Cell Culture Media & The Emergence of Blood-Free Components

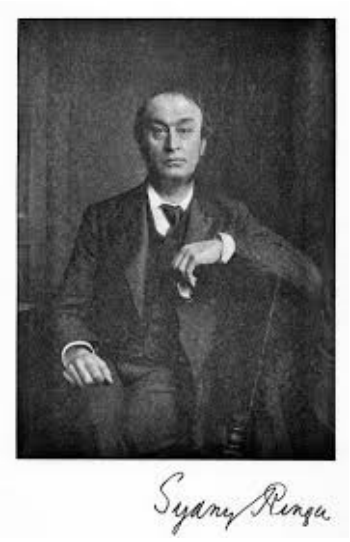
Application Note

The Evolution of Cell Culture Through Medicinal Advancements

In the past two decades, pharmaceuticals have seen a dramatic change as they have transitioned from preventative, synthetic pills and vaccinations to curative biologics and biosimilars. While preventative medicines still exist, gene therapy, cell therapy, and RNA therapeutics set a new standard for the pharmaceutical/biotechnology industry.

Today's biopharmaceuticals date to 1665 with the discovery of the cell by Robert Hooke. Under a microscope, the specimens reminded Hooke of cells within a monastery: giving rise to their accepted nomenclature.[1,2] Cells are the home to life's building blocks. Since their discovery, the ability to manipulate the building blocks of life—DNA, plasmids, genes, RNA, small molecules, antibodies, etc.—has become increasingly important to optimizing therapeutic efficacy.

To produce the necessary components for biopharmaceutical treatments, scientists leverage cell culture to optimize the production of viruses, viral vectors, and cells for their respective applications. Culture medium is designed to offer a biocompatible environment for biologics to proliferate. The demand for media arose in 1882 when the first tissue preservation experiment was performed. Sydney Ringer developed Ringer's solution to keep frog hearts beating after being removed from the body. Ringer's solution was a balanced salt solution designed to maintain physiological pH and osmolality specifications. Numerous other synthetic solutions were designed following the release of Ringer's experiments; however, embryologist, Ross Harrison, began to leverage natural media in 1907 with the use of a lymph platform. Harrison isolated pieces of frog embryonic tissue and monitored nerve fiber growth in lymph – fluid of the lymphatic system containing white blood cells.[2,3] This successful experiment paved the way for the use of natural media to improve the maintenance and proliferation of cell lines.



Throughout the history of cell culture, media have evolved and expanded to encompass serum-containing media, serum-free media, xeno-free media, protein-free media, chemically defined media, and blood-free media. These forms of media ultimately decrease or eliminate the inclusion of serum and serum derived proteins. Reducing serum in culture is helpful for many reasons: decreased supply chain risk, improved consistency, fewer regulatory/safety concerns, etc. More recently, companies have managed to eliminate the inclusion of serum and serum

derived proteins in their media while maintaining performance and consistency from lot-to-lot; the elimination of serum and serum derived proteins defines blood-free media. For this reason, the concerns associated with serum have, in theory, been mitigated. In practice, many pharmaceutical and biotechnology companies continue to incorporate serum and/or serum derived proteins in one or more of their manufacturing processes steps; therefore, the concerns remain. For the remainder of this paper, the types of media are going to be evaluated based on the benefits and risks associated with each.

Serum-Containing Medium

Serum-containing medium refers to any animal derived plasma used as cell culture media. More commonly, fetal bovine serum (FBS), human serum, and chicken embryo serum are used for cell culture applications; however, serum can be harvested from many animals. Serum was initially used for cell culture applications because it provides the basic nutrients for cell expansion: tissue extracts, hydrolysates, growth factors, hormones, carrier proteins, lipids, transition metals, vitamins, polyamines, reductants, detergents, and adhesion factors.[2,4,5] Because serum is a bodily fluid, it is inherently biocompatible; therefore, it readily maintains the pH and osmolality of the human body. The disadvantages associated with serum are a result of contamination from adventitious agents, high protein content (hindering product purification), lot-to-lot variability, supply-chain sourcing, and ethical concerns. All human derived serum used to culture cells must be from an AB+ male donor as AB+ is the universal plasma donor. Only 2% of the universal population is male AB+; therefore, it is difficult for the supply of human serum to meet industry demand. For these reasons, cell culture users experience difficulties with consistency, regulations, and patient safety.[6]

Serum-Free Medium

Serum-free medium (SFM) lacks mammalian derived serum; however, serum derived proteins still exist within the media as supplements. The commonly used protein supplements include bovine serum albumin (BSA), human serum albumin (HSA), α-globulin, and/or β-globulin. SFM are typically developed for specific cell types to optimize the growth of independent cell-lines (e.g., HEK293, T-Lymphocyte, VERO) by varying inclusion rates of necessary culture components. While SFM more directly optimizes cell growth by catering to single cell types, the risks associated with serum-containing media are often still present in serum-free media because of the remaining presence of mammalian-derived components. Furthermore, it is worth noting that impurities in SFM can often have a stronger effect on the cultured cells than the impurities in serum-containing media because SFM lacks the toxic-neutralizing agents that serum possesses.[2,4,6]

Xeno-Free Media

Xeno-free media, like serum-free media, were created to combat some of the challenges associated with serum-containing media. Xeno-free media eliminate animal derived components; however, the media are still supplemented with human-sourced components like human serum albumin (HSA), human transferrin, human platelet lysate (hPL), and human

hydrolysate. Therefore, xeno-free is not animal-free, xeno-free is not blood-free, and xeno-free does not alleviate all the serum-based disadvantages introduced above. Human sourced components are screened for viruses and inactivated by pasteurization; however, the risk of contamination via adventitious agents (e.g., dormant virus, unknown virus, Creutzfeldt-Jakob) persists. Xeno-free media do not mitigate or eliminate issues associated with serum-free media. The same risks stemming from contamination and lot-to-lot variability are present when sourcing human derived components for the manufacturing of xeno-free media.[2,4,6]

Protein-Free Media

Protein-free media were produced for the benefit chemical definition and animal component-free status. Protein-free media are defined as forms of cell culture media that do not contain large proteins but is instead supplemented with small protein fractions such as peptides and hydrolysates.[7] In CHO cell culture systems in particular, the bioreactor scaled culture system does not contain any proteins because of the difficult downstream processing associated with isolating monoclonal antibodies from proteins inherent to the media (e.g., albumin, transferrin, insulin). While protein-free media simplify downstream processing of biopharmaceuticals, they are not as robust as media containing proteins. Media are designed to mimic an in vivo environment; therefore, the best performance with respect to cell expansion is observed from protein containing media. For some companies, however, ease of downstream purification is a higher priority than optimized performance — hence the creation of protein-free media.[4]

Chemically-Defined Media

Chemically defined media are free of animal and human derived components. This ensures the proteins and supplements included within the media contain no undefined components (e.g., HSA, BSA, hPL). Instead, non-mammalian sources such as yeast and *E. coli* are used to generate recombinant proteins for media supplementation at varying inclusion ranges to support the proliferation of specific cell types. Chemically defined media should not be confused with defined media which is an interchangeable term to encompass serum-free and xeno-free media. Chemically defined media were initially developed to cater to suspension cell culture systems, eliminating the need for mammalian derived attachment factors required in adherent cultures. Recombinant proteins and growth factors derived from plant or bacterial sources (e.g., yeast, *E. coli*) are used to supplement base media to provide the cells with the necessary metabolites for proliferation while maintaining a chemically defined profile. Chemical definition offers reproducibility and consistency that serum and serum derived components lack; however, in terms of supply chain continuity, producing recombinant protein in *E. coli* or yeast is not scalable to meet the needs of cell culture media.[8] Relying on a non-mammalian expression system (e.g., plants) offers a highly scalable, consistent, and reproducible approach to manufacturing recombinant proteins for the purpose of generating chemically defined cell culture media.[8]

Blood-Free Media

InVitria invented and specializes in the production of blood-free, chemically defined media using recombinant serum proteins. The term blood-free was coined by InVitria and can be associated

with its non-mammalian, non-bacterial expression system to manufacture its recombinant proteins. The presence of InVitria's recombinant serum proteins eliminates the need to include mammalian and human derived serum and serum proteins in cell culture media; therefore, unlike all other media, blood-free medium has successfully eliminated all mammalian serum and serum proteins from cell culture media (i.e., animal and human). Blood-free media are not only successful in eliminating patient risks, but they also improve therapeutic biopharmaceutical yields (e.g., viral vectors, cells).

InVitria produces blood-free recombinant albumin and blood-free recombinant transferrin, both of which are provided to biopharmaceutical manufacturers prioritizing the reduction/elimination of BSA, HSA, FBS, human transferrin, etc. in their cell culture systems. By leveraging its recombinant proteins, InVitria also develops, optimizes, and manufactures complete media for many cell lines, including HEK293t, VERO, and T-Lymphocyte. These products allow ease of integration into current biopharmaceutical manufacturing and final formulation processes from a regulatory perspective given their chemical definition and blood-free profile. These properties optimize patient safety by eliminating the risks associated with contamination from adventitious agents present in mammalian and human derived serum and serum components. Furthermore, by manufacturing consistent, quality material, lot-to-lot variability is mitigated. This level of consistency directly translates to improved performance by way of increased cell expansion and titer. Leveraging a non-mammalian expression system also allows InVitria to easily scale manufacturing, unlike serum and serum proteins.

Overall, throughout the history of cell culture, media has vastly improved both manufacturing performance and product safety; however, elimination of risk from adventitious agents requires complete replacement of serum and serum proteins—an unlikely occurrence. Therefore, the optimal way in which the cell culture media industry can support biopharmaceutical manufacturing and final formulation practices is by appropriately educating the end user. It is critical for cell culture media manufacturers to educate their customers on products harnessed in their manufacturing and/or final formulation processes. It is necessary that media companies provide complete transparency as to the level of chemical definition their media offers. Through these actions, end users can take all necessary precautions required to mitigate regulatory concerns; this ensures the patients' health and safety remain a top priority in the manufacturing and final formulation of today's biopharmaceutical.

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