USE OF ETHANOLAMINE FOR ENHANCING CELL GROWTH IN MEMBRANE SYSTEMS

The present invention is based, in part, on the discovery that cell growth, cell viability, and antibody production in a membrane system such as hollow fiber system can be enhanced by culturing cells in the presence of serum on the cell side and in a medium containing ethanolamine in the absence of serum on the non-cell side. Accordingly, the present invention provides methods for the enhanced growth of cells while minimizing the use of serum.
USE OF ETHANOLAMINE FOR ENHANCING CELL GROWTH IN MEMBRANE SYSTEMS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 60/551,119 (filed March 8, 2004) entitled “Use of Ethanolamine for Enhancing Cell Growth in Membrane Systems”. The entire content of the above-referenced application is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Culturing mammalian cells is an essential technique for research into cellular processes, production of recombinant therapeutic proteins, and generation of expanded cells for transplantation purposes. Cell culture studies have led to the determination of numerous metabolic processes and the identification of growth factors, hormones and their receptors (Bio Techniques, 5:534-542, 1987). In particular, membrane systems such as hollow fiber systems provide a powerful tool for culturing cells for the production of proteins, and, in particular, culturing hybridomas for the production of antibodies. Membrane systems such as hollow fiber systems provide improved cell retention and high density cell growth. The use of a semi-permeable membrane to retain high molecular weight proteins on the cell side allows for more efficient use of expensive medium components while producing a highly concentrated product (e.g., antibody).

The composition of media used to culture cells is of paramount importance because of its influence on cell survival and cell response to various effectors. Conventional cell culture media comprise basal nutrient media supplemented with serum from various sources, most often fetal bovine serum, horse serum or human serum. However, the use of serum is undesirable for several reasons. Growth media containing serum may vary in composition, hormone content, and contaminants, thereby introducing extraneous factors and/or infections agents into the culture system (Bio Technology, 11:49-53, 1993; Pharm. Technol., 48:56, 1987). In addition, serum is expensive and impractical for large-scale production of therapeutics. Further, variance between serum lots and laboratory protocols is also a problem. Recent concerns by the FDA, the European community, and others about serum quality, contamination (i.e.,
bovine spongiform encephalopathy, bovine immunodeficiency virus), and increased demand have generated significant interest in the development and utility of serum-free growth media.

Significant advances have been made in the development of serum substitutes and serum-free media that address the inadequacies of media containing serum (J. Tissue Cult. Meth., 14:45-50, 1992; Biochem. Pharmacol., 38:3723-3729, 1989; J. Tissue Cult. Meth., 12:13-16, 1989). Serum-free media provide many important advantages over serum-containing media, including lot-to-lot consistency, biological uniformity, and freedom from adventitious agents. Serum-free media are generally cost effective and low in protein content. All of these factors contribute to a more controlled examination of cellular, molecular and metabolic processes (ATCC Connection, 16(2):5-6, 1996).

Serum-free technology is not entirely problem free. Many serum-free media are highly specific to a particular cell type, are for short-term culturing only, or contain components extracted from serum. Very few available products can support primary cell cultures.

Ethanolamine has been reported to aid in the growth of hybridomas in serum-free medium in T-flasks (Murakami et al. (1982) PNAS 79:1158-1162). In addition, the use of ethanolamine in serum free medium has been reported (Glassy et al. (1988) Biotechnol. Bioeng. 32:1015-1028). In some cases, when a defined serum-free medium is used on the cell side of the membrane, the smaller components such as ethanolamine are included on both sides of the membrane (van Erp et al. (1991) Biotechnol. 20:235-248). In one case, a purified serum component, bovine serum albumin, was added to serum free medium containing ethanolamine to analyze antibody production in a hollow fiber system (Omasa et al. (1995) Biotechnol. Bioeng. 48:673-680). In one other case, ethanolamine (at 20 μM) was added to the basal medium on the non-cell side, while hybridoma cells were quickly weaned off serum on the cell side (Klerx et al. (1988) J. Immunol. Meth. 111:179-188). In one trial performed by Klerx et al., cells were maintained at 10% serum concentration of total media components for 7 days and then subsequently weaned to 0% of total media components over the course of the ensuing 8 days. In another trial performed by Klerx et al., cells were weaned from 10% to 2% serum concentration of total media components over the course of approximately two weeks. These studies did not demonstrate or identify the enhanced cell growth or cell viability achieved by the presence of ethanolamine on the non-cell side. Furthermore,
Klerx et al. conclude that antibody production is optimized when a serum free medium is used on the cell side.

Overall, the enhanced growth of cells in the presence of serum on the cell side and in the presence of ethanolamine without serum on the non-cell side has not been demonstrated. When using serum as a supplement on the cell side, the tendency has been to use a standard basal medium on the non-cell side. None of the standard basal medium such as Dulbecco’s Modified Eagle’s Medium (DMEM), Basal Media Eagle (BME), Minimum Essential Eagle’s Medium (MEM), Ham’s F12, RPMI, Iscove’s Modified Dulbecco’s Medium (IMDM), McCoy’s, or Waymouth’s contain ethanolamine.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides methods for enhancing cell growth in a membrane system having a cell side and a non-cell side, by culturing cells in the presence of serum at an initial concentration on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side has a medium containing ethanolamine, wherein the serum concentration on the cell side is maintained at greater than 20% (e.g., at least about 25-50%, 50-75% or 75-100%) of the initial concentration, and wherein the cell growth is enhanced compared to growth in the absence of ethanolamine. Examples of suitable membrane systems include hollow fiber systems, such as hollow fiber bioreactors.

In another aspect, the present invention provides methods for culturing cells in a membrane system having a cell side and a non-cell side, by culturing cells in the presence of serum at an initial concentration on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side comprises a medium containing ethanolamine, and wherein the serum concentration on the cell side is maintained at greater than 20% (e.g., at least about 25-50%, 50-75% or 75-100%) of the initial concentration.

In yet another aspect, the present invention provides methods for enhancing cell viability in a membrane system having a cell side and a non-cell side, by culturing cells in the presence of serum at an initial concentration on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side has a medium containing ethanolamine, wherein the serum concentration on the cell side is maintained at greater than 20% (e.g., at least about 25-50%, 50-75% or 75-100%) of the initial concentration.
and wherein cell viability is enhanced when cells are cultured in the presence of ethanolamine compared to when cells are cultured in the absence of ethanolamine.

In yet another aspect, the present invention provides methods for enhancing antibody production in a membrane system having a cell side and a non-cell side, by culturing antibody producing cells in the presence of serum on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side comprises a medium containing ethanolamine, and wherein antibody production is enhanced when cells are cultured in the presence of ethanolamine compared to antibody production when cells are cultured in the absence of ethanolamine.

In certain embodiments of the preceding aspects of the invention, the non-cell side medium contains less than about 100 mg/L ethanolamine. In another embodiment, the medium contains less than about 10 mg/L ethanolamine. The ethanolamine can be added to the non-cell side medium at these concentrations or can be added at higher concentrations and allowed to reduce to these levels.

In other embodiments of the invention, the non-cell side medium containing ethanolamine also contains basal medium. The basal medium may include Ham’s F-12. In addition, the basal medium may include Ham’s F-12 and a second basal medium selected from the group consisting of Dulbecco’s modified Eagle’s medium (DMEM), Essential modified Eagle’s medium (EMEM), RPMI-1640, and Iscove’s modified Dulbecco’s Medium (IMDM). The Ham’s F-12 and the second basal medium may be present in about a 1:1 ratio (v/v).

In other embodiments, the medium containing ethanolamine contains at least one supplement. The supplement may be, but is not limited to, an amino acid, glucose, glutamine, methionine, cystine, cysteine, tryptophan, sodium bicarbonate and/or combinations thereof. In a particular embodiment, the medium contains glucose (e.g., less than about 10 g/L), glutamine (e.g., less than about 5 g/L), methionine (e.g., less than about 100 mg/L), cystine (e.g., less than about 100 mg/L), tryptophan (e.g., less than about 100 mg/L) and sodium bicarbonate (e.g., less than about 10 g/L). In yet another embodiment, the medium also contains fats or insulin.

In one embodiment, the cells may be cultured in the presence of serum on both the cell side and the non-cell side (e.g., for less than 24 hours), prior to culturing the cells in the presence of serum on the cell side and in the absence of serum on the non-cell side.
Typically, the serum concentration is maintained between about 2-15% of the total media components on the cell side, more preferably, between about 8-12% of the total media components. In certain embodiments, the serum concentration is maintained at greater than 2% of the total media components for at least 7 days (e.g., at least 7-14 days, 14-28 days or more, or for the entire duration of the culturing step). In other embodiments, the serum concentration is maintained at about 50%, at least about 75%, at least about 90%, or at about 100% (i.e., without weaning) of the initial serum concentration. In further embodiments the serum concentration on the cell side is maintained at at least about 25% of the initial serum concentration for at least about 14-28 days or more, or for the entire duration of the culturing step.

In other embodiments, the cultured cells may be hybridoma or heterohybridoma cells (e.g., human, sheep or rodent hybridomas or heterohybridomas, such as rat, murine, rabbit, and hamster hybridomas or heterohybridomas). In a particular embodiment, the cells may be Chinese hamster ovary (CHO), NS/O, SP2/O or murine hybridomas or heterohybridomas.

Methods of the present invention serve to enhance cell viability and growth in the absence of serum on the non-cell side of a membrane system. For example, where the cultured cells (e.g., hybridomas) produce an antibody (e.g., idiotype), the methods of the invention serve to enhance cell growth and, thus, antibody production when cells are cultured in the presence of ethanolamine compared to when cells are cultured in the absence of ethanolamine. In various embodiments, antibody production is enhanced by at least about 20% or by at least about 50%.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1** depicts the effect of bioreactor extracapillary space (EC) medium supplements on cell growth in a micro bioreactor. Rho 1D4 cells were inoculated in 10% fetal bovine serum-supplemented medium at 5x10⁶/ml in the bioreactor intracapillary space (IC) (0.2 ml culture space), and were harvested three days later. The EC reservoir (non cell side) contained 16 ml basal medium (Basal), or basal medium supplemented with 10% fetal bovine serum (FBS), 10% newborn calf serum (NCS), 10% adult bovine serum (ABS) or a serum-free medium supplement SITE+3 (SITE+3). Data shown are the averages and standard deviations of duplicate bioreactors.
**Figure 2** depicts the effect of removing components of the serum-free medium supplement. Rho 1D4 cells were inoculated in 10% FBS-supplemented medium at 5x10^6/ml in the bioreactor IC (0.2 ml culture space), and were harvested three days later. The EC (non-cell side) reservoir contained 16 ml basal medium (Basal), the basal medium plus the complete SITE+3 supplement (All), or the basal medium with the SITE+3 supplement minus insulin (-I), transferrin (-T), selenium (-S), ethanolamine (-E) or the BSA/fatty acid complex (-BF). Data shown are the averages and standard deviations of duplicate bioreactors.

**Figure 3** depicts the effect of ethanolamine, bovine serum albumin (BSA), and fatty acid addition to medium. Rho 1D4 cells were inoculated in 10% FBS-supplemented medium at 5x10^6/ml in the bioreactor IC (0.2 ml culture space), and were harvested three days later. The EC reservoir (non-cell side medium) contained 16 ml basal medium (Basal), or basal medium supplemented with BSA (B), the BSA/fatty acid complex (BF), ethanolamine (E), BSA with ethanolamine (BE), or the BSA/fatty acid complex with ethanolamine (BFE). Data shown are the averages and standard deviations of duplicate bioreactors.

**Figure 4** depicts the effect of various supplements in a 5-ml micro-bioreactor. Rho 1D4 cells were inoculated in 10% FBS-supplemented medium at 5x10^6/ml in the bioreactor IC (0.2 ml culture space), and were harvested three days later. The EC reservoir (non-cell side medium) contained 5 ml basal medium (Basal), or basal medium supplemented 10% FBS (FBS), ethanolamine (E), or the BSA/fatty acid complex with ethanolamine (BFE). Data shown are the averages and standard deviations of duplicate bioreactors.

**Figure 5** depicts the T-flask growth curve of rho 1D4 cells. Cells were grown in 10% FBS-supplemented medium with (closed circles) or without (open circles) 4 mg/L ethanolamine. Data shown are the averages and standard deviations of triplicate T-flasks.

**Figure 6** depicts the effect of different media on cell growth in the ResCu Primer-HF. Rho 1D4 cells were inoculated in the EC space of three bioreactors at 5x10^6/ml with 10% FBS. The IC medium reservoir contained either basal medium, basal medium supplemented with 10% FBS, or basal medium supplemented with 4 mg/L ethanolamine. GUR is short for glucose uptake rate.
**Figure 7** depicts the effect of different media on cell growth in the AcuSyst-Maximizer. Rho 1D4 cells were inoculated in the EC space of two bioreactors at 5x10^6/ml with 10% FBS. The IC medium contained basal medium with (closed circles) or without (open circles) ethanolamine. The abbreviations are as follows: glucose uptake rate (GUR), lactate production rate (LPR), and oxygen uptake rate (OUR).

**Figure 8** depicts the growth of three other murine hybridomas in the micro-bioreactor. Cells were inoculated in 10% FBS-supplemented medium at 5x10^6/ml in the bioreactor IC (0.2 ml culture space), and were harvested three days later. The EC reservoir contained 5 ml basal medium (Basal), basal medium supplemented with 10% FBS (FBS), or basal medium supplemented with 4 mg/L ethanolamine (Basal+E). Data shown are the averages and standard deviations of duplicate bioreactors.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is based, in part, on the discovery that cell growth and cell viability in a membrane system, such as a hollow fiber system, can be significantly enhanced using a cell culture medium containing ethanolamine, even when serum is present on the cell side. In view of the well known fact that serum alone can fully support cell growth, the discovery that adding ethanolamine significantly increases cell growth and viability was unexpected.

In particular, by way of the present invention, it was found that when including ethanolamine in a serum free medium on the non-cell side of a membrane system, with serum on the cell side maintained at greater than 20% of its initial concentration, unexpected improvements in cell growth and viability are achieved. Therefore, the methods of the present invention provide the advantage of minimizing the use of expensive serum components on both sides of the membrane and reducing the risks, such as exposure to contaminants, extraneous factors and infectious agents, that come with excessive use of serum. Minimizing or, alternatively, eliminating entirely the use of serum on the non-cell side of a membrane system in accordance with the methods disclosed herein is particularly advantageous in that media components on the non-cell side of membrane systems are known to be consumed at a far greater rate than components on the cell side.
Ethanolamine is an important element for optimal cell growth. Without wishing to be bound to any particular theory, cell growth requires the synthesis of various cellular components, including, in particular, phospholipids. The phosphate group of a phospholipid is capped with either ethanolamine, choline, serine, glycerol and inositol. Choline, serine and inositol are generally present in standard basal media. In addition, cells readily synthesize sufficient glycerol to support cell growth. However, while cells can synthesize ethanolamine, cells generally cannot synthesize sufficient ethanolamine to support optimal cell growth. Accordingly, the addition of ethanolamine to standard basal media is beneficial for cell growth.

**DEFINITIONS**

As used herein, the following terms shall have the definitions referred to below.

The term "cell side," as used herein, refers to the region or side of the membrane system on which cells are cultured. Conversely, "non-cell side," as used herein, refers to the region or side on which cells are not cultured. Generally, the cell side will be separated from the non-cell side by a membrane. For example, in a hollow fiber system characterized by hollow fibers that traverse through the system, the cell side and non-cell side regions are defined by the hollow fibers themselves. In such cases, cells are usually cultured in the extracapillary (EC) space outside of the hollow fibers. Accordingly, the EC space will be considered the cell side while the intracapillary (IC) space will be considered the non-cell side. However, in certain embodiments, when cells are cultured in the IC space, the IC space will be considered the cell side while the EC space will be considered the non-cell side.

The term "serum," as used herein, includes sera derived from both human and animal sources. Suitable sera include, but are not limited to, fetal bovine serum (FBS) (also known as fetal calf serum (FCS)), newborn calf serum (NCS), adult bovine serum (ABS), bovine calf serum (BCS), horse serum (HS), human, chicken, goat, porcine, rabbit, bovine, fetal, calf and sheep sera. Serum can be obtained from a variety of commercial sources such as Sigma Aldrich Co. (St. Louis, MO). Serum may be used at any concentration easily determinable by one skilled in the art so as to optimize culturing conditions. In various embodiments, the concentration of serum may be less than about 50, 45, 40, 35, 30, 25, 20, 15, 10, 5, 4, 3, 2, or 1%. In a particular embodiment, the concentration of serum may be between about 5% and about 25% (for example, at about
5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25%). In a particular embodiment the concentration of serum is about 10%. The concentrations of serum as used on the cell side and the non-cell side may differ. In addition, the concentrations of serum as used on a particular side may differ over time.

The term "basal medium," as used herein, refers to a medium containing several art-recognized (non-serum derived) nutrients for culturing cells and supporting cell growth. The basal medium may support cell growth on its own or, in some instances, may require additional components, including serum or serum-derived components, to support cell growth. The basal medium may be any of a variety of basal media known in the art, including, but not limited to, Iscove's Modified Dulbecco's Medium (IMDM), Dulbecco's Modified Eagle's Medium (DMEM), Essential Modified Eagle's Medium (EMEM), Minimal Essential Medium Eagle (MEM), Basal Medium Eagle (BME), Click's Medium, L-15 Medium Leibovitz, McCoy's 5A Medium, Glasgow Minimum Essential Medium (GMEM), NCTC 109 Medium, Williams' Medium E, RPMI-1640, and Medium 199. A medium specifically developed for a particular cell type/line or cell function, e.g., Madin-Darby Bovine Kidney Growth Medium, Madin-Darby Bovine Kidney Maintenance Medium, various hybridoma media, Endothelial Basal Medium, Fibroblast Basal Medium, Keratinocyte Basal Medium, and Melanocyte Basal Medium may also be utilized (all available from Sigma Aldrich Co.; St. Louis, MO).

The term "presence of serum," as used herein, refers to the presence (e.g., cell side or non-cell side) of a sufficient number of components derived from serum that together are able to support cell growth and/or cell viability. Thus, the term "presence of serum" does not exclude the presence of some serum components (e.g., that are able to traverse the membrane and diffuse from the cell side to the non-cell side). However, these components are not alone sufficient to support cell growth. The term "absence of serum," as used herein, refers to the absence of a sufficient number of components derived from serum that together are able to support cell growth and/or cell viability.

The term "ethanolamine," as used herein, refers to the chemical well-known in the art. Ethanolamine is commonly referred to as 2-aminoethanol, monoethanolamine, beta-aminoethanol, beta-aminoethyl alcohol, ethylolamine, glycinol, oamine, MEA, UASF EK-1597, colamine, beta-hydroxyethylamine, all of which are encompassed by the term "ethanolamine." Ethanolamine has the molecular formula, C₂H₄NO, and the structural formula, H₂N(CH₂)₂OH. Ethanolamine is available from numerous
commercial suppliers, including Moravek Biochemicals (Brea, CA). Ethanolamine, as used herein, is also intended to encompass other known forms of ethanolamine including, but not limited to, phosphoethanolamine and phosphatidyl ethanolamine.

The term “cell viability,” as used herein, refers generally to the lifetime and physical condition of cultured cells.

The term “cell growth,” as used herein, refers to the division and proliferation of cultured cells.

The term “hybridoma,” as used herein, refers to a B cell obtained from an animal, e.g., a human or rodent, fused to an immortalized cell. Hybridomas are often used for the production of monoclonal antibodies.

**Membrane Systems**

Membrane systems provide an efficient and effective means for culturing cells, often for the subsequent production of proteins. In particular, membrane systems are ideal for culturing hybridomas for the production of antibodies. Membrane systems provide improved cell retention and high density cell growth. The term “membrane system,” as used herein, refers to a cell culture system that has a growth region (e.g., compartment) separated, by a membrane, from a medium region (e.g., compartment). The membrane system often has a permeable or semi-permeable membrane (e.g., hollow fibers or capillaries) that allows for the bi-directional diffusion of gases, ions and small molecules for optimal cell culturing conditions. Generally, the membranes have a molecular weight cut-off (usually less than 100 kD, and most often in the range of 10 to 30 kD) designed so as to, in part, allow smaller components to pass through, while retaining growth factors and product (e.g., antibodies secreted by the cultured cells).

Hollow fiber systems (e.g., hollow fiber bioreactors such as hollow fiber micro-bioreactors or large scale batch hollow fiber bioreactors) are examples of membrane systems. Perfused tanks and flask systems (e.g., CL-1000 from Integra Biosciences, Chur, Switzerland) are other examples of membrane systems.

Generally, hollow fiber systems have a plurality of semi-permeable hollow fibers potted in a housing to create a space inside the fibers (intracapillary or IC space) separate from that outside the fibers (extracapillary or EC space). Communication between the IC and the EC space is entirely through diffusion through fiber pores. Cells are usually placed on the EC side of the fiber, but, in other embodiments, cells may be placed on the
IC side. Cells are retained better when grown in the EC space. Additionally, when cells are cultured for the purpose of collecting a secreted product (e.g., antibodies), growing the cells in EC space is preferred. However, if cells are cultured with the intention of ultimately recovering the cells, growing the cells in IC space is preferred.

**NON-CELL SIDE MEDIUM**

Medium circulating on the non-cell side whether in the EC space or the IC space, serves as the primary nutrition source for the cells. The non-cell side medium may be circulated at a fast rate so as to provide sufficient oxygen, which diffuses through the porous fibers into the cell mass. Conversely, carbon dioxide created from cell respiration and acid neutralization diffuses from the cell mass, across the fibers and ultimately is carried away in the non-cell side medium. In addition to providing oxygen, the non-cell side medium provides the bulk of nutrition for components small enough to pass through the fibers.

In various embodiments of the invention, the non-cell side of the membrane system contains a medium that lacks serum, but includes ethanolamine. Ethanolamine is added or included in the serum-free medium in an amount necessary to support cell growth, as can be determined by a skilled practitioner in light of various factors including, but not limited to, culturing conditions, the nature of the cell being cultured, and the nature of the medium components. In one embodiment, the serum-free medium contains less than about 100 mg/L ethanolamine. In another embodiment, the medium contains less than about 10 mg/L, less than about 5 mg/L, less than about 2 mg/L, less than about 1 mg/L, or less than about .5 mg/L ethanolamine. The ethanolamine can be added and maintained at these concentrations or added at greater concentrations and allowed to be reduced by consumption down to these concentrations.

According to the invention, ethanolamine uniquely maintains and enhances cell growth in a non-cell side environment that lacks sufficient serum components to support cell growth. In certain embodiments, however, the cells may first be cultured in the presence of serum on both the cell side and non-cell side prior to culturing the cells in the absence of serum on the non-cell side. By doing so, the serum on the non-cell side can become adsorbed to, for example, the silicone surfaces used in tubing and oxygenation to optimize the initial cell culture conditions. For example, without wishing to be bound to any particular theory, the silicone surfaces often used in membrane
systems are hydrophobic and interact with hydrophobic fats (Gramer et al. (1998) *Biotechnol. Prog.* 14:203-209). The silicone surfaces on the non-cell side attract fats away from the serum on the cell side and deplete the cell side serum of necessary fats. However, when the non-cell side is initially exposed to serum, the serum coats the silicone surfaces and blocks the surface with fats. Upon further culturing, additional fats will not be drawn to the non-cell side and will remain on the cell side, thereby enhancing the culturing conditions. Thus, utilizing medium containing serum on the non-cell side for a limited time is often advantageous. Following this initial period, the serum on the cell side is sufficient to support serum feeding. Furthermore, continued use of serum beyond this initial period is undesirable in light of the unpredictability (in terms of its components), risks (potential contaminants), and costs associated with serum use. In various embodiments, the cells are cultured in the presence of serum on the non-cell side for less than 120 hours, 96 hours, 72 hours, 48 hours or 24 hours. In certain embodiments, serum is added to the non-cell side at times less than 120 hours, 96 hours, 72 hours, 48 hours or 24 hours after inoculation. In a particular embodiment, serum is added on the day of inoculation. In yet another embodiment, serum is added only on the day of inoculation. According to the invention, the serum may be added during any of the initial days of culturing (e.g., only during the day of inoculation) and subsequently allowed to gradually dilute out of the non-cell side.

In various embodiments, the non-cell side medium contains basal medium including, but not limited to, any of the basal media described above. In a particular embodiment, the combination of Ham’s F-12 and DMEM in a 1:1 ratio (D/F12) is used. D/F12 is commercially available; for example, a modified form of D/F12 containing trace elements and pyruvate is available from Sigma Aldrich Co. as Product No. D0547. In particular embodiments, where trace elements and pyruvate are not present in the basal medium, such components may be added as necessary. Other combinations of basal media may be used in the practice of the present invention. Preferably, the combinations include Ham’s F-12, regardless of the identity of the other basal medium component, preferably in a 1:1 ratio. Without wishing to be bound to any particular theory, Ham’s F-12 contains important components for cell growth not necessarily provided for by the other basal media. For example, it is believed that particular components of Ham’s F-12 can serve as substitutes for particular low molecular weight serum components.
In another embodiment of the invention, the non-cell side medium includes at least one supplement. The term “supplement,” as used herein, refers to additional components that supplement other media components so as to promote cell growth. Suitable cell culture supplements are well known in the art and include, for example, glucose, sodium bicarbonate, and amino acids such as glutamine, methionine, cystine, cysteine and tryptophan. Each or a combination of supplements are added as necessary. As is well known in the art, factors to be considered in determining the nature and the concentration of supplements to be added include culturing conditions, the nature of the cell being cultured, and the nature of the basal medium. For example, a skilled practitioner must account for the various supplements, such as glucose and glutamine, already present in certain commercially available basal media formulations (e.g., D/F12 available from Sigma Aldrich). A balanced medium may require additional supplements.

In a particular embodiment, the non-cell side medium includes less than 10 g/L glucose, less than about 5 g/L glutamine, less than about 100 mg/L methionine, less than about 100 mg/L cystine, less than about 100 mg/L tryptophan, and/or less than about 10 g/L sodium bicarbonate.

In another embodiment, the non-cell side medium is as described below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/F12 (DMEM and Ham’s F-12 in a 1:1 (v/v)) (Product No. D0547 available from Sigma Aldrich Co.)</td>
<td>N/A</td>
</tr>
<tr>
<td>Glucose</td>
<td>1 g/L</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>3.4 g/L</td>
</tr>
<tr>
<td>Glutamine</td>
<td>.51 g/L</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>4 mg/L</td>
</tr>
<tr>
<td>Methionine</td>
<td>7.3 mg/L</td>
</tr>
<tr>
<td>Cystine</td>
<td>9.4 mg/L</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>8.16 mg/L</td>
</tr>
</tbody>
</table>

Additionally, the non-cell side medium may contain fats or insulin as necessary to support and enhance cell growth. Without wishing to be bound to any particular theory, the hydrophobic components of fats and insulin aid in cell growth in a membrane system, in part, by becoming adsorbed to the silicone surface area used in tubing and for oxygenation on the non-cell side. As described above, by coating the non-cell side
silicone surfaces with fats and insulin, fats will remain on the cell side associated with serum as necessary for optimal cell growth.

The non-cell side medium can be supplemented with a variety of growth factors, cytokines, serum, etc., depending on the cells being cultured. Examples of suitable growth factors include: basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), transforming growth factors (TGFα and TGFβ), platelet derived growth factors (PDGFs), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), insulin, erythropoietin (EPO), and colony stimulating factor (CSF). Examples of suitable hormone additives are estrogen, progesterone, testosterone or glucocorticoids such as dexamethasone. Examples of cytokine medium additives are interferons, interleukins or tumor necrosis factor α (TNFα). Salt solutions may also be added to the media, including Alsever's Solution, Dulbecco's Phosphate Buffered Saline (DPBS), Earle's Balanced Salt Solution, Gey's Balanced Salt Solution (GBSS), Hanks' Balanced Salt Solution (HBSS), Puck's Saline A, and Tyrode's Salt Solution.

**CELL SIDE MEDIUM**

According to the present invention, cells are typically cultured on the cell side in the presence of serum. Any suitable animal derived serum may be used, as is well known in the art. Suitable sera include, but are not limited to, fetal bovine serum (FBS) (also known as fetal calf serum (FCS)), newborn calf serum (NCS), adult bovine serum (ABS), bovine calf serum (BCS), horse serum (HS), human, chicken, goat, porcine, rabbit and sheep sera. Serum replacements may also be used, such as controlled process serum replacement-type (CPSR; 1 or 3) or bovine embryonic fluid. Specific purified growth factors or cocktails of multiple growth factors can also be added. Specific factors or hormones that promote proliferation or cell survival can also be used. In a particular embodiment, the cell side also contains ethanolamine. In other embodiments, the cell side also contains basal medium. Other medium components including, but not limited to, those listed above (e.g., fats, insulin, supplements, etc.) may be added as necessary. The selection of the cell side media and culture conditions (including temperature, gas exposure, etc.) varies depending, in part, on cell type and may be empirically determined by one skilled in the art.

In one embodiment, the serum level is maintained at more than about 20% of its
initial concentration. As used herein, the terms “maintain” and “maintained” refer to sustaining serum concentration above or at a particular concentration. In various embodiments, the serum level is maintained at about 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99 or 100% of the initial concentration. In other embodiments, the serum is maintained at greater than 2% of the total media components, for example, between about 2-15%, preferably between about 8-12%, of the total media components. The serum level is preferably maintained at these levels for at least 7-14 days (e.g., 15, 16, 17, 18, 19, 20, 21, 22 or 28 days) or the entire duration of the culturing step.

Based on the present discovery that the growth and/or viability of cells are enhanced by culturing cells in a membrane system that includes, on the non-cell side, ethanolamine but not a sufficient number of serum components to support cell growth, the methods of the invention are particularly suited for culturing a wide variety of cells including, in particular, cells that produce antibodies (e.g., monoclonal antibodies).

Membrane systems, such as hollow fiber bioreactors, are particularly suited for the mass production of antibodies by culturing hybridomas or heterohybridomas. Suitable hybridomas include, but are not limited to, rodent hybridomas (such as murine, rat, hamster and rabbit), sheep hybridomas and human hybridomas. In a particular embodiment, the hybridoma cells are Chinese hamster ovary (CHO) hybridoma cells, NS/O hybridomas, SP2/O hybridomas, or murine hybridoma cells.

The culturing techniques of the present invention serve to enhance antibody (e.g., idiotype) production. Without wishing to be bound to a particular theory, the enhanced growth and/or enhanced viability of the cells results in enhanced antibody production. In various embodiments of the present invention, antibody production is enhanced by at least about 200%, 150%, 100%, 75%, 50%, 40%, 30%, 20%, 10% or 5%.

The following examples are expected to be illustrative of the invention and in no way limit the scope of the invention:
EXEMPLARYIFICATION

Example 1: Use of Ethanolamine in Culturing Murine Hybridomas in Hollow Fiber Bioreactors

In this study, a 10 kD MWCO hollow-fiber micro bioreactor was used to determine if a less expensive form of serum other than 10% fetal bovine serum (FBS) or a more defined supplement could be added to the medium reservoir (non-cell side) of the bioreactor to optimize growth of a murine hybridoma. Neither 10% newborn calf serum nor 10% adult bovine serum served as a replacement for 10% FBS in the medium reservoir. However, cell growth was comparable when either 10% FBS or a common serum-free medium supplement containing selenium, insulin, transferrin, ethanolamine, and bovine serum albumin (BSA) with oleic and linoleic acid was added to the medium reservoir. A series of screening experiments determined that the only important components of this serum-free medium supplement were ethanolamine and the BSA/fatty acid complex. As the ratio of the medium reservoir to cell side volume decreased, only ethanolamine was required to replace FBS in the medium reservoir. Contrary to the micro bioreactor studies, ethanolamine had no effect on the growth of cells in 10% FBS in a T-flask. Experiments in large scale hollow-fiber systems demonstrated enhanced growth and a 50% increase in antibody productivity when ethanolamine was added to the basal medium. Results for three additional murine hybridoma cell lines in micro bioreactors paralleled those of the first cell line tested; cell growth was greatly enhanced by the addition of ethanolamine or 10% FBS to the medium reservoir, and growth with these two supplements was comparable. These results suggest a general requirement for ethanolamine for optimal growth of murine hybridomas in hollow-fiber systems.

In a previous study, the micro hollow-fiber bioreactor was used to demonstrate that components in serum that can pass through a 10 kD membrane are important during the initial growth phase of the rho 1D4 murine hybridoma cell line (Gramer et al. (1998) Biotechnol. Prog. 14:203-209). Optimal growth was attained when 10% fetal bovine serum (FBS) was used in both the IC and EC compartments. For economic reasons, serum is usually added only to the cell side of the bioreactor which reduces serum usage by a factor of about 100. In this study, the hollow-fiber micro bioreactor was used to determine if a less expensive form of serum or a more defined supplement could be
added to the non-cell side of the bioreactor. The results are generalized by examining a total of four different murine hybridoma cell lines.

**MATERIALS AND METHODS**

**Media**

Basal medium (Sigma Chemical Company) consisted of DME/F12 with trace elements and 2.5 mM L-glutamine, and was additionally supplemented with 1.5 mM L-glutamine, 2.44 g/L sodium bicarbonate, 0.066 g/L penicillin G (Irvine Scientific), and 0.144 g/L streptomycin sulfate (Irvine Scientific). Serum-supplemented media were prepared by adding fetal bovine serum (FBS), newborn calf serum (NCS) or adult bovine serum (ABS) (all from Sigma) to basal medium. The serum-free medium supplement SITE+3 from Sigma was used as a 1:50 dilution to give final concentrations of 10 μg/L selenium, 20 mg/L insulin (bovine pancreas), 11 mg/L transferrin (human), 4 mg/L ethanolamine, 1 g/L bovine serum albumin (BSA), 9.4 mg/L linoleic acid, and 9.4 mg/L oleic acid. These components were also purchased individually from Sigma for the screening experiments, but were always used at the concentrations listed here.

**Cells**

The four murine hybridoma cell lines including rho 1D4 (Molday et al. (1983) *Biochemistry* 22:653-660), MH370, MH372, and MH373 were obtained from the National Cell Culture Center (Minneapolis, MN). Cells were routinely propagated in 10% FBS-supplemented medium at 37°C in a humidified incubator with 5% CO₂. Cells were passaged every 1 to 3 days.

**Assays**

The viable and total cell concentrations were determined with a hemacytometer using trypan blue. Glucose and lactate concentrations were measured with a YSI 2700 Select Bioanalyzer (Yellow Springs Instruments). Antibody concentrations were determined by sandwich ELISA with a polyclonal goat anti-mouse IgG antibody (heavy and light chain) and polyclonal goat anti-mouse IgG antibody labeled with peroxidase (Sigma); color was developed with ABTS. Dissolved oxygen concentration and pH were measured using an AVL 990 blood-gas analyzer (AVL Scientific).
Hollow-Fiber Micro Bioreactor

The micro bioreactors were constructed and used as previously described (Gramer, 1998). Briefly, the bioreactor fibers were made of regenerated cellulose (cuprophane) with a 10 kD MWCO. The bioreactors were flushed before use to remove the wetting agent in the fibers and residuals from the sterilization process. The IC (0.2 ml) was then primed with 10% FBS-supplemented medium, and the EC (5 or 16 ml depending on the diameter of silicone tubing used) was rinsed and filled with basal medium plus other components as described in the Results section. Cells at about 5x10^5/ml were pelleted by centrifugation at 200xg, resuspended in fresh 10% FBS-supplemented medium at 5x10^6/ml, inoculated in the IC compartment of the micro bioreactor, and placed in an incubator at 37°C with 5% CO₂. Cells were harvested 3 days later by injecting air in one IC port with a 1-ml syringe while collecting the effluent from the opposite port with another 1-ml syringe.

T-Flask Growth Curve

Cells (rho 1D-4) were cultured for one week in 10% FBS-supplemented basal medium which contained an additional 1 g/L glucose and 1 g/L sodium bicarbonate with or without 4 mg/L ethanolamine. Cells at about 5x10^5/ml and 95% viability were pelleted at 200xg and resuspended in the same fresh medium at about 5x10^4/ml. Cells (5 ml) in each of the two media were inoculated into thirty T-25 flasks. Three flasks were harvested each day for cell counts and antibody determination until the viability dropped below 25%.

Large-Scale Batch Hollow-Fiber Bioreactor

A simple hollow-fiber system (ResCu-Primer HF, Cellex Biosciences) was operated as previously described (Gramer, 1998). Briefly, the bioreactor fibers were 10 kD MWCO Hemophan which is similar to Cuprophan, except that a portion of the glucose polymer free hydroxyls are substituted with a DEAE group which adds a positive charge to the fiber. The total surface area based on fiber ID was 2200 cm^2 and the EC volume was 85 ml. The hollow-fiber system was housed in a CO₂ incubator for temperature and gassing control. Cells (2x10^8) were inoculated into the EC
compartment in 10% FBS-supplemented medium. Basal medium supplemented with 4 mg/L ethanolamine was circulated from the IC medium reservoir, through the oxygenator, through the fiber IC compartment, and was returned to the bottle. The EC medium was changed 3 times per week by adding 20 ml of 10% FBS-supplemented medium through the bottom port while harvesting 20 ml through the top port. The IC medium bottles were changed daily to keep the glucose concentration above 1.5 g/L. The IC reservoir initially contained 200 ml of medium, and this volume was increased up to 2 L to support the glucose demand, after which the medium was supplemented with an additional 1 g/L glucose and 1 g/L sodium bicarbonate. The results from control bioreactors (basal medium or 10% FBS-supplemented medium in the IC) were generated previously (Gramer, 1998).

Large-Scale Continuous Hollow-Fiber Bioreactor

An automated hollow-fiber system was used (AcuSyst-Maximizer, Cellex Biosciences). The bioreactor fibers were made of cellulose acetate with a 10 kD MWCO. The total surface area based on fiber ID was 1.1 m² and the EC volume was about 100 ml. Cells (5x10⁸) were inoculated simultaneously into the EC compartment of two instruments in 10% FBS-supplemented medium. Basal medium was circulated in the IC which contained an additional 1 g/L glucose and 1 g/L sodium bicarbonate; in one instrument, this medium also contained 4 mg/L ethanolamine. The pH was controlled at pH 7.2 and the temperature was controlled at 37°C. Fresh basal medium was continually added to and removed from the IC medium reservoir. The rate of IC medium addition was increased daily from 25 ml/hr up to 400 ml/hr based on off-line glucose analyses to keep the glucose above 2 g/L. EC cycling was initiated on day 3 (70 ml transfer volume in 15 minutes). On day 4, 10% FBS-supplemented medium was added to the EC circuit at a rate of 1 ml/hr; this rate was increased at about 1:100 of the rate of IC medium addition up to 4 ml/hr. The rate of antibody harvest from the EC compartment matched the rate of EC medium addition. Cells were removed from the EC chamber 2-3 times per week, and the medium harvested from this procedure was pooled with the regular harvest.
**RESULTS**

**Alternatives to Supplementation of FBS in the Reservoir**

The rho 1D4 murine hybridoma cell line was inoculated at 5x10^6/ml with 10% fetal bovine serum (FBS) into the IC compartment (0.2 ml volume) of a series of micro bioreactors. The EC reservoir contained either 16 ml of basal medium or 16 ml of basal medium supplemented with 10% FBS, 10% newborn calf serum (NCS), 10% adult bovine serum (ABS), or a serum-free medium supplement SITE+3. Cells were harvested and counted after three days of growth in an incubator. As expected, growth was poor in the bioreactor containing only basal medium in the EC reservoir, while growth was very good in the bioreactor with 10% FBS in the EC reservoir (Fig. 1). Somewhat surprisingly, neither NCS nor ABS substituted for FBS in the EC reservoir, while growth in bioreactors containing the SITE+3 supplement in the EC reservoir was similar to that with FBS.

**Effect of Removing Components from the SITE+3 Supplement**

The finding of a defined supplement to replace FBS in the reservoir of the bioreactor was fortunate, but the supplement cost was still quite high. The SITE+3 supplement contains insulin, transferrin, selenium, ethanolamine, and BSA with oleic and linoleic acid. Of these components, selenium (in the trace elements) and a small amount of linoleic acid are already found in the basal medium. Selenium, ethanolamine, insulin, and the fatty acids (the equilibrium portions not bound by BSA) are small enough to pass through the 10 kD hollow-fiber membrane. Transferrin and BSA cannot pass through the membrane, but these proteins are buffers of potentially toxic metals and hydrophobic substances, respectively (Glassy et al., 1988). To determine which of these components are most critical to cell growth, each component was selectively removed from the EC supplement, and the micro bioreactor experiment was repeated. As expected, cell growth was poor in the basal EC medium control bioreactor and very good in the bioreactor containing the complete SITE+3 medium supplement in the EC reservoir (Fig. 2). Cell growth was not reduced by removing insulin, transferrin, or selenium from the SITE+3 supplement. However, cell growth was greatly suppressed by the removal of ethanolamine or the BSA/fatty acid complex.
Effect of Ethanolamine, BSA, and Fatty Acid Addition

The above experiment suggested that ethanolamine and BSA with bound fatty acids are the only components in the serum-free EC medium supplement required by the cells. To confirm this finding, these components were added to basal EC medium in a stepwise fashion, and the experiment was repeated. As expected, cell growth with the basal EC medium control was poor (Fig. 3). Addition of just BSA or BSA with the fatty acids to the basal EC medium resulted in slightly better cell growth compared to the control. Addition of just ethanolamine to the basal EC medium resulted in good cell growth, while the combination of ethanolamine and BSA was only slightly better than ethanolamine alone. However, the combination of ethanolamine and BSA with fatty acids in the basal EC medium resulted in very good cell growth, similar to that seen in Figs. 1 and 2 for the complete medium supplement. Fatty acid-free BSA was used in this experiment which is still relatively expensive. An additional experiment with crude BSA demonstrated results essentially identical to that shown in Fig. 3 (not shown). This was somewhat surprising since crude BSA was expected to already carry fatty acids.

Supplementation of basal EC medium with ethanolamine and the fatty acids without BSA resulted in essentially complete cell death, presumably due to the toxicity of the free fatty acids (not shown).

Effect of Micro Bioreactor EC Volume

A previous study (Gramer, 1998) demonstrated that cell growth was dependent on the size of the EC medium reservoir in the micro bioreactor; with basal EC medium, a 5-ml EC volume bioreactor supported better cell growth than a 16-ml EC volume bioreactor, presumably due to the lesser degree of dilution in the smaller bioreactor (the serum-containing IC cell compartment in both bioreactors was 0.2 ml). This phenomenon was further explored with the defined medium components identified in this study. As expected, when the EC contained only basal medium, growth was better in the 5-ml bioreactor compared to the 16-ml bioreactor, while growth was similar in the 5-ml and 16-ml bioreactors when the EC medium contained FBS (compare Fig. 4 and Fig. 1). Somewhat surprisingly, ethanolamine alone was nearly sufficient as a substitute for FBS in the EC medium of the 5-ml bioreactor (Fig. 4). The addition of BSA with the fatty acids to the EC medium of the 5-ml bioreactor resulted in only a marginal increase in cell growth (Fig 4).
Ethanalamine in T-Flasks

Cells were grown in 10% FBS-supplemented medium in a T-flask with or without 4 mg/L ethanalamine. There was very little difference in the viable cell density (Fig. 5), total cell density (not shown), and the antibody concentration (Fig. 5) over the 6-day growth period. The discrepancy between the T-flask and hollow-fiber bioreactor data suggests that T-flasks should not be used to optimize the medium formulation for cells grown in a hollow-fiber system.

Scale-Up

Dialysis experiments using the micro bioreactor in a previous study demonstrated that components in serum essential for optimal cell growth can cross the 10 kD membrane; at least one hydrophilic component remained soluble in the medium while at least one hydrophobic component was adsorbed to the silicone tubing in the micro bioreactor (Gramer, 1998). The results presented here do not necessarily identify those components, but it is interesting to note the one soluble component (ethanalamine) and one hydrophobic component (fatty acids bound by BSA) can serve as a substitute for serum on the non cell side of the hollow-fiber bioreactor. In results presented here, reducing the size of the EC medium reservoir did not reduce the need for ethanalamine, but the need for fatty acids was reduced. The reduction of the EC medium reservoir also results in a reduction of the silicone surface area. Taken together, these data suggest that the ratios of cell side volume to reservoir volume and silicone surface may be important scale-up parameters.

Two types of production-scale hollow-fiber systems were used to test the preceding hypothesis. The first was a simple hollow-fiber system (ResCu-Primer HF) placed in an incubator that is operated through batch changes of IC and EC media. The second was an automated system (AcuSyst-Maximizer) that provides automatic temperature and pH control with continuous IC and EC media addition and removal. The ratios of initial non cell side volumes and silicone surface areas to cell side volumes are compared in Table 1 to the same ratios for the micro bioreactors (note that the cells are placed in the IC in micro bioreactors and in the EC in the large hollow-fiber bioreactors). Based on the results in this paper, the two larger production systems should not require the BSA/lipid supplement on the non-cell side of the membrane since the
ratio of silicone to cell side volume is smaller than that for the 5-ml bioreactor. However, the two production scale bioreactors could still potentially benefit from the ethanolamine supplement.

Results for three simple batch operated production systems are shown in Figure 6. The cell side of the bioreactors (EC compartment) contained basal medium supplemented with 10% FBS, while the reservoir side (IC compartment) contained either basal medium, basal medium supplemented with ethanolamine, or basal medium supplemented with 10% FBS. As shown in Figure 6, cell growth was very poor with only basal medium in the reservoir. However the addition of either ethanolamine or FBS to the medium reservoir resulted in good cell growth (as estimated by the glucose uptake rate), and growth with the two supplements was essentially identical.

The metabolic activity of cells grown in the automated systems was more extensively characterized (Fig 7). Cells were inoculated on the EC side in basal medium supplemented with 10% FBS. The IC (non-cell) side contained basal medium with or without 4 mg/L ethanolamine. In this case, the cells grew fairly well without the addition of ethanolamine to the IC medium reservoir. However, the metabolic activity increased at a much faster rate when ethanolamine was added to the basal medium. At steady state, the two cultures consumed medium at the same rate and displayed similar glucose uptake rates and lactate production rates. However, the oxygen uptake rate and antibody production rate were about 70% and 50% higher respectively for the bioreactor with ethanolamine relative to the bioreactor without ethanolamine.

Other Murine Hybridoma Cell Lines

Optimal growth of the rho 1D4 murine hybridoma cell line in a hollow-fiber system required the addition of ethanolamine or FBS to the basal medium reservoir. Three other murine hybridoma cell lines were tested in 5-ml micro bioreactors to determine if this is might be a general requirement for this cell type. As shown in Figure 8, the results for these three additional cell lines paralleled those of the rho 1D4 cell line; cell growth was greatly enhanced by the addition of ethanolamine or FBS, and growth with these two supplements was comparable. The rho 1D4 cell line was created with the NS-1 fusion partner while the three cell lines in Fig. 8 were created using the Sp2/0 fusion partner. These results suggest that ethanolamine should automatically be added to
the basal medium of murine hybridoma cell lines for optimal growth in hollow-fiber systems.

**DISCUSSION**

A novel hollow-fiber micro bioreactor was used as a screening tool to look for less expensive alternatives to FBS supplementation on the non-cell side of the membrane. Less expensive forms of serum such as newborn calf serum and adult bovine serum did not substitute for FBS (Fig 1). In micro bioreactors that had a 0.2 ml cell-side volume and 16-ml non-cell side volume, FBS could be replaced by the addition of ethanolamine and BSA with fatty acids on the non cell side of the membrane (Fig. 3). In bioreactors that had a 0.2 ml cell-side volume and 5-ml non-cell side volume, FBS could be replaced by the addition of just ethanolamine to the basal medium (Fig. 4). In a large-scale batch hollow-fiber system, the cells required either FBS or ethanolamine on the non-cell side to grow (Fig. 6). In a large-scale automated system, cells could grow without the addition of ethanolamine or FBS on the non-cell side (Fig. 7). However, the addition of ethanolamine increased the initial growth rate in the bioreactor and also increased the antibody production by about 50% without increasing the medium consumption cost.

The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

**EQUIVALENTS**

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.
CLAIMS

We claim:

1. A method for culturing cells in a membrane system having a cell side and a non-cell side, comprising culturing cells in the presence of serum at an initial concentration on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side comprises a medium containing ethanolamine, and wherein the concentration of serum on the cell side is maintained at greater than 20% of the initial concentration.

2. A method for enhancing cell growth in a membrane system having a cell side and a non-cell side, comprising culturing cells in the presence of serum at an initial concentration on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side comprises a medium containing ethanolamine, wherein the concentration of serum on the cell side is maintained at greater than 20% of the initial concentration, and wherein the cell growth is enhanced compared to the growth in the absence of ethanolamine.

3. A method for enhancing cell viability in a membrane system having a cell side and a non-cell side, comprising culturing cells in the presence of serum at an initial concentration on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side comprises a medium containing ethanolamine, wherein the concentration of serum on the cell side is maintained at greater than 20% of the initial concentration, and wherein cell viability is enhanced when cells are cultured in the presence of ethanolamine compared to when cells are cultured in the absence of ethanolamine.

4. The method of any one of the preceding claims, wherein the medium containing ethanolamine comprises basal medium.

5. The method of any one of the preceding claims, further comprising the step of adding ethanolamine to the medium on the non-cell side.

6. The method of any one of the preceding claims, wherein ethanolamine is also present on the cell side.
7. The method of any one of the preceding claims, further comprising culturing the cells in the presence of serum on both the cell side and the non-cell side, prior to culturing the cells in the presence of serum on the cell side and in the absence of serum on the non-cell side.

8. The method of claim 7, wherein the cells are cultured in the presence of serum on the non-cell side for less than 24 hours.

9. The method of any one of the preceding claims, wherein the cells are hybridoma or heterohybridoma cells.

10. The method of claim 9, wherein the hybridoma or heterohybridoma cells are selected from the group consisting of rodent, sheep and human hybridomas or heterohybridomas.

11. The method of claim 10, wherein the hybridoma or heterohybridoma cells are rodent hybridoma or heterohybridoma cells selected from the group consisting of rat, murine, rabbit and hamster hybridomas or heterohybridomas.

12. The method of claim 9, wherein the hybridoma or heterohybridoma cells are selected from the group consisting of NS/O, SP2/O, CHO and murine hybridomas or heterohybridomas.

13. The method of any one of the preceding claims, wherein the membrane system comprises a hollow fiber system.

14. The method of claim 13, wherein the hollow fiber system comprises a hollow fiber bioreactor.

15. The method of claim 4, wherein the basal medium comprises Ham’s F-12.
16. The method of claim 4, wherein the basal medium comprises Ham’s F-12 and a second basal medium selected from the group consisting of Dulbecco’s modified Eagle’s medium (DMEM), Essential modified Eagle’s medium (EMEM), RPMI-1640, and Iscove’s modified Dulbecco’s Medium (IMDM).

17. The method of claim 16, wherein the Ham’s F-12 and the second basal medium are present in about a 1:1 ratio (v/v).

18. The method of any one of the preceding claims, wherein the medium containing ethanolamine comprises at least one supplement.

19. The method of claim 18, wherein the at least one supplement is selected from the group consisting of an amino acid, glucose, glutamine, methionine, cystine, cysteine, tryptophan, sodium bicarbonate and combinations thereof.

20. The method of claim 18, wherein the medium containing ethanolamine comprises glucose, glutamine, methionine, cystine, tryptophan and sodium bicarbonate.

21. The method of claim 20, wherein the medium containing ethanolamine comprises less than about 10 g/L glucose, less than about 5 g/L glutamine, less than about 100 mg/L methionine, less than about 100 mg/L cystine, less than about 100 mg/L tryptophan, and less than about 10 g/L sodium bicarbonate.

22. The method of claim 20, wherein the medium containing ethanolamine comprises between about 4 g/L to about 5 g/L glucose, less than about 1 g/L glutamine, between about 14 mg/L to about 15 mg/L methionine, between about 40 mg/L to about 41 mg/L cystine, between about 17 mg/L to about 18 mg/L tryptophan, and between about 4 g/L to about 5 g/L sodium bicarbonate.

23. The method of any one of the preceding claims, wherein the medium containing ethanolamine comprises less than about 100 mg/L ethanolamine.
24. The method of any one of the preceding claims, wherein the medium containing ethanolamine comprises about 4 mg/L ethanolamine.

25. The method of any one of the preceding claims, wherein the medium containing ethanolamine comprises fat or insulin.

26. The method of any one of the preceding claims, wherein the serum concentration is maintained at about 50% or more of the initial concentration.

27. The method of any one of the preceding claims, wherein the serum concentration is maintained at about 75% or more of the initial concentration.

28. The method of any one of the preceding claims, wherein the serum concentration is maintained at about 90% or more of the initial concentration.

29. The method of any one of the preceding claims, wherein the serum concentration is maintained at about 100% of the initial concentration.

30. The method of any one of the preceding claims, wherein the serum concentration is maintained for more than about 7 days.

31. The method of any one of the preceding claims, wherein the serum concentration is maintained for more than about 14 days.

32. The method of any one of the preceding claims, wherein the serum concentration is maintained for more than about 28 days.

33. The method of any one of the preceding claims, wherein the serum concentration is maintained for at least the duration of the culturing step.

34. A method for enhancing cell growth in a membrane system having a cell side and a non-cell side, comprising culturing cells in the presence of serum at a concentration greater than 2% of total media components on the cell side and in the absence of serum
on the non-cell side, wherein the non-cell side comprises a medium containing ethanalamine, and wherein the cell growth is enhanced compared to the growth in the absence of ethanalamine.

35. The method of claim 34, wherein the cells are maintained at a serum concentration of greater than 2% for more than about 7-28 days.

36. The method of claim 34, wherein the serum concentration is between about 2% and about 15%.

37. The method of claim 34, wherein the serum concentration is between about 8% and about 12%.

38. A method for culturing cells in a membrane system having a cell side and a non-cell side, comprising culturing cells in the presence of serum on the cell side and in the absence of serum on the non-cell side, wherein the non-cell side comprises a medium containing ethanalamine, and wherein serum is maintained at a concentration of greater than 2%, without weaning.
Figure 1
Figure 3
Figure 4
Figure 6
Figure 7
Figure 8