI-38	5000/cm ²	+++	+++	+	+++	ND
Suspension	SP20	75000/ml	-	-	-	-	++++
Suspension	HFN	75000/ml	-	-	-	-	++++

Note: Number of "+" indicates strength of application data results; ND = Not determined.

Note: MegaCell is not recommended for 3T3 or MDCK cells.

MegaCell Media can be Supplemented with Calf or Adult Bovine Serum

Due to the increasing cost and decreasing availability of fetal bovine serum (FBS) the possibility of supplementing MegaCell media with less expensive and more readily accessible adult bovine serum (ABS) and/or calf serum (CS) was explored. ABS and CS are characteristically far less nutritionally rich than FBS; therefore it is not usually possible to use these sources of serum to achieve superior cell growth. Serum titrations were completed using both ABS and CS in the MegaCell media using the same assay design as employed in the previous studies. MegaCell media supplemented with either ABS or CS were able to perform as well as if supplemented with 3% FBS. The levels of the ABS and CS that were required to reach this performance level varied from 6%-10% depending on the version of the media and the cell type tested. Figures 3 and 4 illustrate examples of the performance of the MegaCell media supplemented with ABS and CS.

MegaCell Outperforms the Competition

Media from the MegaCell product line were evaluated for their ability to support cell growth relative to other commercially available fortified media designed for use with reduced levels of serum supplementation (GIBCO's Advanced D-MEM, Advanced MEM and Opti-MEM from Invitrogen). Attachment dependent and suspension cell lines were grown in MegaCell media and a comparable fortified medium with reduced levels of serum (3%) and a comparable non-fortified classical medium with 10% FBS. Example growth curves for attached (Figure 5) and suspension (Figure 6) cell lines are shown. In all cases, the MegaCell media outperformed the other media tested.

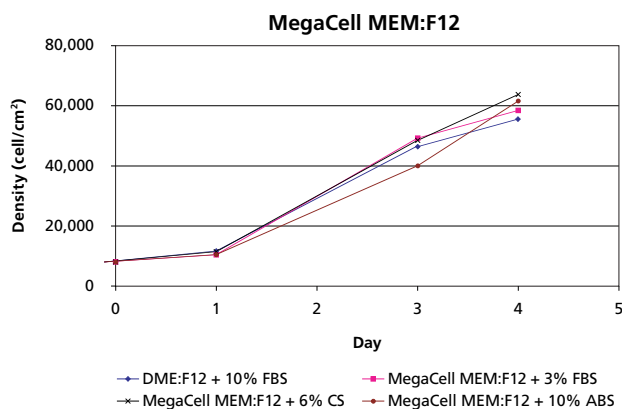


Figure 3. MegaCell MEM:F12 was supplemented with 6% calf serum (CS) or 10% adult bovine serum (ABS) and MRC-5 cell growth was compared to DME:F12 + 10% FBS and MegaCell MEM:F12 + 3% FBS. Both the CS and the ABS-supplemented media performed as well as the MegaCell with 3% FBS and as well as the classical medium plus 10% FBS.

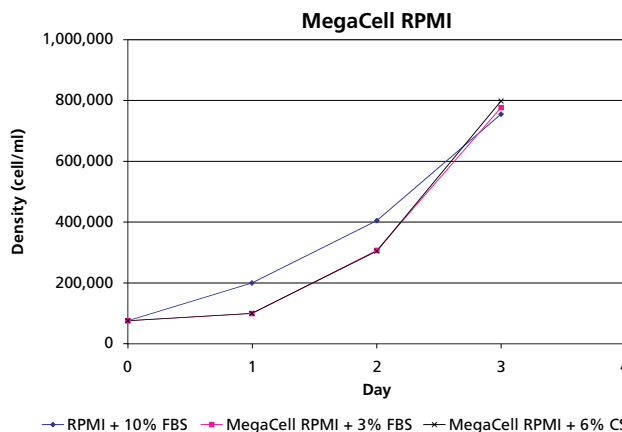


Figure 4. An example of the suspension data. The best serum substitute for FBS in MegaCell RPMI when growing HFN 7.1 cells was 6% calf serum (CS). The adult bovine serum (ABS) did not support growth as well as compared to the CS (data not shown).

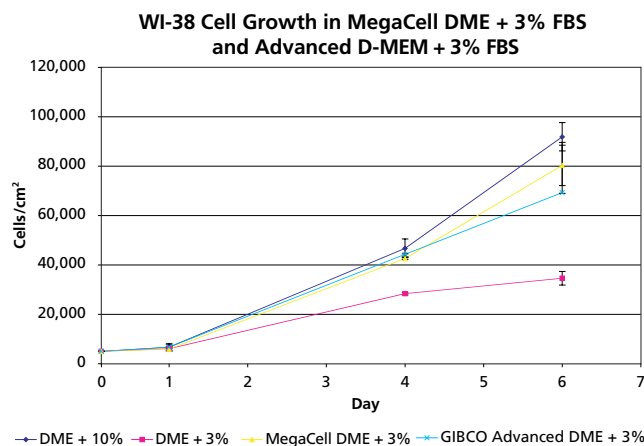


Figure 5. When testing the Advanced D-MEM with attached WI-38 cells the MegaCell DME performs better than the Advanced D-MEM. Average of 2 wells.

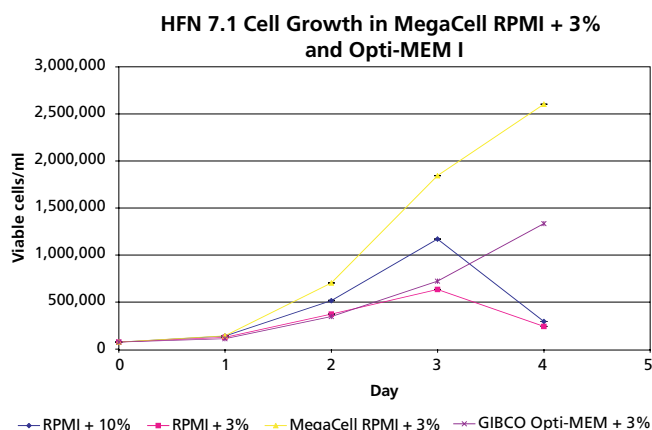


Figure 6. MegaCell RPMI + 3% FBS outperforms GIBCO's Opti-MEM I + 3% FBS. The Opti-MEM I growth curve shows a large lag phase, while the MegaCell RPMI-1640 allows for exponential growth starting almost immediately after inoculation. Average of 2 wells.

Summary

The MegaCell media product line is a group of fortified media based on classical media formulations that have been enriched to support cell growth under reduced serum conditions. When supplemented with 3% FBS these media have been shown to support cell growth comparable to non-fortified classical media supplemented with 10% serum without the necessity of weaning or adaptation. Additionally, the fortifications incorporated into the MegaCell media products have been found to allow the use of adult bovine and/or calf serum in place of more expensive and increasingly scarce fetal bovine serum. Recognizing the diversity of cell lines in use, Sigma has developed a total product line of media with a broad range of formulations and nutritional components to better serve our customers.

Ordering Information

Product	Description	Unit
M 3942	MegaCell DME	500 ml
M 4067	MegaCell MEM	500 ml
M 4317	MegaCell MEM:F12 Ham	500 ml
M 3817	MegaCell RPMI-1640	500 ml
M 4192	MegaCell DME:F12 Ham	500 ml

Sigma-Aldrich uses the MegaCell trademark pursuant to an agreement with Cortex Biochem, Inc.