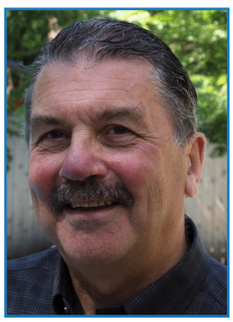
Editorial

Pharmaceutical BIOPROCESSING

Pharm. Bioprocess.
(2013) 1(4), 1–4

Serum-free media: standardizing cell culture systems



Michael Butler
418 Buller Building, Department of Microbiology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
E-mail: butler@cc.umanitoba.ca

> The disadvantages of the use of serum in cell cultures have now led to a strong demand in both research and industry for cell culture formulations that are serum-free media.

Keywords: bioactive, fetal bovine serum, growth factors, MRC-5, peptide hydrolysates, Plackett–Burman, serum-free, Vero, WI-38

Historical development of basal media

The first attempts at culturing animal cells *in vitro* made use of biological fluids, such as serum or tissue extracts. It was in the 1950s that a scientific approach was adopted to determine the defined nutrients required for mammalian cell growth. The idea of a chemically defined media was pioneered by Eagle, who determined the minimum ingredients that were essential for the growth of a number of human cell lines. This led to the development of Eagle's minimal essential media that consisted of 13 amino acids, 8 vitamins and 6 ionic species [1]. This formulation appeared to provide the requirements for the growth of a number of isolated cell lines if supplemented with animal-sourced serum. Higher cell densities were obtainable by increasing the component concentrations of Eagle's minimal essential media and became established through basal formulations such as Dulbecco's modification of Eagles medium (DMEM). Clonal cell growth of selected cell lines was obtained by enrichment with an enhanced range of nutritional components, largely through the early work of Ham to produce the well-known Ham's F-12 medium, Sato had the ingenious idea of combining these two approaches to blend a basal media formulation – DMEM/F-12 – that has become widely used for the growth of multiple cell lines to high density [2]. However, despite the inclusion of up to 70 components in these well-defined basal media formulations, supplementation with dialyzed serum (~10%) is necessary to provide sustained growth of most cell lines.

Serum-based media

Bovine serum (particularly fetal bovine serum [FBS]) contains many components required for cell growth such as growth factors, hormones and trace elements. The high albumin content acts as a carrier of micronutrients as well as protecting the cells from adverse pH fluctuations or shear forces. However, despite the excellent growth support there are significant disadvantages in using serum as a culture additive. These include:

- Cost and availability: as a media component, serum can account for 90–95% of the overall cost. FBS varies in availability, particularly from favored countries such as New Zealand, and can cost anything from US$500–1000/l;
- Ethical concerns: the procedure needed to obtain serum from a bovine fetus causes concern regarding animal suffering and welfare;

FUTURE SCIENCE

10.4155/PBP.13.45 © 2013 Future Science Ltd ISSN 2048-9145

- Batch-to-batch variation in composition: the composition of serum is variable and undefined, which leads to inconsistent growth and productivity. Each batch can vary in content depending upon the diet and condition of the donor cows. This variation can cause significant differences in the growth-promoting characteristics, and ultimately causes significant differences in productivity of the cell-culture process;

- A high protein content hinders product purification: the cells grown in a bioreactor secrete the product of interest (normally a protein) into the culture medium. If the culture medium contains serum at 10% v/v, its protein concentration is already high, often approaching 10 g/l. In comparison, the concentration of a targeted protein secreted by the cells may only reach 0.1–1 g/l, thus a difficult purification process is required to separate the product from the serum protein. If the product of interest is a monoclonal antibody, it may well be mixed with any other antibodies present in the serum and these are very difficult to separate;

- The potential for product contamination: the threat of contamination arises from unwanted viruses and mycoplasma that may be present in serum as well as prions associated with bovine spongiform encephalopathy. One estimate indicates that 20–50% of commercial FBS may be virus positive [3]. The incidences of new variant Creutzfeldt–Jakob disease (the human version of bovine spongiform encephalopathy) that have originated from meat products are a source of concern for the manufacture of injectable biologicals. Although there have been no proven cases of such contamination getting into a final product, no one wants to take the chance of such contamination in a manufactured biopharmaceutical.

Serum-free media

The disadvantages of the use of serum in cell cultures have now led to a strong demand in both research and industry for cell culture formulations that are serum-free media (SFM). For research, serum-free formulations are important to ensure consistency of performance and reduced batch-to-batch variability. These factors are also important for large-scale manufacturing processes, particularly to minimize the risk of contaminating final product biopharmaceuticals with prions or viruses. Regulatory authorities have demanded the use of serum-free processes for biopharmaceutical manufacture when possible.

/// The objective in formulating a serum-free media is to supplement a basal medium such as DMEM/F12 with essential components such as growth factors, vitamins, trace elements, hormones and any other micronutrients not provided by the basal media. ///

The challenge of developing SFM is to be able to substitute serum with equivalent growth-promoting factors. The requirement for such factors varies considerably between cell lines with some producer cells requiring an extensive profile of bioactive components. It has not been possible to design a universal serum-free formulation as a serum substitute suitable for all cell lines. Even different clones of a cell line may have different requirements for optimal growth.

The objective in formulating a SFM is to supplement a basal medium such as DMEM/F12 with essential components such as growth factors, vitamins, trace elements, hormones and any other micronutrients not provided by the basal media. Early attempts to develop SFM formulations incorporated such animal-sourced components as insulin, transferrin, albumin and cholesterol. However, these first-generation formulations still had the disadvantages of containing relatively high protein content and components that were derived from animal sources. There followed two important, but separate, criteria for SFM: protein-free (PF) and animal-derived component-free (ADCF). ADCF media may contain recombinant proteins and protein hydrolysates derived from non-animal sources. For PF media, proteins may be replaced by low-molecular-weight components including peptides, hormones and inorganic salts. However, in many cases commercially available media described as PF contain minimal levels of recombinant proteins.

Peptide hydrolysates of plant and microbial origin used widely in the food industry have become valuable sources of non-animal components to promote mammalian cell growth. These are often supplemented to cultures as optimal blends from various sources such as soy, wheat gluten and yeast designed by statistical design of experiment protocols [4]. The

consensus is that there may be a combination of nutritive components and growth factors in these hydrolysates to promote cell growth [5]. There are clear advantages in being able to identify these bioactive components because they then can be included in a completely chemically defined (CD) media, which potentially could be manufactured from pure ingredients to a consistent standard. There has been some concern over the batch-to-batch variation in the content of these hydrolysates, although the method of processing can be improved to minimize this variability [6]. The chemical identity of all the bioactive components needed for cell growth leading to a fully CD media is a desirable goal, but proves elusive, particularly for some fastidious cell lines. Although there are ADCF, CD culture media formulations available, many show a decreased performance with the higher degree of chemical definition [7].

Design of serum-free formulations

It is reported that there are over 450 different SFM formulations available commercially for various cell lines and at various degrees of chemical definition [8]. Unfortunately, many of these formulations are proprietary and, unlike the basal media, are not in the public domain. This means that the user of a SFM may be reliant on a specific vendor to maintain defined conditions for their cell line.

Serum-free formulations may be designed using several strategies. Initially, serum should be reduced to a minimum level for cell growth using different combinations of standard basal media. The determination of specific nutrient uptake rates and depletion during culture will aid in metabolic analysis that can identify requirements for supplementation of critical components. Optimal cocktails of critical components for growth and productivity can be determined from replicate cultures using a statistical design of experiments with techniques such as the Plackett–Burman matrix design [9]. An original approach is the identification by microarray analysis of specific receptors expressed during cell growth by analysis of extracted RNA with a microarray of cDNA. The media is then supplemented with the corresponding ligands to stimulate the associated signaling pathways [10]. Using these techniques it is possible to design a serum-free formulation with optimal properties for any desired criteria, which may include high growth rate, cell yield, productivity or defined product quality such as glycosylation.

One major challenge in this area of media design is the formulation of consistent and robust CD media for those fastidious human cell lines that may be anchorage-dependent and used for vaccine production, such as Vero, MRC-5 and WI-38 human cells. It is anticipated that the systematic approaches that are now being used, particularly with the high-throughput methods, will eventually enable even these cells to feed on a vegetarian, CD diet.

Financial & competing interests disclosure

M Butler works for the department of Microbiology, University of Manitoba and and is founder of BiogroTechnologies Inc., a spin-off company involved in the commercialization of serum-free media formulations for mammalian cells. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Eagle H. Amino acid metabolism in mammalian cell cultures. *Science* 130(3373), 432–437 (1959).

2 Jayme D, Watanabe T, Shimada T. Basal medium development for serum-free culture: a historical perspective. *Cytotechnology* 23(1–3), 95–101 (1997).

3 Wessman SJ, Levings RL. Benefits and risks due to animal serum used in cell culture production. *Dev. Biol. Stand.* 99, 3–8 (1999).

4 Kim SH, Lee GM. Development of serum-free medium supplemented with hydrolysates for the production of therapeutic antibodies in CHO

cell cultures using design of experiments. *Appl. Microbiol. Biotechnol.* 83(4), 639–648 (2009).

5 Burteau CC, Verhoeye FR, Mols JF, Ballez JS, Agathos SN, Schneider YJ. Fortification of a protein-free cell culture medium with plant peptones improves cultivation and productivity of an interferon-gamma-producing CHO cell line. *In Vitro Cell. Dev. Biol. Anim.* 39(7), 291–296 (2003).

6 Siemensma A, Babcock J, Wilcox C, Huttinga H. Towards an understanding of how protein hydrolysates stimulate more efficient biosynthesis in cultured cells. In: *Protein Hydrolysates in Biotechnology.* Pasupuleti VK, Demain AL (Eds). Springer, Dordrecht, Germany, 33–54 (2010).

7 Hodge G. Media development for mammalian cell culture. *BioPharm Int.* 54–57 (2005).

8 Van Der Valk J, Brunner D, De Smet K *et al.* Optimization of chemically defined cell culture media – replacing fetal bovine serum in mammalian *in vitro* methods. *Toxicol. In Vitro* 24(4), 1053–1063 (2010).

9 Gonzalez-Leal IJ, Carrillo-Cocom LM, Ramirez-Medrano A *et al.* Use of a Plackett–Burman statistical design to determine the effect of selected amino acids on monoclonal antibody production in CHO cells. *Biotechnol. Prog.* 27(6), 1709–1717 (2011).

10 Allison DW, Aboytes KA, Fong DK *et al.* Development and optimization of cell culture media genomic and proteomic approaches. *BioProcess Int.* 3(1), (2005).