SERUM-FREE MEDIUM ADAPTATION OF MAB-PRODUCING CHO-K1 CELLS: CRITICAL STEPS AND GUIDELINES

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Introduction

Large-scale production of biopharmaceuticals, such as monoclonal antibodies (mAb), commonly requires the use of serum-free (SF) medium for both safety and cost reasons. But since serum is essential for most mammalian cells' growth, its removal becomes very time-consuming. Therefore, cells are usually subjected to a gradual adaptation consisting of a step-wise decrease of serum concentration. To ease this process, other media supplements such as insulin and trace elements can be used. **In this study, strategies for CHO-K1 cell adaptation to SF media using different supplement combinations were assessed, and the most critical steps / problems identified.**

Methods

Adaptation methodology: Cells were grown in DMEM supplemented with gradually reduced serum percentages (10 to 0.625%). Different supplement combinations were tested (Table 1). At 0.625 % serum, cells were sequentially adapted to the chemically-defined SF medium EXCELL CHO DHFR-, and the SF adaptation was continued.

TABLE 1. Composition of the five combinations of media supplements tested

MEDIA SUPPLEMENT	FUNCTION	COMBINATION				
		1	2	3	4	5
rhInsulin	Growth factor	X	X	X	X	X
Copper sulfate ($CuSO_4$)	Proliferation	X	X		X	X
Zinc sulfate ($ZnSO_4$)	Proliferation	X	X		X	X
Sodium selenite (Na_2SeO_3)	Antioxidant	X	X		X	X
Ammonium iron citrate ($FeC_6H_5O_7.NH_4OH$)	Proliferation	X			X	
Ferrous ammonium sulfate ($NH_4Fe(SO_4)_2$)	Proliferation		X			X
Ammonium metavanadate (NH_4VO_3)	Unknown			X	X	X
Nickel chloride ($NiCl_2$)	Unknown			X	X	X
Tin chloride ($SnCl_2$)	Unknown			X	X	X

First experiment: Cultures were initiated in 24-well plates and all combinations were tested. A CHO-K1 cell line transfected with OSCAR™ technology for mAb production was used.

Second experiment: Cultures were initiated in 25 cm^2 T-flasks and combinations C2, C3 and C5 were tested. Three cell lines were used to assess the impact of transfection on the process of adaptation: (A) CHO-K1 non-transfected; (B) CHO-K1 transfected with a common method; and (C) CHO-K1 transfected with OSCAR™ technology.

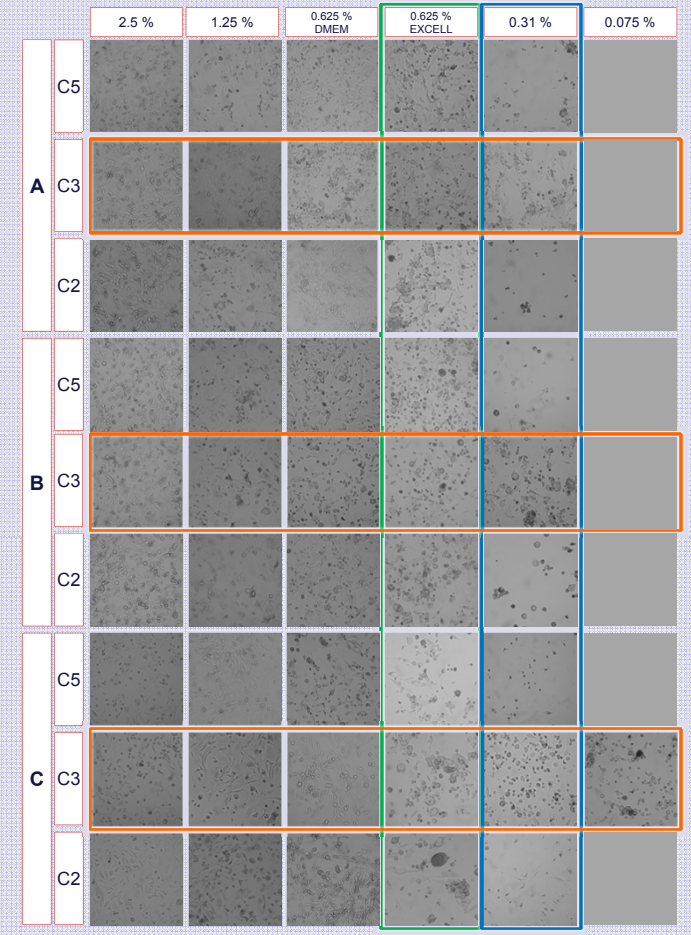
Results

First experiment

- Cells died at: 2.5 % serum for C1 and C4 (ammonium iron citrate); 0.625% serum for C2, C3 and C5. Some procedures should be carefully considered.

- Use higher initial cell concentration to allow higher cell survival
- Avoid harsh procedures to the cells (centrifugation, trypsin)
- Allow enough time for full cell adaptation at each step.

Second experiment



Among the three cell lines assessed, cell line C demonstrated better ability to adapt to SF conditions

Cell adaptation from DMEM to EXCELL is easily achieved, as long as some serum supplementation is maintained.

Combination C3 provides the better cell adaptation for all cell types assessed in the study

Cells start to detach at 0.31 % serum, and become fully detached at 0.15 %, growing in suspension from this point on.

Conclusions

- The use of media supplements impacts cell adaptation to SF conditions. C3 proved to be the best combination in this study
- For the set of conditions evaluated in this study, the mAb-producing cell line C proved to be the one with better ability to adapt to SF medium
- The process of adaptation to SF medium is very demanding to the cells, making them more sensitive to procedures that are commonly used in cell culture (centrifugation and trypsinization)