ADAPTATION OF MAMMALIAN CELL LINES to serum-free culture media

The adaptation of cell lines to serum-free cell culture media and growth in suspension is not always straightforward. Learn about the do-or-die-approach and weaning and get a few helpful tips to make it a success.

You might find yourself in the following situation:

You have developed an adherent cell line (for example based on CHO K1 or HEK293) that produces your product of interest. You plan to scale up your process, for example in order to produce material for clinical studies, and therefore want to switch to suspension cultivation in animal-derived component free (ADCF) cell culture medium.

Challenges associated with the transition from monolayer to serum-free suspension cultivation

By switching from adherent monolayer to suspension culture, the factors limiting cell growth and product formation change. Monolayers are largely limited by the available surface area. In suspension culture, high cell densities can be reached. In consequence, nutrient depletion and toxic metabolite accumulation often become the major limitations. Selection of a suitable, chemically defined culture medium that is proven to support high cell densities, is an important first step. Still, you have to adapt your cells to three new situations:

1. growth in suspension
2. growth without serum
3. growth in a new medium with different substrate concentrations

Growing in a shake flask or bioreactor, cells are exposed to shear stress. The culture medium should therefore contain a suitable surface active and protective agent. Xell's cell culture media do readily contain suitable surfactants. In addition, shaking frequency should be chosen carefully to reduce shear stress to a minimum.

Lack of serum induces another type of stress. Growth factors and other serum proteins are missing. Albumin for example is an abundant serum protein that has the ability to bind and sequester a wide range of ligands, including ions and small molecules. Its absence can change the availability or dose-response relationship of certain media components. This has also an implication on the use of antibiotics. It is well-known that their activity is higher in serum-free media compared to those containing serum. If the use of antibiotics can't be avoided, their concentration should be reduced by at least 80 to 90 %.

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Any form of additional stress, as for example induced by pH and temperature shifts, should be avoided.

Do-or-die approach vs. sequential adaptation

When adapting cells to suspension culture and serum-free conditions you can either follow the do-or-die approach, meaning that you passage the cells directly into chemically defined, serum-free medium. Or you can use a less harsh, but a more time-consuming, step-wise adaptation procedure. If you have previously used a very basal medium like DMEM or RPMI supplemented with serum, you probably want to ultimately use a richer medium that supports high cell densities and high product yields. Here again you have two options. You can either adapt them to the serum-free version of the previously used basal cell culture medium and select a richer medium afterwards, or you can adapt them to the final medium directly. Save some time and start do-or-die approach and sequential adaptation in parallel. After initial experiments, it will become apparent whether the direct approach has been successful or not. Depending on the outcome, you continue the one or the other approach.

What to consider during trypsinization

The first step in any case is the trypsinization of adherently grown cells, followed by a washing step and inoculation of a shake flask. If classical trypsin is used, inactivation or saturation of the enzyme with protein is necessary. Hence, cells should be washed once with fresh, serum-containing medium and once with PBS buffer before they are being transferred to the serum-free growth medium. If Accutase® is used, washing with serum-containing medium is obsolete.

Tips for inoculation and passaging

It almost goes without saying that it is essential to start the adaptation process with a sufficiently high number of viable cells. It is even recommended to inoculate shake flasks with higher cell densities than normal (at least twice as high, appr. 5-6 x 10^6 cells/mL). Cell viability should definitely be higher than 90 %, as it is not uncommon to observe a (transient) reduction in cell growth and viability during the course of the adaptation. Cell metabolism is likely to change significantly in reaction to altered nutrient and growth factor availabilities. Furthermore, not all cells will grow under the new conditions. During the adaptation process, subpopulations will be selected.

Monitor cell density and viability on a daily basis and passage cells every 3-4 days. Cells might still tend to aggregate. If you observe cell clumps while passaging, triturate them carefully (by pipetting up and down). Even if there is very little to no growth in the beginning, the medium should be exchanged completely each time. You can use a suitable pH-dependent dye (phenol-red) as a simple indicator for metabolic activity. When cells grow faster again, make sure to passage them well before they reach the

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stationary phase and adjust the splitting ratio when necessary. To make sure that no subpopulation with disadvantageous properties related to production will be selected, important growth and production parameters, as well as product characteristics should be monitored carefully during the whole process.

Perform do-or-die and sequential approach in parallel

The do-or-die approach is always worth a try. If your cell line is robust enough, it saves a lot of time. However, it surely doesn't work with every cell line. The alternative is to first adapt the cell line to grow in suspension before removing the serum in either one or several consecutive steps. The step-wise reduction is often referred to as weaning.

You can start the weaning process with the previously used serum percentage and reduce it by approximately 20 % of the initial value and passage cells 2-3 times at each step. Bank your cells after completion of each step and inoculate another shake flask with the same serum content. Having a backup culture allows you to continue immediately from there if your cells fail to grow with the next lower supplementation with serum. Consider to make smaller incremental steps in the reduction of serum if necessary.

If your cells require a growth factor, make sure to supplement the culture medium with a suitable recombinant peptide. If you add it to the medium at the recommended concentration from the beginning, the total concentration of growth factors might be too high because of those already contained in the serum. Therefore, it is recommended to gradually increase the concentration of the recombinant growth factor according to the reduction of the serum content.

When is the adaptation complete?

As a rule of thumb, cells should grow stably in serum-free suspension culture for 3-5 passages with high viabilities. In the final stage, it is best to cultivate them in a controlled system such as spinner flasks or other suitable bioreactors.

Typical suspension cultivation conditions

Below you can find some typical cultivation parameters that we usually use for the suspension cultivation of various mammalian cell lines.

Typical parameters:

- shaking diameter: 5cm
- shaking frequency: 125-185 rpm
- temperature: 37 °C
- CO2: 5%

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Typical culture volumes:

- 125 mL, plain vent cap shaker: 20-50 mL
- 250 mL, plain vent cap shaker: 80-150 mL
- 500 mL, plain vent cap shaker: 200-300 mL
- 1000 mL, plain vent cap shaker: 400-600 mL

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