Efficient Cloning Of Single CHO Cells Using A Novel Animal Component-Free Culture Medium Supplement

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Introduction

Over the past decade, the number of biotherapeutic drugs produced in Chinese hamster ovary (CHO) cells has increased dramatically. To achieve consistently high expression of a protein product, monoclonal cultures of transfected CHO cells are generated by single-cell cloning after plating cells at very low densities. Efficient expansion of single CHO cells typically requires the use of medium containing fetal bovine serum (FBS) or alternatively, use of conditioned medium or co-culture with feeder cells. However, these systems are not defined, and batch variation and risk of contamination from adventitious agents make the use of FBS undesirable. Chemically-defined, protein-free media, which are available from many suppliers, can effectively support CHO cell cultures for many passages when cells are plated at high densities. However, cloning efficiency in these media is very low if the cells are plated at the very low densities necessary for limiting dilution cloning. To address these issues, we have developed a defined, animal component-free (ACF) culture supplement that contains only recombinant proteins and synthetic components. The ClonaCell™-CHO ACF Supplement significantly increases the cloning efficiency of CHO cells plated at low density in protein-free media from various commercial suppliers.

Materials & Methods

Cell Culture

CHO-S and CHO-K1 cells were maintained as suspension cultures in ClonaCell™-CHO CD Liquid Medium (STEMCELL Technologies) supplemented with 6 - 8 mM L-glutamine. Cell concentrations and viability were determined using a hemacytometer and trypan blue staining.

Low Plating Density Experiments

CHO-S and CHO-K1 cells were suspended in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS (positive control), or in various protein-free media containing 6 - 8 mM L-glutamine with or without ClonaCell™-CHO ACF Supplement. The following protein-free media were tested: ClonaCell™-CHO CD Liquid from STEMCELL Technologies, PowerCHO™-2 from Lonza, and CD CHO and CD OptiCHO™ from Invitrogen. Individual wells of 96-well plates were seeded at low cell densities of, on average, 1 or 10 cells/well in 200 µL of the respective test media. The plates were maintained in a humidified incubator at 37°C in 5% CO_2 for 12 - 14 days before screening to identify wells containing >100 cells/well using an inverted microscope. Plating efficiency (referred to as cloning efficiency in experiments seeded at an average of 1 cell/well) was defined as the percentage of the total number of seeded wells that contained >100 cells/well after 14 days.

Results

Two CHO lines, CHO-S and CHO-K1 were cultured in ClonaCell™-CHO CD Liquid medium and then seeded at an average of 1 cell/well in 96-well plates in four different protein-free media alone or supplemented with the ClonaCell™-CHO ACF Supplement. The cloning efficiencies measured after 14 days of culture and normalized to the cloning efficiencies obtained in DMEM containing 10% FBS are shown in Figure 1A and 1B for CHO-S and CHO-K1 cells, respectively. The average cloning efficiency in DMEM + 10% FBS was approximately 50% for both CHO cell lines. In protein-free media, the cloning efficiency was much lower, ranging between 0 and 5%. This demonstrates that protein-free media do not efficiently support the growth of CHO cells plated at very low densities. Addition of the ClonaCell™-CHO ACF Supplement significantly improved the cloning efficiencies in all four protein-free media to levels of 25 - 110% of that obtained in DMEM + 10% FBS.

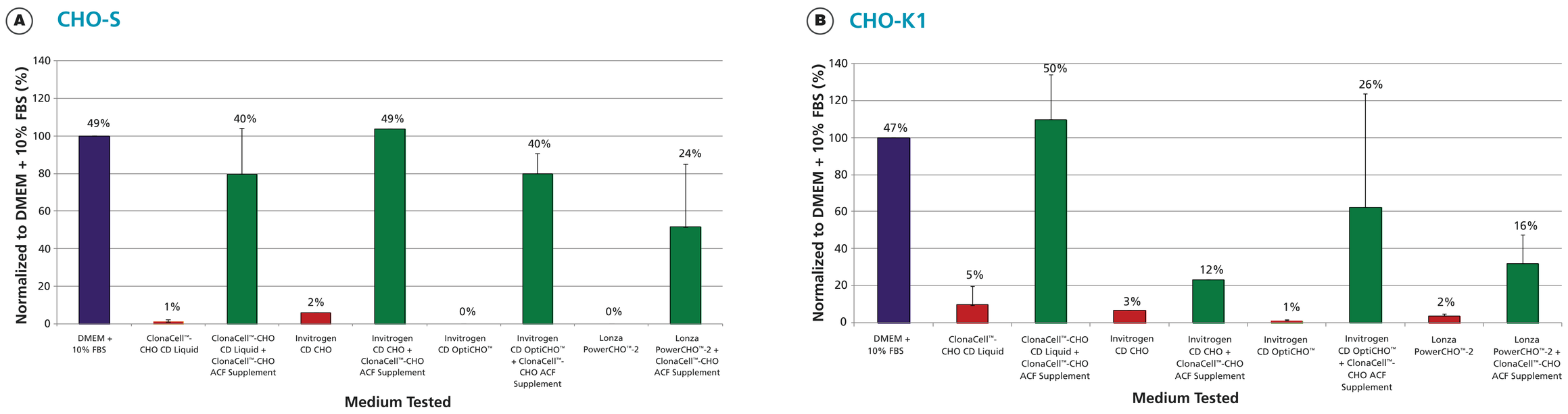

FIGURE 1: Cloning efficiencies of CHO-S and CHO-K1 cells in protein-free media with and without ClonaCell™-CHO ACF Supplement.
CHO-S cells (A) and CHO-K1 cells (B) were plated at, on average, 1 cell/well in DMEM + 10% FBS (purple bars), and in four commercially available protein-free media with (green bars) or without (red bars) ClonaCell™-CHO ACF Supplement. Wells were screened after 14 days and cloning efficiency, defined as the percentage of wells containing >100 cells/well, was calculated. The average cloning efficiencies were normalized to the results obtained in DMEM + 10% FBS with the absolute cloning efficiencies for each condition shown above each bar. Shown are mean + SD values for 3 experiments per condition, except for Invitrogen CD CHO alone or supplemented with ClonaCell™-CHO ACF Supplement, which are from the average of 2 experiments.

To examine how cloning efficiencies are affected by the protein-free medium used to maintain CHO cell cultures prior to cloning, CHO-S and CHO-K1 cells were first cultured in the four protein-free media for a minimum of 5 passages and then plated at low cell density either in the same passaging medium or in ClonaCell™-CHO CD Liquid medium with or without ClonaCell™-CHO ACF Supplement (Table 1). The results indicate that both CHO cell lines exhibit low plating efficiencies in unsupplemented protein-free media, irrespective of the protein-free medium used for routine passaging. The addition of ClonaCell™-CHO ACF Supplement increased the plating efficiencies of both CHO cell lines in all passaging media.

CHO-S

Passaging Medium	Absolute Plating Efficiency (%)	Normalized to DMEM + 10% FBS Control (%)			
	DMEM + 10% FBS	Passaging Medium	Passaging Medium + ClonaCell™-CHO ACF Supplement	ClonaCell™-CHO CD Liquid	ClonaCell™-CHO CD Liquid + ClonaCell™-CHO ACF Supplement
ClonaCell™-CHO CD Liquid	80.9	4.3	97.4		
Invitrogen CD CHO	64.2	3	102	2	48
Invitrogen CD OptiCHO™	61.5	1	14	0	1
Lonza PowerCHO™-2	43.8	1	69	0	104

CHO-K1

Passaging Medium	Absolute Plating Efficiency (%)	Normalized to DMEM + 10% FBS Control (%)			
	DMEM + 10% FBS	Passaging Medium	Passaging Medium + ClonaCell™-CHO ACF Supplement	ClonaCell™-CHO CD Liquid	ClonaCell™-CHO CD Liquid + ClonaCell™-CHO ACF Supplement
ClonaCell™-CHO CD Liquid	15.3	5	364		
Invitrogen CD CHO	20.5	0	100	7	129
Invitrogen CD OptiCHO™	29.9	0	2	1	134
Lonza PowerCHO™-2	21.9	2	267	16	283

TABLE 1: Plating efficiencies of CHO-S and CHO-K1 cells in their passaging medium with and without ClonaCell™-CHO ACF Supplement.
Plating efficiencies of CHO cells passaged in four commercially available protein-free media and then plated at low cell density in the same passaging medium or in ClonaCell™-CHO CD Liquid medium alone or supplemented with ClonaCell™-CHO ACF Supplement. As a control, cells were also cloned in DMEM containing 10% FBS. Shown are the percentage of wells containing >100 cells/well after 14 days normalized to the results obtained in DMEM + 10% FBS.

Summary

Serum-containing medium is often used to support the growth of CHO-S and CHO-K1 cells at low cell density. The use of FBS is undesirable, however, in many applications such as the development of biotherapeutic drugs. Chemically-defined, protein-free media can support the growth of CHO cells in culture for many passages when cells are plated at high densities, but cells expand very poorly in these media if they are plated at the low densities necessary for limiting dilution cloning. The present studies demonstrate that addition of a new ClonaCell™-CHO ACF Supplement to several commonly used, commercially available, protein-free media can increase the cloning efficiencies of CHO-S and CHO-K1 to levels similar to that obtained with serum-containing medium, even when cells are plated at very low density. This completely defined ACF culture system should increase the reproducibility and productivity of CHO cell cultures used for biological drug development.