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## Will we ever find a perfect medium for mammalian cell culture?

/// The development of biosimilars creates additional expectations in terms of media development. ///

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When the biopharmaceutical industry discovered the use of mammalian cells for the production of biotherapeutics, supplementation of simple cell culture media with serum was a standard and titers reached only a few hundred milligrams per liter. Despite the fact that product yields were much below those obtained with current media, they were sufficient to cover the demand of early biotherapeutics which were mainly replacement hormones or other classes of products efficacious at low dose.

The regulatory and financial landscapes since early biotherapeutics have greatly evolved. The breakout of mad cow disease in the late 1980s and viral infections experienced by some biotechnology companies have made regulatory authorities and the industry conscious of the risk of disease transmission between animals used as a source of raw materials and patients. The development of high-dose biotherapeutic blockbusters increased pressure on the industry to develop more efficient manufacturing processes and extend existing production capacities. Nowadays, the development of biosimilars creates additional expectations in terms of media development, that is ensuring appropriate product quality and consistency while maintaining high yields and low cost of goods.

Within the last 20 years, considerable progress in media formulation was made. Serum was replaced by more complex formulations including growth factors, vitamins, plant hydrolysates and higher concentrations of amino acids. This second generation of cell culture media greatly minimized the risk of transmission of viral agents and prions to patients and also allowed for sustained growth of cells in suspension, thus opening the door to very large-scale production in stirred tank bioreactors and high performance fed-batch processes. Consequently, volumes and product yields of production bioreactors have increased considerably.

The major drawback of second generation media was the variability of some of these different supplements. For example, although available in sufficiently large amounts, plant hydrolysates are complex mixtures. Their composition varies because of insufficiently robust manufacturing processes and because of seasonal fluctuations and various origins of the starting raw materials. For the user, this necessitates intensive testing to minimize the potential effects on process performance and product quality consistency. The numerous components found in hydrolysates can be grouped into three categorical functions: desired, neutral and undesired components. The user cannot select which of these components he wants in his process. He has to deal with the sum of all the effects. While the addition of hydrolysates showed great initial improvements, further improvements were hampered by the fixed stoichiometry of all these components.

In order to overcome the drawbacks of the second generation media, the third generation, consisting of chemically defined media, was introduced in the last decade. However,



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the task was difficult. While some of the essential components were not present in many basal second generation media, they were provided by the hydrolysates, which improved the media performance. In a chemically defined medium, the challenge is to ensure that all the essential components are present in the medium in a biologically active form during the entire culture period. Missing just one essential component can have detrimental consequences on the performance of the cell culture.

The first chemically defined media formulations were supporting cell growth and expression, but were limited in terms of process performance. The use of high throughput cell culture screening technologies and advanced experimental designs allowed for a better understanding of the impact of the ratio of essential components. Fully customized media and feed solutions were developed, resulting in high performance processes with multigrams per litre product yields. In addition, the biopharmaceutical industry benefited from an increased control over the raw materials.

However, before initiating large media optimization efforts, the following question arises: how much media development is needed and at what stage of product development?

Before tackling this question one should be aware that the medium plays a central role for all aspects of the upstream manufacturing process. The culture medium represents the environment to which the cells are exposed during all stages of production, from cell bank to harvest of the unprocessed bulk material. During all these steps, the medium directly impacts cell growth and protein expression. In addition, its impact on the cell metabolism influences the quality of secreted proteins. Once the protein is secreted, it is further exposed to the culture medium until it is harvested and purified.

Today, for the development of new biological entities, more and more companies are introducing the concept of technology platforms in order to rapidly generate Phase I clinical trial material. Therefore, predefined process parameters as well as media and feed formulations developed for a given parental cell line are applied in early cell culture development. Alternatively, commercially available media are used and, if necessary, several media are screened with commercial feed supplements sourced from different suppliers. If the performance of existing media is not satisfiable, then rapid optimization can be achieved by using advanced cell culture technologies. In a later stage of product development, the cell culture medium and the process can be further optimized for the supply of Phase III clinical trials and market, providing that the quality of the product is demonstrated to be comparable to the reference material. Thus, the manufacturing process defines product quality.

For the development of biosimilars, it is essential that the quality of the product matches the quality of the reference medicinal product at a very early stage of development. As more and more sophisticated and highly sensitive characterization tools become available, intense development efforts should be allocated to the design of the manufacturing process and especially to the cell culture medium to ensure that all critical quality attributes (CQAs) are within their predefined biosimilarity specifications. Thus, in this case, product quality defines the manufacturing process.

Consequently, the knowledge of effects of the medium components on process performance and CQAs is essential whatever drives the development efforts (e.g., the financial pressure for increasing the process performance, its consistency and robustness, the regulatory pressure for minimizing the impact of a process change on product quality, the quality pressure for ensuring a high degree of biosimilarity, and so forth).

Several factors simplify media optimization using empirical testing. First, all the components generally used for the preparation of a chemically defined medium are commercially available, of appropriate quality and can be individually tested by the user. Second, automated liquid handling systems combined with shaken multi-well plates allow one to run many cultures simultaneously and to test several hundreds of media compositions in parallel. Third, sophisticated statistical tools cover complex experimental designs from the planning phase through data acquisition to complete statistical analysis and modeling. When correctly applied, high-throughput approaches represent a powerful tool for improving the medium and feeds as a function of effect on performance and CQAs.

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Will we soon get a perfect medium? What would we expect from a perfect medium? No doubt there is a long list of criteria to be fulfilled when designing optimal cell culture media. A fit-for-purpose medium depends on the actual process (fed-batch or perfusion), the recombinant product (new biological entity or biosimilar), the cell line (CHO, NS0, nontransformed cells, stem cells and artificial organs), and so forth. A medium that is optimal for the production of tons of a product is unlikely to be adapted for stem cell cultures. Even specific applications, such as large-scale manufacturing, typically use several types of media for cell cloning, freezing, expansion or production.

Chemically defined media typically contain 50–100 different components. Each individual component can have an impact on growth, viability, productivity, product quality, cloning, apoptosis, robustness, manufacturing, media stability, purification method, environment, production costs and risk for patient. However, the understanding related to media components and interactions with other components and the product is still generally limited. Despite this lack of profound understanding, the empirical approach was very successful for improving the media and feeds [1–2]. Further improvements are expected since there are no strictly known limits that cannot be surpassed.

However, improving further media formulations will require developing innovative solutions to address certain hurdles, such as limited solubility of specific components or excessive accumulation of particular metabolic waste products. In general, there is still a lot of room for improvement as there are new options to identify and overcome the limitations. New analytical tools help to better characterize the raw materials, to measure the consumption rate and to identify strongly consumed components before they are depleted. Moving from knowledge to understanding will require additional tools such as genomics and metabolomics, which can give further insight into the differences between cell lines and the component fluxes during the cultures and, thus, might in the long term become indispensable tools to support cell line selection and cell culture media design.

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