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(54) Title: METHOD FOR PRODUCING CELLS

(57) Abstract

A process for growing cells in a nutrient medium utilizing an electrically conductive material contacting the cells and passing an electrical current or voltage through the material contacting the cells.

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METHOD FOR PRODUCING CELLS

Field of the Invention

The present invention relates to a method for stimulating and/or growing cells. More particularly, the invention is concerned with a method for culturing of cells using electrically conductive polymeric material and an electrical current or voltage.

Background of the Invention

It has long been known that electrical stimulation of plant and animal cells may cause them to exhibit a change in growth characteristics, motility, and production of bioactive molecules. Instruments have even been devised to deliver small continuing amounts of electrical current to sites of bone fracture in order to stimulate healing.

Recently, it has been shown that fibroblasts on a collagen matrix can be stimulated to grow by passing an electric current through the media in which the fibroblast and collagen preparation is placed. Other workers have shown that cells grown on evaporated gold electrodes may be used as biosensors to monitor cell growth.

It has been postulated that cellular responses to light such as UV and lasers may involve the transport of electrons through cell membranes by proteins similar in function to the retinal proteins. Any mechanical deformation of the cell membrane may cause an electric field to develop which may alter the cell's function.

In the past, current could only be applied indirectly through the media or support of the cell culture system or by means of the "patch - clamp"

system for individual cells. The individual cell method is obviously impractical, and the apparatus needed to apply current by other means is clumsy and difficult to use.

It is known that electric currents and electromagnetic fields can influence cellular growth and synthetic responses in the absence of applied mechanical stresses. In particular, there has been increased interest in electrical stimulation of osteogenesis.

In a report by Raphael C. Lee, et al., entitled "A Comparison of In Vitro Cellular Responses to Mechanical and Electrical Stimulation" in The American Surgeon, November 1982, pp. 567-574, incorporated herein by reference, states that the passage of a sinusoidal alternating current through a membrane support at current densities of between 1 to 1,000 nanoamps/mm^2 produced qualitatively similar changes in biosynthetic activity of chrondrocytes. Electrical current was passed through cell-laden membranes and media. The elastin membrane supports utilized were not electroconductive.

The article of Bryant, et al., entitled "Electromechanical Stresses Produced in the Plasma Membrane of Suspended Cells by Applied Electrical Fields" in J. Membrane Biology 96, pp. 129-139 (1987), discloses that cells are deformed in a uniform electrical field. A membrane-solution system was utilized in which no appreciable current flows across the membrane. The applied electric field induces surface charges of opposite signs on each face of the membrane but the membrane is non-conductive and no appreciable current flows across the membrane.

It is known that the growth of many anchorage dependent cells are dependent upon the function of attachment and spreading. Other stimulus such as agitation and the type of carrier promote the growing of cells.

U.S. Pat. No. 4,335,215 to Folbert, et al., which is incorporated herein by reference, discloses nutrient systems and growth environment for continuously growing and harvesting cells which can be used in connection with the present invention.

It is also known that sporulation of microorganisms can be promoted by the addition of chemicals to the culturing medium. U.S. Pat. No. 3,672,957 to Scharpf, which is herein incorporated by reference, discloses culturing conditions and culturing media which are useful in connection with the present invention.

In a paper entitled "Electrochemically Prepared Polypyrroles from Aqueous Solutions" by Qian, et al., Polymer Journal, Vol. 19, No. 1, pp. 157-172 (1987), which is herein incorporated by reference, there is disclosed electrically conductive polypyrroles as well as other electrically conductive polymers which may be utilized in the process of the present invention.

Reaction based on the interaction of amino groups of amino acids, peptides or proteins with the glycosidic hydroxyl groups of sugars are well known. Aldoses when heated in methanol, ethanol or water with compounds containing a primary amino group yield solid glycosidic amines that separate upon cooling or addition of ether. If excess acid is present the glycosylamine will rearrange to the 1-amino-1-deoxy-2-ketose derivative (Amadori arrangement). W-amino acids appear to react best, lysine and ornithine being

the most reactive. It has now been found that similar reactions can be used to provide a combination of an antibody with a linking molecule. Any molecule containing a terminal amino group, including but not limited to lower alkyl diamines, may be utilized. It is further possible to couple the antibody-linking molecule to a conducting polymer by modifying the polymer to contain a carboxyl group and coupling at the amine and carboxyl groups.

Summary of the Invention

The present invention provides a method of culturing cells and promoting cell growth through the utilization of electrical energy which is applied to the support on which the cells are cultivated, to the polymer formed on the cell surface, to the conducting polymer latexes surrounding the cell or ingested by the cell. More particularly, the process relates to the promotion of the proliferation of cells, including the germination of spores of sporulating microorganisms, through the use of electrically conductive mediums and the application of an electrical energy.

In accordance with one embodiment of the invention, cells which are cultivated in a nutrient media by anchorage to a support are grown in the present invention by attachment to an electrically conductive film or gel, which is subjected to an electrical current of between about 0.5 microamps to about 1 milliamp.

In accordance with another embodiment of the invention, the germination of spores is accelerated by the passage of an electrical charge through an

electrically conductive gel or macromolecular material which comes into contact with the spores.

In accordance with a further embodiment of the invention, the growth of cells is promoted and the cells altered by the application of an electrical charge through the electrically conductive medium upon which the cells are grown or an electrical charge through the media in which the cells are cultivated.

In accordance with another embodiment of the invention, the growth of cells is promoted by surrounding the cells with a latex of conducting polymers and the passage of an electrical charge through the latex and to the cell membrane.

In accordance with yet another embodiment of the invention, latex particles of conducting polymers are ingested by the cells and an electrical current is applied so as to stimulate the cells.

In accordance with still another embodiment of the invention, the conducting polymers are linked to an antibody molecule either by direct polymerization or by a linking group and either oriented on the surface of the membrane or spanning the cell membrane. An electrical current or microwave stimulus is then applied to the polymer.

In still another embodiment, a combination of cells having ingested polymers and/or antibodies attached to the conducting polymers are used to form a battery configuration.

Detailed Description of the Invention

The cells which can be used in the process of the present invention may be animal, plant or microorganism. The animal cells include mammalian,

avian and amphibian. Among cells which may be grown in accordance with the invention are various mammalian cells which can be cultured in vitro, particularly tumor cells and hybridomas. The microorganisms include bacteria, fungi and viruses. Cells of fungal origin include aspergillus and rhizopus. Bacterial microorganisms include Bacillus and Clostridium genera. The method of the invention is especially useful in the cultivation of E. Coli and yeast which are used in the production of chemical compounds through biotechnological procedures. That is, not only can naturally occurring microorganisms and cells be employed, but also microorganisms and cells which have been modified by genetic engineering techniques, such as transformation, DNA insertions, transduction, fusion and the like.

The invention is especially useful with human diploid cells. The invention is adaptable to all types of animal cells including, for example, mammalian, avian and amphibian cells. Primary cells taken from embryonic, adult or tumor tissues as well as of established cell lines can thus be used. Examples of typical cells are primary rhesus monkey kidney cells, baby hamster kidney cells, pig kidney cells, mouse embryo fibroblasts, normal human lung embryo fibroblasts, HeLa cells, primary and secondary chick fibroblasts, and various cells transformed with SV-40 or polyoma virus.

After suitable growth of the cells, the cells can be harvested and further treated for the production of desired products by various means. For example, human diploid foreskin fibroblasts cultured by the method of this invention can be treated for the production of angiogenic factor, plasminogen

activator and interferon. Angiogenic factor can be isolated from the growth medium or from the cells. Plasminogen activator can be harvested from a serum-free maintenance medium during a period of aging after the cells have reached their maximum density. Interferon can be induced in the aged fibroblasts and its production enhanced due to the high cell density.

Cells can be employed in the present process in which DNA replication is substantially inhibited or terminated, but metabolism continues for relatively long periods of time. The cells continue to express genes, other than the blocked genes involved with DNA replication. Where the cells have been transformed with exogenous genes, these genes will be expressed to provide the desired product.

By preventing DNA replication, the nutrients are used more efficiently for the functioning of the microbiological reactor. The inhibition of DNA replication can be achieved in a variety of ways, such as chemical inhibitors, temperature sensitive mutants, mutants lacking an intermediate in the biosynthetic pathway to DNA replication, or the like, and the growth facilitated by the present process.

The nutrient medium employed will be dependent upon the microorganism or cell involved, and the product desired. For example, the nutrient medium will be adapted to the particular microorganism or cell. Besides nutrients, other substances may be included to support growth and/or cell differentiation. For example, a natural product such as an excreted protein, e.g., enzymes, hormones, lymphokines, toxins, immunoglobulins, or the like or a non-proteinaceous organic compound resulting from transformation of a substrate, such as by expoxidation, hydroxylation, esterification, e.g,

acetate, phosphate, uronate, or sulfate, reduction, methylation, etherification with sugars or the like, may be added.

The sporulating microorganisms may be cultivated in accordance with the invention utilizing standard fermentation techniques and physiological germinants together with the passage of electrical energy through the conductive support according to the present invention.

The physiological germinants that may be used in the practice of this invention include sugars, amino acids and nucleosides. Examples of sugars include glucose, maltose, lactose and sucrose. Examples of amino acids include tyrosine, L-alanine, DL-valine, DL-cysteine, methionine, glutamic acid, L-arginine, L-phenylalanine, L-leucine, L-tryptophane, aspartic acid, glycine, lysine, L-isoleucine, histidine, serine, threonine and proline. Examples of nucleosides include inosine, guanosine and adenosine. Glucose, L-alanine and inosine are preferred.

Some of these physiological germinants are more specific for germinating certain spores and, depending on the spore to be germinated, faster times for the germination phase are obtained. For example, the spores of B. subtilis and B. stearothermophilus are germinated faster employing glucose or L-alanine or a combination thereof. However, in the case of B. megaterium spores, a faster germination time is obtained employing the physiological germinant inosine.

The amount of the physiological germinant utilized according to this invention will depend, to some extent, upon the particular germinant and the spores to be germinated. An effective concentration

of the physiological germinant is at least .001 mg per 10^3 to 10^9 spores. In most instances, it was found that an amount below .001 mg does not sufficiently decrease the time required for the germination phase. Generally, the preferred range is from about 0.01 mg to about 10 mg per 10^3 to 10^9 spores. Although the physiological germinants may be used in amounts above 10 mg, for example 25 mg, usually it is without commensurate advantage. In the case of L-alanine, for example, an amount of about 2 to about 5 milligrams/10^3 to about 10^9 is especially preferred. In the case of glucose and inosine, about 0.05 to about 0.4 mg/10^3 to 10^9 spores is especially preferred.

The electrically conductive materials which may be utilized in the present invention are the natural and synthetic polymers, including gums, which are normally utilized in the cultivation of cells that are electrically conductive or have been made electrically conductive by incorporation or intercalation of other polymers or materials. The aforementioned article of Qian, et al., discloses such materials and how the material can be made electrically conductive. One way of preparing conductive polymers is by polymerizing in situ acetylene, pyrrole, or thiophene in a flexible matrix as described by Qian, et al. Molecular composites with polyacetylene, polypyrrole, polythiophene, polystyrene, and the like may be prepared with nylon, polyvinyl chloride, polyvinyl alcohol, polyvinyl acetate, polyvinyl butyral, polyethylene glycol, gelatin, collagen, guar gum, elastin, glycoproteins, carotenoids, hemins, diazobenzyloxymethyl, nitrocellulose, paper, and the like.

The following examples are illustrative of the present invention. It will be apparent to those skilled in the art that many modifications, both of materials and methods, can be made without departing from the spirit and scope of the invention.

Example 1: Formation of Polypyrrole and Cellulose Conducting Polymer Film

A polysaccharide matrix, cellulose dialysis membrane, is suspended in a solution prepared by dissolving approximately 5.4 ml of pyrrole in 200 ml of 0.10 M NaCl. The duration of the suspension (from seconds to days) and the temperature of the solution (from 0x to 20xC) do not significantly affect the subsequent formation of the conducting polymer film. The oxidizing solution is prepared by dissolving 4.6 g of ammonium persulfate, $(NH_4)_2S_2O_8$, in 200 ml of 0.10 M NaCl. The oxidizing solution is then added to the solution containing the monomer and the matrix. Over a period of several seconds (less than 1 minute), the solution changes color from clear to aqua to black. At this time the matrix is removed and washed with copious quantities of 0.10 M NaCl. The wash process removes excess conducting polymer from the conducting polymer film. Loosely adhering polypyrrole and electrolyte are further removed by gently wiping the conducting polymer film. The resulting material is allowed to air dry. Conductivity is between 0.1 to 1 S/cm. The polymer is suitable for growing animal cells and sporulating microorganisms.

Example 2: Formation of Polyaniline and Cellulose Conducting Polymer Film

A polysaccharide matrix, cellulose dialysis membrane, is suspended in a solution prepared by dissolving approximately 20 ml of aniline in 300 ml of 1.0 M HCl. The duration of the suspension (from seconds to 12 hours) and the temperature of the solution (from 0x to 20xC) do not significantly affect the subsequent formation of the conducting polymer film. The oxidizing solution is prepared by dissolving 11.5 g of ammonium persulfate, $(NH_4)_2S_2O_8$, in 200 ml of 1.0 M HCl. The oxidizing solution is then added to the solution containing the monomer and the matrix. Over a period of several seconds (less than 1 minute), the solution changes color from clear to aqua to green. At this time the cellulose matrix is removed and washed with copious quantities of 1.0 M HCl. The wash process removes excess conducting polymer from the conducting polymer film. Loosely adhering polyaniline and electrolyte are further removed by gently wiping the film. The resulting material is allowed to dry. The conductivity is between 0.1 to 1 S/cm. The stability of this material can be increased by exposure to base. This polymer has been used for growing fungal microorganisms.

Example 3: Preparation of Polypyrrole-Polyvinyl Alcohol Grafted Polymers

1.2 g of polyvinyl alcohol (PVA) (Aldrich #18,966-a) average molecular wt 88,000 was dissolved in 200 ml of 0.1 M NaCl and then heated to 65xC. When all the PVA granules dissolved, the solution was

cooled to room temperature, after which 10.8 ml of pyrrole was added while stirring the solution. After the pyrrole dissolved, the solution was left overnight at 4xC. A 200 ml portion of 0.2 M ammonium persulfate cooled to 4xC was added slowly to the PVA/pyrrole solution with stirring. The color of the solution turned from light green to dark green to dark brown and was left stirring for 15-30 minutes at room temperature. The solution was then filtered through glass wool. The dark filtrate contained polypyrrole polyvinyl alcohol (grafted) polymer in solution. The filtrate (50 ml) was then poured slowly into 1 liter of acetonitrile on a large dish (depth of solvent was 1-1.5 cm) and left undisturbed for 1-2 hours. The casting of polymer on the organic solvent resulted in thin film of the polymer (black in color). The solvent was discarded and the black film was washed three times with 100 ml acetonitrile and dried.

Lower molecular weight PVA (mol. wt. 2000) was polymerized in the same fashion to yield similar results. The resistance, when measured by a two prong probe, in a polymer sample approximately one cm long, was 0.08 - 0.1 k ohms.

In a similar manner there may be prepared conductive polymers comprising polystyrene-pyrrole, polyvinylbutyral-pyrrole, polyethylene-pyrrole and polypropylene-pyrrole.

Example 4: Preparation of DEAE-Sephadex Attached to Polypyrrole

4 g of DEAE-Cellulose (Cellex^{TM} from BioRad) was added to 200 ml of 0.1 M NaCl, to which 10.8 ml of pyrrole was added, stirred gently for one and one-

half hours and left overnight at 4xC. A 200 ml portion of 0.2 M ammonium persulfate in 0.1 M NaCl solution was added to the Cellex - monomer solution with rapid stirring. The resulting solution was stirred for an additional 2 hours at room temperature. The solution was then filtered and the precipitate washed several times with 0.1 M NaCl and then with water. The resulting black powder was air dried. The resistance of a one cm section of polymer was 0.25 -0.3 ohms.

Example 5: Preparation of Poly (organophosphazenes)-Polypyrrole Grafted Polymer

Hexachlorocyclotriphosphazene (phosphonitrillic chloride trimer) $(NPCl_2)_3$ obtained from Aldrich produced a high molecular weight dichlorophosphazene polymer when heated at 250xC. The uncrosslinked polymer was then dissolved in a suitable organic solvent that also dissolved polypyrrole. Upon reacting, the highly reactive dichlorophosphazene polymer generated a grafted polymer.

However, an alternate route can be envisioned, in which the trimer is reacted with polypyrrole, or the trimer is dissolved in a solvent, to which pyrrole is added, where pyrrole will replace the chloride ion in the trimer. The next step is to polymerize the pyrrole on the phosphazene trimer by adding oxidants such as ammonium persulfate to the solution. After the pyrrole has polymerized on the trimer, the trimer can be heated to form the uncrosslinked polyphosphazene polypyrrole polymer.

In lieu of the trimer there may be utilized polyvinyl alcohol to obtain polyvinyl alcohol-polypyrrole copolymer.

Example 6: Attachment of Polypyrrole to Microgranular Cellulose

10 g of microgranular cellulose powder (Whatman, cc41, cat #4061 - 050) was suspended in 200 ml of monomer solution (10.8 ml of pyrrole/0.1 M NaCl), overnight at 4xC. The suspension of cellulose powder was then stirred gently for 2 hours, after which 200 ml of 0.2 M ammonium persulfate in 0.1 M NaCl was added in a dropwise manner, with stirring. Stirring was continued for 1 hour, then the powder was washed three times with NaCl (0.1 M) and then several times with water, until the supernatant was free of chloride ions. The aqueous suspension of cellulose was cast in petri dishes (square and circular) and left overnight to dry. The resistance of the cast cellulose was measured by two pin probes attached to a multimeter. The average resistance of the material was between 0.1 to 0.7 k ohms when the probes were placed about 1 to 2 cm apart.

Example 7: Procedure for Attaching Polypyrrole to Collagen

Acid soluble collagen (4 mg) from Sigma (type III, acid soluble, No. c-3511) was dissolved in 2 ml of acetic acid (diluted 1: 1000) at 4xC for 48 hours, then spun for 1 hour at 23,000 RPM. To the supernatant, 1/6 volume of 0.1 N NaOH was added (1 ml of supernatant mixed with 166 microliters of NaOH). A white precipitate resulted. The mixture was centrifuged at 1500 RPM for 20 minutes. The white pellet which formed was then suspended in 1 ml of pyrrole (0.1 M pyrrole in 0.1 M NaCl) solution overnight at 4xC. Next, 0.1 M ammonium persulfate

solution (in 0.1 M NaCl) was added dropwise to the collagen/pyrrole solution, vortexed and left to incubate at 4xC, for 1 hour. The resulting black suspension was centrifuged for 20 minutes at 1500 RPM. The supernatant was discarded, the pellet washed three times with 0.1 M NaCl and then several times with distilled water. To the black pellet, 1 ml of (1:1000) acetic acid was added and the mixture was left overnight at 4xC. The suspension was centrifuged for 30 minutes. Unbound polypyrrole and collagen was removed in the supernatant. The collagen/polypyrrole was made into a thin film by dissolving the complex in an ethanol solution then spraying onto a glass surface. The organic solvent vaporized leaving the biomolecule on the surface.

Example 8: Proliferation of Fibroblasts on Conducting Polymer Films

Folllowing the procedure of Rabinovitch and de Stefano J. Cell Biol. 59, 165 (1973), the effect of cell spreading using BHK fibroblasts was observed.

Polystryene-pyrrole dishes having a 5 cm diameter, prepared according to the invention, were inoculated with BHKC13 at 10^5 cells per dish in 3 ml of medium. Two sorts of medium were compared: serum-free minimal salts buffer containing 1 M magnesium acetate in SBK (saline-bicarbonate-potassium) and complete medium with 10% serum.

Four conducting posts to which two platinum wires were anchored were placed in one dish. A constant potential of 0.5 microamps was applied. The other dish was used as a control.

Spreading was assayed as the percentage of cells which ceased to be rounded, and became uniform or

polygonal in shape, having distinct corners. Cells which merely increased in size without becoming angular were not regarded as "spread".

After 30 minutes the negative surface charge density on the dishes were assayed by dye binding. The surfaces were equilibrated with a solution of crystal violet cationic dye (0.1 mM in distilled water) and washed with distilled water. The remaining dye, bound to the fixed negative charges, was eluted with acid-ethanol and determined colorimetrically.

The percent spread of cells measured in charges per 10 cm^2 square for the control was 0.6. The percent spread of cells for the electrically stimulated cells was 70.

Fibroblasts, yeasts, hybridomas, bacteria and fungi can also be grown following this procedure.

Example 9: Attachment and Spreading of HeLa Cells on Polypyrrole/Collagen Films

Late log-phase cultures of HeLa cells growing on plastic dishes were harvested by a 5-min exposure to EGTA (2 mM) and trypsin (0.05%) in PBS, the cells collected by centrifugation at 200 g for 5 min and suspended in MEM containig 10% foetal calf serum. One ml aliquots of this cell suspension were then put into 35 mm plastic tissue culture dishes containing gels of native collagen fibers as a control and the gel of Example 7 to give an initial number of 10^4 cells per dish. The dishes were incubated at 37xC in a humidified CO_2 incubator for 120 minutes. The dish containing the pyrrole-collagen had a current of 0.5 mA constantly delivered through platinum wires connected to conductive posts inserted in the gel.

The total cell number was estimated by dissolving the gel with collagenase (Sigma, C-2139, 0.2 mg/ml) in serum-free MEM and counting the cells with a Coulter electronic particle counter.

Total cell number and the percentage of cells detached by exposure to trypsin were determined in parallel dishes by a 2-step procedure. First, dishes were washed 3 times with Hanks' balanced salt solution, incubated with trypsin (0.25%) in PBS for 10 min and the number of detached cells counted; the gels were then dissolved by exposure to collagenase and the number of remaining and the number of remaining attached cells determined as above. Total cell number was estimated by this second procedure by the addition of the number of cells detached by trypsin and those remaining attached to the gel. This figure always agreed within 10% with the total cell number obtained by simple collagenase digestion of the gel.

It was observed that in the electrically stimulated dishes cell adhesion was rapid and extensive. Approximately 95% of the cells attached and spread. In the control, attachment was about 70%.

Example 10: Preparation of Bacillus Stearothermophilus Spores

A control slant culture of B. stearothermophilus is transferred to 100 ml of nutrient broth in a 500 ml Erlenmeyer flask. The flask is placed on a rotary shaker at 300 RPM for 24 hours. Sterile nutrient agar 100 x 15 mm plates are inoculated with 0.5-1.0 ml of the contents of the flask and held for 7 days

at 55xC during which time the organisms sporulate. The spore concentration is 10^8 spores/ml of solution. Bacillus spores were prepared as above except that the 100 x 15 plates were coated with polypyrrole-polyvinyl alcohol following the procedure of Example 3. After a 2 day incubation under a constant potential of 0.8A, the spore concentration was 10^{10} spores/ml of solution.

Example 11: Preparation of Polyvinyl Alcohol - Pyrrole Latex

Pyrrole (Aldrich Chemicals) was vacuum-distilled and stored under argon in a refigerator at 4xC. Water was double-distilled from an all-Pyrex apparatus. Ferric chloride ($FeCl_3 \cdot 6H_2O$; BDH), potassium iodide (BDH; "AnalaR" grade), iodine (BDH), and boric acid (BDH, "AnalaR" grade) were all used as received.

Two samples of partially hydrolyzed poly-(vinyl acetate) were used as received: (i) 88% hydrolyzed material (BDH), having a nominal relative molar mass of 125,000 (PVA-88); (ii) 96% hydrolyzed material (Aldrich Chemicals), having a nominal relative molar mass of 96,000 polyvinyl alcohol (PVA-96).

Dispersion polymerization reactions were carried out in 500 ml flasks. Initially 0.3 g of PVA plus 8.83 g (0.033 moles) of $FeCl_3 \cdot 6 H_2O$ were dissolved in 100 ml of water. To this solution was added 1 ml (0.014 moles) of purified pyrrole monomer, and the mixture was stirred magnetically at 20xC for 18-14 h. It was shown in all cases that greater than 95% conversion of monomer was achieved within this time. Within a few seconds of adding the pyrrole to the reaction mixture, the color changed from orange to

brown-black, indicative of the onset of polymerization, but no precipitate was formed. At the end of the reaction period the dispersions formed were centrifuged at 15,000 rpm for about 1 h. This led to a black sediment and a pale green, but transparent supernatant. Indeed, it was subsequently shown by visible absorption spectroscopy that the supernatant contained no polypyrrole. The sediment could be readily redispersed by shaking in pure water. The dispersions formed in this way showed no tendency to aggregate on standing. The aqueous dispersions were freeze-dried to yield a fine, black powder.

Various other examples and modifications of the foregoing examples will be apparent to the person skilled in the art after reading the present disclosure without departing from the spirit and scope of the invention and it is intended that all such examples and modifications be included within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. In a process for stimulating and/or growing cells in a nutrient medium the improvement which comprises providing an electrically conductive material contacting the cells and passing an electrical current or voltage through said medium in an amount to promote growth and proliferation of said cells.

2. The process of claim 1 wherein said electrical current is between about 0.5 microamps to about 1 milliamp.

3. The process of claim 1 wherein said cell is selected from the group consisting of mammalian cells, avian cells, amphibian cells, microorganism and plant cells.

4. The process of claim 1 wherein said medium comprises a polymeric support.

5. The process of claim 4 wherein said polymeric support is electrically conductive.

6. The process of claim 4 wherein said polymeric support includes a polymer selected from the group consisting of collagen, elastin, guar gum and gelatin.

7. The process of claim 6 wherein said polymer has been made electrolytically conductive by the incorporation of or association with an electrically conductive polymer.

8. The process of claim 7 wherein said electrically conductive polymer is selected from the group consisting of polyacetylene, polypyrrole, polypyrrole-cellulose, polyaniline-cellulose, polypyrrole-polyvinyl alcohol and polypyrrole-collagen.

9. The process of claim 1 comprising surrounding cells with a latex of a conducting polymer and passing an electrical charge through said latex.

10. The process of claim 1 wherein said electrically conductive medium is a polymer and said polymer is ingested by said cells.

11. The process of claim 1 wherein said electrically conductive medium is a polymer and said polymer is linked to an antibody through a linking group.

12. The process of claim 11 wherein said linking group is an alkyl diamine.

13. An apparatus for growing cells, comprising:

an electrically conductive material, and

means for passing an electrical current through the material.

14. The apparatus of claim 13 wherein the electrically conductive material is a support material in association with an electrically conductive polymer.

15. The apparatus of claim 14 wherein the support material is selected from the group consisting of nylon, polyvinyl chloride, polyvinyl alcohol, polyvinyl acetate, polyvinyl butyral, polyethelene glycol, gelatin, collagen, guar gum, elastin, glycoproteins, carotenoids, hemins, diazobenzyloxymethyl, nitrocellulose, and paper.

16. The apparatus of claim 14 wherein the electrically conductive polymer is selected from the group consisting of polyacetylene, polypyrrole, polythiophene, and polystyrene.

17. The apparatus of claim 13 wherein the electrically conductive material is capable of supporting cells which grow by attachment.

18. The apparatus of claim 13 wherein the material is ingested by the cells to be grown.

19. The apparatus of claim 13 wherein the electrically conductive material is linked to an antibody specific for the cell to be grown.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/03737

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): C12N 13/00 C12M 3/00

U.S. 435/173 435/284

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/173,177,178,179,180,181,182,240.2,240.21,240.23, 240.241,240.243,240.4,242,243,284,285,286,317.1, 530/387,389,390,391,811,812,813,814,815,816 attachment.

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

CAS,BIOSIS databases, searched with Keywords: conductiv?, polymer, pyrrole, aniline, acetylene, see attachment.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 3,871,961 (Gianessi) 18 March 1975, see column 1, lines 26-39 and 53-63 and the claims.	1,3,13
X Y	US, A, 4,085,254 (Atkins) 18 April 1978, see column 3, line 28 - column 4, line 14; column 6, line 14 - column 7, line 17 and claims 1 and 11.	13,18 1,3-5, 9,10, 14-17
Y	US, A, 4,521,450 (Bjorklund et al) 05 June 1985, see column 1, line 63 - column 2, line 59.	4-8, 14-17
Y	US, A, 4,604,427 (Roberts et al.) 04 August 1986, see column 2, line 64 - column 4, line 61.	4-8, 14-17
Y	US, A, 4,687,808 (Jarrett et al.) 18 August 1987, see column 5, line 25 - column 8, line 52.	11,12,19

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
08 February 1989	15 MAR 1989
International Searching Authority: ISA/US	Signature of Authorized Officer: Elizabeth C. Weimar ELIZABETH C. WEIMAR

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, 16 with indication, where appropriate. of the relevant passages 17	Relevant to Claim No 18
X	WO, A, 87/02705 (Sweeney) 07 May 1987 see pages 2 and 3 and the claims.	1-3,13
X Y	SU, A, 0552353 (Shigaeva) 05 May 1977, see abstract and figure.	1,3,13 2,4

Attachmentto PCT/ISA/210
II. Fields Searched

U.S. Classification Symbols:

204/180.1, 131

252/500

429/2

47/1.3

Keywords: collagen, elastin, guar, gelatin, cellulose, nylon, thiophene, support, matrix, latex, culture, microbe, bacteria, yeast, animal, plant, growth, stimulat?, electric?, current, alkyl, diamine, linker, antibod?, poly vinyl alcohol, PVA.