Lipids in Cell Culture Media

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Introduction

Requirements for better performance, a desire to avoid serum, and improved understanding of culture systems are inspiring fresh interest in supplementing cell-culture media with identified lipids.

Surprisingly, there is no universally accepted definition of "lipid." Originally, scientists considered all naturally occurring compounds that are soluble in non-polar solvents such as benzene, to be lipids. For cell culturists, a more practical definition is "water-insoluble biomolecules, biosynthetically or functionally related to fatty acids and their derivatives." This definition includes fatty acids, sterols, triacylglycerols, glycerophospholipids, and sphingolipids, but excludes steroid hormones, fat-soluble vitamins, and petroleum products. There is logic to both definitions, and either will suffice in this article. By any definition, cell culturists have a special concern about lipids: Their limited solubility in media makes adding sufficient quantities a challenge.

Lipids function in three roles. They serve as energy stores, as structural components of cellular membranes, and in transport and signal systems. Cell and organelle membranes contain much of a cell's lipids. Major categories of biological lipids include glycerophospholipids, e.g., phosphatidyl choline; sterols, e.g., cholesterol in animals and phytosterols in plants; sphingolipids, e.g., ceramides; and various lipoprotein complexes. Structural functions of many lipids, such as the requirement of cholesterol for animal cell membrane fluidity, have been extensively described. Many signaling systems rely upon lipid-containing complexes, such as the familiar ABO blood type determinant from the lacto- and neolacto-series of glycosphingolipids.

Cellular Requirements

In any living system an "essential nutrient" is a compound that the organism requires for growth and reproduction, and which the organism cannot produce. Cells can synthesize most of the dozens of lipids they require for primary cellular functions. In mammals, though, two fatty acids (linoleic and -linolenic acid) have been proven to be essential.

For decades scientists have reported on the lipid requirements of particular cell lines as cultured in an excess of lipids, usually from FBS. With the more widespread use of serum-free and chemically defined (CD) media, researchers are now looking at the requirements of cells maintained in minimal complements of identified lipids. Also, a distinction is emerging between required media components and optimal components and levels. Apparently, much of the work performed with cells adapted to media with high lipid levels provided an inaccurate picture of the actual requirement of cells in culture. Many known serum-free media (SFM) formulations lack one or both of linoleic and -linolenic acid, yet sustain indefinite cell growth and full function. It appears that these "essential" fatty acids are not essential in most animal cell culture (Grammatikos et al. 1994).

Supplementation of cell culture systems with particular non-essential fatty acids, phospholipids, and sterols significantly improves performance. Providing cells with appropriate preformed lipids, even when not essential, reduces the need for their biosynthesis by the cell. The result is more efficient metabolism. This is especially evident where the rate of cell division is important, or where the cell produces high levels of transgenic product (Manwaring, et al., in press). Some cultured cells are truly auxotrophs for particular lipids, meaning that these lipids are "essential" to them. For example NS0, a common myeloma cell line, requires large amounts of cholesterol but is incapable of producing it. In this case, the phenotype is caused by NS0's loss of an enzyme in the cholesterol synthesis pathway.

Media Supplementation

People have been devising heuristic formulas for working with lipid dispersions for millennia. Churning butter and adding an egg to a failing recipe are examples of applying simple techniques without necessarily understanding the chemistry behind them. At the other extreme, modern industries such as pharmaceuticals, food processing, agrochemicals, and cosmetics often approach oil and water dispersion issues with sophisticated technologies. Interfacial and colloidal chemistry, hydrocarbon chain packing, and lyotropic and thermotropic mesophase behavior have all been brought to bear against lipid dispersion issues. (Larsson, 1994).

Whatever approach or combination is used, the goal of cell-culture media supplementation is to disperse select lipids such that they are nontoxic, are taken up by the cells in a controlled fashion, can be micro-filtered, and remain stable upon storage at 5 to 50°C for up to a year.

These requirements can be met in three ways:

1. Adsorb the lipid to a soluble carrier molecule,
2. Devise a formula that drives lipid self-assembly to the required particle size, and
3. Disperse and stabilize a lipid mixture to a particle of sufficient transient size and stability.

Each of these approaches is a science in its own right, and all have been used in developing cell culture media (Table 1).

Adsorption to a Carrier

Animal serum, the original vehicle for providing lipids to cells in culture, uses proteins as carriers of every lipid required by mammalian systems. FBS, the most common serum in cell culture, contains very high levels of lipids. For example, FBS contains approximately 300 µg/mL cholesterol and 30 µg/mL oleic acid. The major lipid carrier proteins in sera include: (1) Albumin, a globular protein with many distinct hydrophobic moieties and which represents over 50% of the protein in serum, and (2) Four classes of apoprotein-containing lipoproteins: chylomicrons, carrying trya cylglycerides; very low-density lipoproteins (VLDL), carrying fatty acids; and low- and high-density lipoproteins (LDL and HDL) that are principally involved in cholesterol transport. The high level and diversity of lipids in sera is sometimes detrimental. For example, cells cultured in serum are constantly exposed to a number of steroids, making it difficult to determine the specific effects of each.

Serum extracts and lipid-rich fractions are popular products for adding high concentrations of serum lipids to media. Commercially available fractions of animal serum contain a number of serum lipids, including cholesterol, fatty acids, and phopholipids bound to select serum proteins. While the ratio of lipids remains fixed,

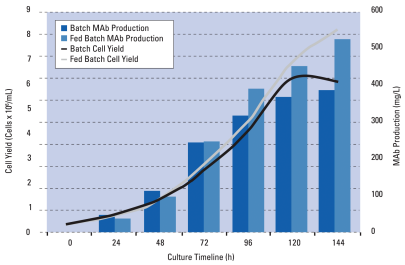

Figure 1. Cyclodextrin-based culture feeding. rNS0 seeded at 2.5 x 10^5 cells/mL in SFM4MAb™ plus LS250 in a 10 L Celligen® bioreactor, with and without feeding with LS1000 lipid supplement.

these IgG-free and albumin-reduced supplements allow variation of the total lipid concentration, and combination with other supplements. They are of significant value in some serum-dependent systems by, for example, reducing material costs and improving performance. Notably, they work well with some SFM, but not at all with others.

Bovine serum albumin (BSA) is a common vehicle for lipids. It is commercially available in a variety of purities and formats. All basic preparations contain high levels of serum lipids, especially fatty acids and lecithin. It is possible to create or purchase a reduced lipid BSA. Such a preparation has the capacity to adsorb a significant amount of added lipids of choice. Cyclodextrins, naturally occurring circular polymers of glucopyranose, increase the solubility of lipids. Their function is similar to protein adsorptive systems, in that they encase or chelate lipids in a more water-soluble molecule and thereby increase the lipids' aqueous solubility. The addition of various side groups to the cyclodextrin molecule increases the cyclodextrin's solubility in water to nearly 50% w/v, while leaving the hydrophobic, lipid-active central cavity intact. Two newer products, Thermo Scientific HyClone LS1000™ and Thermo Scientific HyClone LS250™, successfully utilize cyclodextrin to solubilize cholesterol and fatty acids for supplementation of cell culture media. LS1000 is designed to be added at culture initiation, or as a component in fed batch cultures (Figure 1). LS250 is optimized for media supplementation at culture initiation. Dilutions in media may be filtered and are stable in appropriate storage for months.

Lipid Dispersions

Liposomes, emulsions, and microemulsions are forms of lipid dispersions used in media supplementation.

Table 1. Various lipid dispersion technologies; their potential and limitations.

	Physical Stability	ADCF Potential	PF Potential	CD Potential	Formulation Adjustable
Serum	High	No	No	No	Minimally
Serum Extracts	High	No	No	No	No
Albumins	High	rAlbumin	No	Nearly	Somewhat
Emulsions	Low	Yes	Yes	Yes	Significant
Micelles	High	Yes	Yes	Yes	Somewhat
Liposomes	Med	Yes	Yes	Yes	Somewhat
Cyclodextrin	High	Yes	Yes	Yes	Yes

Liposomes

Spheres of lipids in the lamellar phase with an aqueous core are variously referred to as lipid bilayer vesicles or liposomes. All nutrient lipids carried by liposomes must be components of the lipid lamellae. It is sometimes possible to combine lamellae-forming polar lipids with nutrient lipids such that the phase behavior and intrinsic curvature of the mixture generates liposomes of suitable size and stability. For example, cholesterol or fatty acids can intercalate within the acyl chains of polar lipids, such as phosphatidyl choline. The temperature, pH, and tonicity of cell culture media usually remains within a narrow range. This allows the use of such principles as steric hindrance, hydrogen bonding, and electrostatic charge to be exploited in developing stable preparations. Although mixtures of polar and nutrient lipids so formulated are conceivable, published attempts mostly report failure.

Emulsions and Microemulsions

These two related forms of filterable dispersions can have a term of physical stability comparable to that of the media itself.

Emulsions are kinetically (non-equilibrium) stable dispersions. They are produced by first reducing particle size through introduction of hydrodynamic force, and then stabilizing the surface of the particles. Stabilization is accomplished by the addition of such amphophiles as polar lipids and certain peptides or polymers, or by modulating the particle surface charge.

Microemulsions may be formed by using detergents to generate mixed micelles containing the lipid of interest at their core. The key to developing such microscale and thermodynamically stable dispersions is to find a surfactant that is neither too toxic nor too disruptive to cell membranes. The surfactant and lipids must form filterable structures of acceptable size and stability at normal temperatures. Concepts such as the critical micelle concentration (CMC) and hydrophilic/lipophilic balance (HLB) are useful here.

A number of SFM in current use have their lipid components dispersed by these means. In either case, the amphiphile surrounds and stabilizes a particle of lipid, presenting its hydrophilic region to the aqueous media.

Materials

Nutrient lipids are available from a variety of sources and vendors. Enriched lipid fractions extracted from such diverse starting materials as animal serum, sheep's wool, fish oil, and soybeans are commercially available. Individual lipids purified from these naturally occurring rich sources are also available. Chemical synthesis and derivitization is used to produce those lipids that are either rarely found in nature, or that are abundant only in unacceptable sources. For example, in formulating animal component free media, cholesterol, commonly obtained from sheep's wool, must instead be synthesized from non-animal origins.

Tween® 80, a non-ionic detergent; pluronic acid, a block co-polymer; phosphatidyl choline (or lecithin); and protein hydrolysates, such as those from beef or soy, have proven to be the most popular materials for dispersing non-polar lipids in serum and protein-free (PF) cell culture media. Lipid particle surface and interfacial energies must be overcome in both the generation of metastable emulsions and in accelerating the equilibrium of microemulsions. This is accomplished in instruments capable of generating extreme hydrodynamic force while minimizing heat production (e.g., the Emulsi Flex-C50, Avestin, Ottawa, ON). The addition of chemical antioxidants, such as -lipoic acid and -tocopherol, can reduce the peroxidation of polyunsaturates in the formulation. Procedures that limit the introduction of free oxygen also help in this regard. Interestingly, many lipids seem to be exquisitely protected from oxidation when complexed with cyclodextrin (Kim, et al., 2000).

Applications

Lipids currently referred to in the literature for supplementation include cholesterol; cod liver oil; soybean oil; and oleic, linoleic, and palmitic acid. Applications for lipid-based media supplementation include essential and performance-enhancing lipids in most CD formulations, special requirements such as the need for high levels of cholesterol in NS0 based transgenic producers, and fed-batch procedures in bioreactors (Mahadevan, 2003).

Conclusion

Systems for delivering lipids have evolved as the culture requirements of research and industry changed. Earlier, supplementation with animal sera provided an effective answer. As concerns about animal-derived products increases, media producers move further towards serum-free, animal-derived component free, and CD technologies. The elimination of serum, with its complement of natural lipid carriers, initially posed significant problems to media supplementation. Fortunately, these are being solved through the approaches and techniques outlined in this paper.