Custom Cell Culture Media

The Life Science Group Ltd (LSG) team have significant experience in the field of cell culture, with Managing Director, Jenny, personally working in the field cell culture sera and media for over 40 years. LSG is ideally placed to support customers in the production of their bespoke media and liquid products, through assistance with development and stability trials to final manufacture and scale-up. The Team can advise on sourcing of excipients, the final presentation and quality release testing to meet regulatory requirements.

The following is an overview of the cell culture media development process required to bring a successful cell culture media development to a final fit-for-purpose product.

Cell Culture Media formulations – an overview

Cell-culture media formulations have been around for many years, since the development of Ringer's Solution, a balanced salt solutions (BSS) devised in 1885 by Sydney Ringer. Media comprise the ingredients required by cells to survive, grow, and function as required for their function in vitro.

These media formulations vary dependent on the cell type and the application. Very small changes in media composition can result in large impacts on the cellular viability and how they function

Cell Culture Media components

Cells rely on many different chemicals and excipients in order to provide the environment for them to grow and thrive. For example, nutrient supply - glucose or other sugars in addition to amino-acids (energy sources), vitamins (for enzyme functions), peptides (to support protein production), lipids (membrane construction). pH and osmotic balance - salts (to maintain osmotic balance), bicarbonate or HEPES and trace elements (for a variety of functions, including acting as enzyme catalysts).

More recently developed media tend to be fully defined and free from animal or human components that can create batch-to-batch variability. These media will be enhanced by the addition of antioxidants, recombinant growth factors and often specific components that support a very specific function required by the cells.

The trick in the development of cell-specific culture media is the ability to maintain balance since having the right composition of components directly impacts the performance of the cells in the media. If there are imbalances in glucose/amino acid levels, then this could result in elevated levels of lactate and ammonium in the media.

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This can result in the 'Crabtree Effect', this is a phenomenon where respiration is frequently inhibited when high concentrations of glucose or fructose are added to the culture medium – this has been observed in numerous cell types, particularly in proliferating cells.

Maintaining balance of all the excipients, to maintain metabolism, pH and osmotic balance is tricky but obtaining the correct balance enables the creation of consistent and reproducible cell-culture and happy cells.

Type of Cell Culture Media

Different cell cultures have different requirements, with the cells having different biological processes. These differences dictate the requirements of the media. For instance, hybridomas will have very different requirements to, HeLa, Jurkat, CHO-S, iPSCs etc. There are notable differences between cells producing a biopharmaceutical protein, viruses produced by the cell disruption and situations where the cell itself is the product, such as is the case in CGT. The media requirements are entirely different when you are looking for a specific output – cells used for viral vector production may be similar to those cells used for monoclonal production or recombinant proteins, but they require media that support high-density cell growth, low doubling time and high-titre production.

Cell type also makes a difference

Different cell types may have very different basal nutritional needs and this needs to be taken into account when looking at product yield, quality, stability and maximising cell growth.

Primary cells and immortalised cells also have different requirements. Primary cells require more in the media to mimic *in vivo* environments whereas immortalised cell lines are frequently not as fastidious and do not always require the same high concentrations or serum and growth factors.

CHO, the Chinese Hamster Ovary cell lines, are well established and a range of media have been developed to provide the correct environment, and these media are available off the shelf by most large media providers.

Insect cell lines and mammalian cell lines, including HEK293, a widely used immortalised mammalian cell line derived from human embryonic kidney cells, may be used as hosts for production, for CGT and vaccine manufacture. For these types of cell lines, the platforms are relatively new and frequently the available media are not well-defined, nor are they animal-component-free.

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The specific demand of cells utilised in cell therapies is to ensure that quality parameters are maintained to retain the therapeutic potential for the life of the cell. It is important that there is a complete, in depth understanding of both the cell type and the cell requirements before starting the process of media development. Small deviations can result in catastrophic effects. This results in specific challenges for developing media for these applications that may not be so critical for other production purposes.

Process conditions also matter

A further aspect of cell culture is the process and this will differ dependent upon whether the cells are suspension, or adherent cells – both cell types will require different media conditions.

Suspension cells, cells freely growing suspended in media, typically operate at higher densities than adherent cells. The requirement of the media to support these cells will be subtly different to contain excipients to stop cells clumping.

Adherent cells, those that grow while attached to a culture vessel or substrate, form a monolayer which will require enzymatic or mechanical detachment for subculturing. These typically grow at lower densities than suspension cells, but they will require specific components to enable the cells to adhere.

It is also necessary to remember that the same cells may have different media requirements for each part of the production process. This can include media to support transfection, media for cell-passaging and media for the production phase.

Cost

Cost is something to consider in the development of your cell culture media. What is affordable in the laboratory setting may become prohibitively expensive when it comes to large-scale production. Cell culture media being developed for scale-up should always be developed with consideration to budgetary constraints for the production process. In the path to develop a bespoke media formulation that provides high production titres, cell stability and high cell growth, it must be remembered that low cost should also be part of the developmental process. This may result in compromises between productivity and cost, and this is likely to result in lively debate. It is vitally important that the choice of excipients for the development of the media formulation is made with consideration of the regulatory framework under which the media will be employed.

It is also necessary to factor in the cost of small-scale initial production to develop manufacturing data and 'prove' stability, reliability and repeatability.

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AI and cell culture media

As can be seen, it is necessary to consider many factors when developing bespoke media products, including cell type, cell environment, productivity, media components, the presence or not of biologicals such as serum and other materials and cost.

Experimental design will need to take all of the above points into consideration. There are now computational and experimental design methods based on AI and machine learning that allow for the systematic evaluation and optimisation of cell culture media components and conditions. These systems will allow for the concentration of components to be varied and data to be generated rapidly to determine the optimum media formulation that is fit for purpose and, more importantly, to increase yields.

LSG works with trusted partners who offer this service.

From idea to production formulation

A typical approach for the development of a successful cell culture media is as follows:

- the adoption of a familiar basal media
- the optimisation of the developing media formulation through the addition of different components to the basal media
- the evaluation of the media and components at differing concentrations
- the generation of performance data of cells in production using the nominated formulations
- the choice of the final formulation with excipients.

It should also be remembered that the formulation will need to be manufactured at scale, so ease of manufacture is also a consideration, with the simplest being a single powder formulation that can be rehydrated easily with the addition of complementary reagents and supplements as a single step.

It is important that the final media formulation is tested for stability and repeatability. Shelf-life studies may be employed on the final formulation, both accelerated and real-time, to demonstrate the stability of the media over a selection of time points.

Packaging of the final media product should also be taken into consideration during the development of the media – how is the media to be used? Is it a requirement to have the product packaged in single use bags for ease of use with the production environment? Parties should be mindful of this when conducting initial trials and stability trials to ensure that this is captured at all data generation points.

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LSG are a trusted partner to academia, biotech, pharma, and research sectors. As a specialist UK-based full service CDMO for liquid solutions, we combine scientific expertise with agile, reliable, accredited manufacturing. LSG services include custom media, high-quality reagents and serum, biospecimens, antibody generation, and bespoke kitting services.

For further information concerning availability and detailed costings please contact sales@lifesciencegroup.co.uk

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