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PATENTS AND LITERATURE

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The objective of this section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnological opportunities. In addition to protein engineering and site-directed mutagenesis covered in the last issue, two more subject areas will be surveyed in 1986: mammalian cell culture and microbial transformations. The subject of this, the fifth Patents and Literature Section of 1986, is mammalian cell culture.

Mammalian Cell Culture

Patents

This section identifies and gives a brief description of patents from the US patent literature from March 1985 through March 1986. The search headings were mammalian, animal, cell, and tissue, with the cross-term culture. Both patent titles and abstracts were searched. Copies of the US patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Baker, P. E.
SERUM-FREE CELL CULTURE MEDIUM AND PROCESS FOR MAKING SAME
US 4,560,655, Dec. 24, 1985
Assignee: Immunex Corp.

A defined, serum-free medium, capable of growing a wide range of suspension and monolayer cells, including a serum substitute composed of fetuin, transferrin, phosphatidylcholine, (1-oleoyl-2-palmitoyl-phosphatidylcholine), linoleic acid, and cholesterol, is described. The me-

dium also includes various inorganic salts, carbohydrates, amino acids, buffering agents, vitamins, and compounds to simulate the natural cell environment.

Crespi, C. L., and Thilly, W. G.
MUTATION ASSAYS INVOLVING BLOOD CELLS THAT METABOLIZE TOXIC SUBSTANCES
US 4,532,204, Jul. 30, 1985
Assignee: Massachusetts Institute of Technology

A line of human blood cells that has high levels of oxidative activity (such as oxygenase, oxidase, peroxidase, and hydroxylase activity) is described. Such cells grow in suspension culture and are useful to determine the mutagenicity of xenobiotic substances that are metabolized into toxic or mutagenic substances. Mutation assays using these cells and other cells with similar characteristics are also described.

Daemer, R. J., Feinstone, S. M., Gust, I. D., and Purcell, R. H.
ISOLATION OF HEPATITIS A VIRUS STRAIN HM-175
US 4,532,215, Jul. 30, 1985
Assignee: The United States of America as represented by the Department of Health and Human Services

Human hepatitis A virus (HAV), taken directly from human clinical specimens, can be isolated and serially passaged in primary African green monkey kidney (AGMK) cell cultures. This strain induced an antibody to HAV in inoculated chimpanzees and is useful for vaccine.

Fabricius, H., and Stahn, R.
HIGH YIELD PROCESS FOR IN VITRO PRODUCTION OF SERUM- AND MITOGEN-FREE INTERLEUKIN-2, USING A ROLLER-BOTTLE CULTURE SYSTEM
US 4,517,293, May 14, 1985
Assignee: Hooper Trading Co., NV

An improved in vitro cell culture process is described for producing, in high yield and purity, a serum- and mitogen-free interleukin-2 (IL-2), containing conditioned supernatant. The incubation steps during stimulation and conditioning of the IL-2-producing cells is carried out with a rapidly rotating roller-bottle culture system. Yields of IL-2 are on the order of 10-fold and higher than those obtained by similar cultivations carried out in flat dishes or tubes or in a roller-bottle culture system rotated at conventional speeds. Improvement of yields in both the static and roller culture systems is also achieved by incubating the IL-2 producing cells under high oxygen concentrations. In addition to using peripheral mononuclear blood cells (PBL) as the source of leukocytes containing the IL-2-producer cells, it is also possible to use buffy coat cells, which are readily available as a waste by-product from blood banks.

Fischer, H., Kickhofen, B., and Vaubel, E.
TRANSPARENT FLUID-BANDAGE MATERIAL AND THE PREPARATION AND USE THEREOF
US 4,556,056, Dec. 3, 1985
Assignee: Max-Planck, Gesellschaft zur Forderung der/Wissenschaften e.V.

Novel bandage materials are described that are comprised of a transparent fluid-material, consisting of hydrophilic, organic, transparent gel in sheet or band form, swollen with an aqueous solution that can contain buffer substances, wound treatment agents, nutrients and/or hormones, and, optionally, a reinforcing mesh. The fluid bandages are made by dissolving a monomer and a gellable, hydrophilic, high-molecular substance in aqueous medium. The gel-forming reaction is started with an initiator for the polymerizable monomer. The bandages can also be utilized as carrier compositions for cell cultures, from which metabolites are obtained.

Healy, G. M., and Curry, K. D.
TISSUE CULTURE AND CELL-GROWTH-PROMOTING MATERIAL AND ITS METHOD OF MANUFACTURE
US 4,520,107, May 28, 1985
Assignee: Polydex Chemicals Ltd.

A novel, cell-growth-promoting material and the method for its preparation is described for use as a supplement to basal tissue culture media for the in vitro cultivation of animal cells. An infusion consisting of growth-promoting material from adult, calf, or fetal bovine blood clots is used as a supplement to basal media for the in vitro cultivation of animal cells.

Horodniceanu, F., and LeFur, R.
PROCESS FOR GROWING CELL CULTURES OF DIPLOID CELLS ON CELL SUPPORTS
US 4,537,790, Aug. 27, 1985
Assignee: Institut Pasteur

A process is described for growing cell cultures of diploid cells. The process includes the steps of: (a) introducing cell culture medium into a container containing cell support; (b) adding human or animal diploid cells to the culture medium of step (a) to form a cell-containing culture medium; and (c) maintaining the cell-containing culture medium of step (b) under cell growth conditions. The cell support is prepared by applying a solution of thermosetting plastic material prepared by the reaction of an aldehyde with a polyvinyl alcohol to glass or polysaccharide base support and then subjecting the coated support to heat to permit the hardening and sterilization of the coated support.

Izawa, M., and Tatsukawa, S.
METHOD AND APPARATUS FOR DISLODGING CULTURED CELLS
US 4,556,639, Dec. 3, 1985
Assignee: Olympus Optical Co. Ltd.

A method is described for disloding cultured cells. A culture container is prepared that has a surface on which cultured cells are grown and that is filled with a culture solution in contact with the cultured cells. The culture container is supported on a reciprocally movable pedestal. The reciprocal movement of the culture container in the direction parallel to the growing surface applies an inertial force on the cultured cells, dislodging them from the growing surface.

Jacobson, B., and Raymond, L.
CELL PROLIFERATION INHIBITOR AND METHOD OF PREPARATION
US 4,534,967, Aug. 13, 1985
Assignee: Boston Biomedical Research Institute

An inhibitor of endothelial cell growth is prepared by directly liquifying vitreous gel by forcing it through a small orifice so as to directly convert the gel into a liquid. The insoluble and suspended material is removed and the liquid is chromatographically fractionated. An inhibitor is also prepared by culturing hyalocyte cells in a medium and chromatographically fractionating the medium.

Kasai, S., Akaike, T., and Miyata, T.
SUBSTRATUM FOR CELL CULTURE AND A METHOD FOR CULTURING AND ISOLATING CELLS USING SAME
US 4,559,304, Dec. 17, 1985
Assignee: Koken Co. Ltd.

A substratum for cell culture, comprised of a chemically modified collagen rich in either positive or negative charges when under culture conditions, is described. The substratum is prepared by modifying the amino or carboxyl groups of collagen. The chemically modified collagen enhances the adherence and proliferation of animal cells much more actively than unmodified collagen in the presence or absence of bovine fetal serum. The cultured animal cells can be detached efficiently from the chemically modified collagen. This allows for highly selective isolation and recovery of the cultured animal cells, which can be accomplished without cell injury.

Maldonado, R. L., and Rosanoff, K. A.
TISSUE CULTURE MEDIUM
US 4,533,634, Aug. 6, 1985
Assignee: AMF Inc.

A growth factor containing serum, derived from natural bovine serum, is

described. A first solids fraction is precipitated from natural bovine serum by adding ammonium sulfate to about 25% saturation to give a first solids fraction and a first supernatant; separating the first solids fraction from the first supernatant; precipitating a second solids fraction from the first supernatant by adding ammonium sulfate to 40% saturation to give a second solids fraction and a second supernatant; and separating the second solids fraction from the second supernatant. The first solids fraction and the second supernatant are then combined and desalted.

Mears, D. C.
REGENERATION OF LIVING TISSUE BY GROWTH OF ISOLATED CELLS IN POROUS IMPLANT AND PRODUCT THEREOF
US 4,553,272, Nov. 19, 1985
Assignee: University of Pittsburgh

A method to repair patient tissues using an implant having a porous, open structure and containing a living-cell sample is described. The cell sample may be cultured in the implant. The implant is secured to the patient by surgical implantation. The implant portion that receives the cells has a pore size of about 25–75 μm. A second pore size of about 100–400 μm may be provided for receipt of blood vessels and osteogenus cells through ingrowth after introduction into the patient. The cell sample may be selected from the group consisting of cartilage cells, tendon cells, ligament cells, and musculotendinous cells. The implant member may be advantageously used in bone or joint reconstruction surgery and in other forms, such as artificial tooth implantation.

Meyers, W. E., and Beck, L. R.
METHOD AND DEVICE FOR CELL CULTURE GROWTH
US 4,546,083, Oct. 8, 1985
Assignee: Stolle Research & Development Corp.

A cell culture device is described for the cultivation of animal, plant, microbiological, or artificial cells. The device involves a three-dimensional arrangement of fibers within a housing arranged to provide maximum exposed fiber surface flow channel diameter, while also reducing the tortuosity of the flow path. Cells are bound to the fibers to allow them to contact nutrient fluid solution and to remove any substances originating in the cells, such as viruses and pharmaceuticals.

Montagnon, B. J., and Fanget, B. J. C.
PROCESS FOR THE LARGE-SCALE PRODUCTION OF A VACCINE AGAINST POLIOMYELITIS AND THE RESULTING VACCINE
US 4,525,349, Jun, 25, 1985
Assignee: Societe Anonyme dite: Institut Merueux

A process is described for large-scale production of each type of poliomyelitis virus, using the following steps. A cell strain, using a cell stock, is

multiplied by culturing the same in a liquid nutritive medium on microcarriers in suspension and by successive passages into biogenerators of increasing volumes. The last biogenerator is one having a capacity of at least 150 L, and the nutritive medium contains serum. This operation is carried out with stirring at a rate not greater than 40 rpm. At the end of the final passage the liquid nutritive medium is withdrawn and replaced by one that is serum-free. The biogenerator used for the last passage is then inoculated with virus, which is permitted to develop, again with stirring at a rate not greater than 40 rpm. After culturing the virus, the liquid suspension is withdrawn, filtered, concentrated at least 150 times by ultrafiltration, and subjected first to gel filtration then ion exchange chromatography. The resulting concentrated suspension is diluted with a serum-free medium and then inactivated. The suspensions of the respective types used are mixed, from which individual dosages are prepared.

Nees, S.
PROCESS FOR THE CULTIVATION OF MATRIX-BOUND BIOLOGIC CELL SYSTEMS
US 4,542,101, Sep. 17, 1985

An improved process is described for cultivation of matrix-bound biologic cell systems on microcarrier particles within a replenishable nutrient medium. A step of providing controlled three-dimensional displacement of a culture vessel and its contents is described to effect uniform cell exposure to available nutrient material.

Nobuhara, M., Yamaguchi, K., and Mochida, E.
METHOD OF INTERFERON PRODUCTION
US 4,548,900, Oct. 22, 1985
Assignee: Mochida Pharmaceutical Co. Ltd.

A method is described for the mass production of interferon, in which cultured cells are brought into contact with at least one polyhydric alcohol. This achieves a remarkable increase in the production of interferon from the cultured cells.

Noll, L. A.
CELL CULTURE USING A MONOLITHIC SUPPORT
US 4,514,499, Apr. 30, 1985
Assignee: Corning Glass Works

A novel immobilized cell composite used in an apparatus for cell culture is described. The composite is comprised of: (a) a high-surface-area monolithic support having a multiplicity of mutually parallel channels passing through it, the channels having walls formed of a medium-insoluble, nontoxic composition and the support having at least about 20 channels per square inch of cross-sectional area; and (b) a population of plant or animal tissue cells anchored to the porous channel walls.

O'Connell, D. M.
ADJUSTABLE HEIGHT MAGNETIC STIRRER
US 4,512,666, Apr. 23, 1985
Assignee: Corning Glass Works

The present invention relates to suspended magnetic stirrers. It describes a stirrer having a suspended magnetic impeller, the height of which is adjusted by a movable bearing. The invention is particularly useful in applications in which solids must be suspended in a liquid medium with a minimum of shear force, such as in microcarrier tissue cell culture.

Ooi, K., Morita, M., Suzuki, K., Hashizume, S., and Yoshizawa, H.
ATTENUATED SMALLPOX VACCINE STRAIN
US 4,567,147, Jan. 28, 1986
Assignee: Chiba Prefectural Government

An attenuated smallpox vaccine strain exhibiting antibody production similar to conventional strains, but without postvaccinal side effects, is described. The vaccine is prepared by attenuating a Lister strain of a vaccinia virus by cell culture and selecting a suitable strain that shows relatively small and uniform pocks on the chorioallantoic membrane of an embryonated egg.

Peterson, A., and Walum, E.
PERFUSION-CULTIVATION OF ANIMAL CELLS AND EQUIPMENT THERFOR
US 4,530,907, Jul. 23, 1985

The invention aims at replacing animal experiments for determining acute toxicity (LD_{50} values) by utilizing stable perfusion-cultivated cell cultures. A cultivating chamber that is movable during operation is also described for determining various parameters.

Rotman, M. B.
CYTOTOXICITY ASSAYS IN CELL CULTURING DEVICES
US 4,559,299, Dec. 17, 1985
Assignee: Brown University Research Foundation, Inc.

Methods and devices for assaying the sensitivity of biopsied cells to therapeutic agents are described. Cells are cultured in artificial organs and then contacted with a fluorogenic substrate such that living cells accumulate a characteristic amount of fluorescence. The agent is then introduced into the organ and changes in the fluorescence released by the cells serve as an indicator of the sensitivity of the cells to the agent.

Tolbert, W. R., Feder, J., and Lewis, C., Jr.
STATIC CELL CULTURE MAINTENANCE SYSTEM
US 4,537,860, Aug. 27, 1985
Assignee: Monsanto Co.

A method and apparatus is described for maintaining animal cells in vitro in a substantially arrested state of proliferation, with continuous secretion of cell product. The cells are retained within a reactor vessel chamber in a semi-rigid matrix having interstices for passage of fluid nutrient medium. Fresh nutrient medium is supplied by perfusion into the matrix through low-porosity tubes that are suspended in the reactor chamber and traverse the matrix; expended medium and cell product is withdrawn through high-porosity tubes, which also are suspended in the reactor chamber and traverse the matrix; and oxygenated gaseous medium is supplied by perfusion into the matrix through a selectively permeable membrane in the reactor chamber.

Yamane, I., Kan, M., and Minamoto, Y.
CULTURE MEDIUM
US 4,533,637, Aug. 6, 1985
Assignee: Ajinomoto Co. Inc.; Yamane; Isao

New findings demonstrate that cyclodextrin show slight or no cytotoxicity. Lipophilic substances, such as unsaturated fatty acid, and lipophilic vitamins, when present together with, or included in, cyclodextrin show such effects as cell growth promotion and acceleration of the productivity of valuable products. A serum-free or -reduced culture medium, comprised of cyclodextrin and at least one lipophilic nutrient substance in the form of inclusion complex between them, is described.

Literature

This section surveys literature in the area of mammalian cell culture from January 1984 to May 1986. This section includes only selected articles that appeared during this time period.

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