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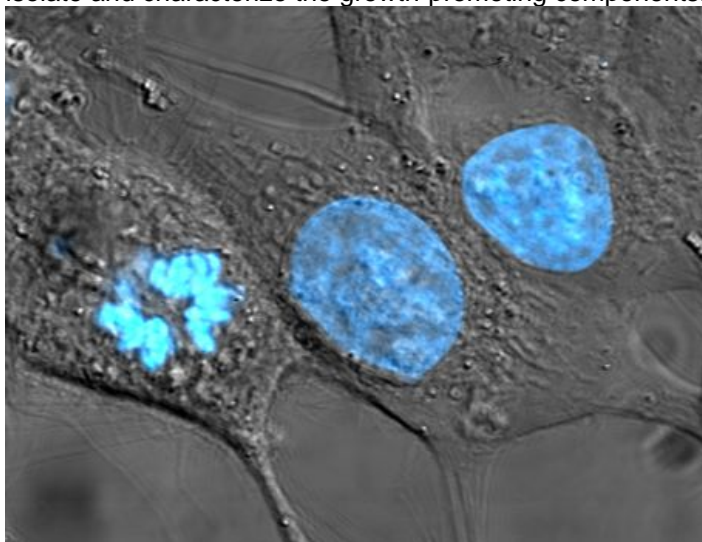
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Protein Hydrolysates: A Magic Elixir for Cultured Cells?

By **K. John Morrow, Jr. President**
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Participants in the Hydrolysates Forum at the recent IBC “Biopharmaceutical Development and Production Week” thrashed out their views on protein hydrolysates as additives for cell culture media. According to Dr. John Birch, PhD, Chief Scientific Officer at Lonza Biopharmaceuticals (www.lonza.com), the group was of two minds concerning their use. “On the one hand, there are those who believe hydrolysates are irrelevant if you already have an optimized protein free medium, but the hydrolysates may offer a cushioning capability for less than ideal conditions,” he stated. “On the other hand, some investigators look upon hydrolysate additives as a starting point, from which they can isolate and characterize the growth-promoting components.



Cultured mammalian cells, from Wikipedia

The trouble with this approach is there may be no single active component, but rather growth promotion may arise from a mixture of peptides.” Hydrolysate as a bolstering component of cell culture medium have been in use since the early days of this technology in the 1950s. In the 1960s there was a move toward delineation of the components in cell culture media and an elimination of unknown factors. A number of serum-free formulations were developed by investigators and reported in the peer-reviewed literature, however most formulations were tested using clonal growth as the criterion for success, meaning that they were not optimized for maximum cell density.

The present-day motivation for the use of hydrolysates is based on the desire to increase recombinant protein production while at the same time eliminating the biosafety risk and expense of animal proteins such as fetal calf serum. In many cases the peptides and proteins present in the hydrolysates do not appear to add significantly to the nutritive potential of basal protein-free nutritive medium. This suggests that their ability to increase cell growth and protein yields may be due to the binding of toxic contaminants in the medium, such as endotoxins, known to exert their negative effects at very low concentrations.

Dr. M. Butler, PhD., professor in the Department of Microbiology, University of Manitoba, CANADA, considered serum-free media with regard to the following issues: elimination of animal products, design of robust, protein-free media and a complete delineation of all substances, including vitamins, amino acids peptides and inorganic compounds. The ideal culture medium could be achieved through the laborious process of item by item optimization, a daunting task when high performance is the ultimate goal. The desired characteristics of media for recombinant protein production are

extensive. However, a well-designed formulation could produce high titres in a bioreactor and obviate problems of downstream processing, since large scale removal of added proteins would not be required.

Dr. Birch elaborated on this argument by showing examples of multiple gel bands associated with harvested media several years ago compared with today’s much simpler picture of single bands associated with antibody products secreted into Lonza’s current chemically-defined media formulation.

Dr. Bryan Monroe, PhD., from Process Science at Invitrogen made the point that the media formulations used today were not designed to provide the high titres expected in today’s culture systems. He argued that there is a lot of excess baggage of irrelevant components in current formulations that could be removed.

Dr. Iman Famili, PhD, of GT Life Sciences advocated a metabolic modeling approach to the development of media. An understanding of the metabolic flux within the cell could be used to provide a balanced nutrient source for the cells.

Geoffrey Francis (Novozyme) advocated the study of cell surface receptors that could lead to the incorporation of cell signaling ligands. This was a reminder of his approach in the development of Long R3-IGF (insulin like growth factor) now an essential component in many serum-free media. The naturally-occurring IGF molecule mimics the effects of insulin, whereas to improve its performance it was engineered with several critical alterations. This robust, growth-promoting 83 amino acid analog of IGF-1 is the complete human IGF-1 sequence with the substitution of an Arginine for the Glutamine at position three, hence R3, and a 13 amino acid extension peptide at the N terminus (thus the

“long” designation).

Dr. Tom Fletcher, PhD., a staff scientist in the R&D Division of Becton-Dickenson outlined his studies on the use of peptones from yeast hydrolysates as bioactive media components. The composition of these hydrolysates are complex and even by sophisticated techniques of fractionation it is difficult to isolate bioactivity in single components.

Dr. Holly Prentice, PhD., from Millipore considered the possibility of single identifiable bioactive peptides which could be differentiated from nutrient additives. However, there have been no reports of success using the high concentrations of single tripeptides that have been reported as growth-stimulatory in the literature.

Dr. Matt Caple, PhD., of SAFC/Sigma described the variability of different lots of peptide hydrolysates. He indicated that the problem of variability was likely to be much less if the hydrolysates were supplemented in well-designed basal media.

The summary statement concluded with the thought that the “holy grail” of medium design is a maximized level of protein production in a chemically-defined and animal-component free formulation. Notwithstanding notable progress, there is still ample scope for improving media formulations that may ease the bottle-neck of production, often considered to be down-stream processing.

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