Basal medium development for serum-free culture: a historical perspective

David Jayme, Toshio Watanabe & Toshiaki Shimada

Life Technologies, Inc., Grand Island, NY USA and Life Technologies Oriental, Tokyo, Japan

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Abstract

The evolution of basal synthetic formulations to support mammalian cell culture applications has been facilitated by the contributions of many investigators. Definition of minimally-required nutrient categories by Harry Eagle in the 1950’s spawned an iterative process of continuous modification and refinement of the exogenous environment to cultivate new cell types and to support emerging applications of cultured mammalian cells. Key historical elements are traced, leading to the development of high potency, basal nutrient formulations capable of sustaining serum-free proliferation and biological production. Emerging techniques for alimentation of fed batch and continuous perfusion bioreactors, using partial nutrient concentrates deduced from spent medium analysis, can enhance medium utilization and bioreactor productivity.

Introduction

Developmental progress of nutrient media, from initial reports in the late 19th century to the present day, has been characterized by evolutionary advances (Jayme and Blackman, 1985; Gruber and Jayme, 1994). The external fluids for mammalian cells and tissues evolved from bathing environments of isosmotic salt solutions to approximations of the native cellular environment through addition of buffering components, more complex salt species, and substrates and co-factors to support intermediary metabolism, such as carbohydrates, amino acids, and vitamins (Ham, 1982).

While these nutrients were adequate to maintain viability and biological function for brief studies, supplemental additives (e.g., serum, tissue extracts, other humoral fluids) were required to support proliferation and to sustain cellular activity for extended periods. Motivation to reduce these ill-defined additives derived from desire to minimize lot-to-lot variation, limited availability, cost, interference with product purification, inability to cultivate certain cell types, and concern with foreign antigens or adventitious viral contaminants (Ham, 1982; Jayme and Greenwald, 1991).

Initial efforts to develop serum-free media exclusively through addition of defined growth factors failed to account for the broader contributions of serum (Jayme, 1991). These properties included both specific biological activities (such as cytokines, supplemental metabolites, nutrient binding and transport factors, and substratum conditioning factors) and bulk protein functions (such as pH buffering, toxin inactivation, protease activity, and protection from shear stress and vessel adsorption) (Jayme and Blackman, 1985; Gruber and Jayme, 1994).

Pioneering efforts to characterize critical serum components and identify biochemically-defined substitutes initially emphasized cell growth and biological function in clonal density cultures. Subsequent applications of cultured cells, however, focused upon biological production and maintenance or directed-differentiation of cell function. These studies evolved more complex nutrient media designed to sustain performance at elevated cell densities and to emphasize metabolic efficiency under culture conditions designed to minimize cell proliferation.

This paper will focus upon the development of basal nutrient formulations used for research and biotechnology applications, with particular emphasis upon research of the late Professor Hiroki Murakami resulting in the eRDF basal synthetic formulation. Innovative techniques currently utilized to optimize nutrient composition and delivery to mammalian cell bioreactors are also described.
Foundational contributions

Primary historical elements for this discussion begin with Eagle’s 1955 publication of a basal nutrient formulation (Eagle, 1955), an ‘isosmotic, pH-balanced mixture of salts, amino acids, sugars, vitamins, and other necessary nutrients’. Four years later, Eagle published a modified formulation (Eagle, 1959), termed ‘Minimal Essential Medium (MEM)’, which augmented the original basal amino acid composition based upon the protein composition of cultured human cells. This modified formulation, while more nutritionally complex than most previous synthetic media, still required supplemental protein in the form of plasma, serum, or tissue extracts to support cell growth. The components of Eagle’s basal medium formulations provided the basis for most nutrient media prepared today (Gruber and Jayme, 1994). Table 1 compares the biochemical composition of selected basal media used for mammalian cell culture applications.

From this point, two parallel lines of investigation emerged. One track elucidated nutrients required to eliminate serum while supporting proliferation of clonal isolates of targeted cell types, particularly examining primary cultures and established lines of finite population expansion. The complementary track focused less initially upon elimination of serum, but instead pursued optimization of nutrient levels for high density culture. The art of synthesis

Future generations of cell culture researchers and biotechnologists may reflect upon the evolution of nutrient medium and recognize the enormous contribution of Gordon Sato and his co-workers (Bottenstein et al., 1979). Perhaps their most frequently cited accomplishment was the merger of these two independent media development tracks into a simple composite, a 1:1 volumetric admixture of DMEM and F-12 media formulations, termed DMEM/F12 medium. By combining the favorable properties of both individual formulations, a fortified basal medium was created which supported both clonal isolation and high density culture (Barnes and Sato, 1980).

Although other combinations of classical formulations (e.g., IMDM/F12, M199/F10) have been implemented for selected applications, DMEM/F12 has emerged as the most widely utilized basal synthetic medium. Supplemented by serum, it was capable of sustaining bioreactor production applications. Augmented by a defined cocktail of peptide and steroid hormones, perhaps in combination with attachment factors or preconditioned matrices, it formed the basis for serum-free media to cultivate a broad range of cell types.

In addition to the direct achievements noted in the publications from Sato’s laboratory, the fertile environment created by his leadership spawned many of the scientists who would carry the evolution of nutrient optimization to its next level. Among the great scientists trained within this environment who would contribute significantly to the development of basal media and to the progress of serum-free culture was Hiroki Murakami.

Murakami focused primarily on the nutrient requirements of various non-adherent cell types (e.g., hybridomas, lymphocytes, human tumor cells) for sustained proliferation and generation of specific monoclonal antibodies (Murakami and Yamada, 1987). Beginning with the basal DMEM/F12 formulation,