

Description

Background of the Invention

The technical field of this invention concerns the encapsulation of living cells for the production of biologically active factors.

There is considerable interest at present in the biologically active products of living cells, including, for example, neurotransmitters, hormones, cytokines, nerve growth factors, angiogenesis factors, blood coagulation factors, lymphokines, enzymes and other therapeutic agents. There is also substantial interest in developing new methods and systems for producing such biological factors as well as in delivering these factors to subjects for therapeutic purposes.

For example, Parkinson's disease is characterized by the degeneration of the dopaminergic nigrostriatal system. Striatal implantation of polymer rods which release sustained amounts of a neurotransmitter, dopamine, has been reported to alleviate experimental Parkinsonism in rodents, indicating that the release of dopamine alone in the proper target structure may be able to correct this functional deficiency.

In contrast to the limited capacity of a polymeric matrix drug release system, encapsulated dopamine-releasing cells have been proposed as a means to provide a continuous supply of neurotransmitters. The encapsulation of neurotransmitter-secreting cells by a permselective membrane which permits diffusion of the biological factor may not only prohibit the escape of mitotically active cells, but also prevent host rejection in the case of cross-species transplantation.

A number of researchers have proposed the use of microcapsules -- tiny spheres which encapsulate a microscopic droplet of a cell solution -- for both therapeutic implantation purposes and large scale production of biological products. However, there are a number of shortcomings to the microencapsulation approach: the microcapsules can be extremely difficult to handle (and retrieve, after implantation); their volume is limited; and the types of encapsulating materials which can be used are constrained (by the formation process) to polymers which can dissolve in biocompatible solvents.

An alternative approach has been macroencapsulation, which typically involves loading cells into hollow fibers and then closing the extremities at both ends with a polymer glue. In contrast to microcapsules, macrocapsules offer the advantage of easy retrievability, an important feature in therapeutic (especially, neural) implants. However, the construction of macrocapsules in the past has often been tedious and labor intensive. Moreover, due to

unreliable closure, conventional methods of macroencapsulation have provided inconsistent results.

The cell-containing beads disclosed in GB-A-2 192 171 are produced using an alginate solution to encapsulate a cell suspension in an aqueous culture medium. The alginate solution is delivered through an annulus formed by two concentric bores, with the cell suspension delivered via the inner bore. The cell suspension and alginate solution are extruded together to form droplets which are blown off the end of the needle by a coaxial air stream. Each droplet falls into a curing bath (e.g. CaCl_2), and only then is the alginate gelled or coagulated to form a firm outer shell.

The curing bath is then changed to a glyoxal-containing buffer which penetrates the alginate shell and cross-links a pre-polymer (e.g. PAAH) present in the cell suspension. The morphology of the encapsulate produced by this methodology is a bead having an inner sphere of a cross-linked cell suspension, concentric with an outer sphere of alginate. The reagent systems are chosen such that both the alginate and polymer components each precipitate slowly, with the curing bath serving to ensure that formation of the alginate shell occurs in the bath.

The need to facilitate precipitation of the alginate shell in the curing bath rather than at the extrusion bore comes from the desire to have the alginate solution entirely surround the cell suspension (e.g. by surface tension) before it is hardened, and thereby produce a completely sealed spherical bead. Consequently, the prior art technique is specifically designed to permit time for the alginate solution to surround the core material before solidification, the morphology of the bead is established during the fall of the droplet from the needle, and is stabilized in the curing bath.

Patent Abstracts of Japan, vol. 11, no. 315 (C-451)(2762), 14 October 1987, discloses that drops of a cell suspension and film forming agent are sprayed from a nozzle into a solution of gelling agent.

GB-A-1538 510 teaches that drops of encapsulate are first allowed to form into spheres before the film material surrounding the capsule is hardened. For example, it is set forth that as the jet stream moves downward it is squeezed or constricted and is cut off drop by drop so that it forms spherical capsules of uniform diameter.

There exists a need for better techniques for macroencapsulation of cells for both therapeutic implantation and industrial production purposes. Encapsulation techniques which can be practiced in an automated fashion, and which permit the usage of a wider range of materials and/or provide more reliable closure would satisfy a long felt need

in the art.

Summary of the Invention

Methods and systems are disclosed for encapsulating viable cells which produce biologically-active factors. The cells are encapsulated within a semipermeable, polymeric membrane by co-extruding an aqueous cell suspension and a polymeric solution through a common port to form a tubular extrudate having a polymeric outer coating which encapsulates the cell suspension.

In one aspect of the invention, methods are disclosed in which the cell suspension and the polymeric solution are extruded through a common extrusion port having at least two concentric bores, such that the cell suspension is extruded through the inner bore and the polymeric solution is extruded through the outer bore. The polymeric solution coagulates to form an outer coating.

Thus more specifically, the invention provides a method of encapsulating viable cells, the method comprising co-extruding an aqueous cell suspension and a polymeric solution through a common extrusion port having inner and outer bores from which the suspension and polymeric solution respectively issue, said solution on co-extrusion through the port coagulating to form a tubular extrudate having a semipermeable polymeric outer coating which encapsulates said cell suspension.

As the outer coating is formed, the ends of the tubular extrudate can be sealed to form a cell capsule. In one illustrated embodiment, the tubular extrudate is sealed at intervals to define separate cell compartments connected by polymeric links.

Strings of cell capsules formed in this manner have a number of advantages over conventional, cell-encapsulating products. The multi-compartment form ensures that breaks in the tubular membrane can be contained to individual cell capsules. Moreover, the design is particularly advantageous in preparing implantable cell cultures for delivery of biologically-active factors to a subject for therapeutic purposes. The string of cell capsules can be coiled, twisted or otherwise deposited in various shapes to provide a dense and compact structure for implantation. Because the cell capsules are connected to each other, they can also be readily retrieved, if necessary, following implantation. The string-like nature of these products is particularly preferable over individual spherical microcapsules which typically are retrieved by aspiration (often resulting in a high percentage of unretrievable capsules and, consequently, inflammation in the subject).

Multi-compartment cell capsule strings can be formed from the tubular extrudate of the present invention by sealing the extrudate at intervals using

various techniques. For example, the extrudate can be sealed by compressing it at intervals using mechanical or pneumatic force. Alternatively, the pressure under which the cell suspension or the polymeric solution is extruded can be modified to collapse the tubular extrudate at intervals and define separate cell compartments. In yet another technique, the flow of the cell suspension can be interrupted or otherwise impeded at intervals to likewise collapse the tubular extrudate and define cell compartments.

The products of the present invention are particularly well-suited for use in therapeutic implant devices, such as those disclosed in U.S. Patent 4,892,538, "In Vivo Delivery Of Neurotransmitters By Implanted, Encapsulated Cells" by Aebischer et al. issued January 9, 1990, herein incorporated by reference. In U.S. Patent 4,892,538, techniques are disclosed for implanting encapsulated neurotransmitter-secreting cells into a target region within a subject's brain, such that the encapsulated cells secrete a neurotransmitter and thereby permit constitutive delivery of a therapeutic agent to treat a neurological deficiency, such as Parkinson's disease. Alternatively, artificial organs capable of secreting other biological factors, such as hormones (e.g., insulin, thymic factors and the like) can also be constructed using the tubular extrudates and multi-compartment cell capsule strings of the present invention.

The cell capsules are also well-suited for use in bioreactors and other *in vitro* culturing systems, for the production of drugs and other useful biological materials. In such applications, cells which produce such materials, either naturally, by mutation or by recombinant design, are encapsulated and allowed to synthesize the materials which can be collected following secretion into a circulating culture medium. Alternatively, the biological materials can be accumulated within the cell capsules (e.g., by appropriate control of the porosity) and then harvested by removing the strands from the culture medium, lyzing the polymeric membranes and recovering the synthesized materials in concentrated form.

The polymeric coating is preferably a semipermeable membrane, that is to say, a porous structure capable of protecting transplanted cells from autoimmune or viral assault, as well as from other detrimental agents in the external environment, while allowing essential nutrients, cellular waste products and cell secretions to diffuse therethrough. As used herein, the term "selectively permeable" or "semipermeable" is used to describe biocompatible membranes which allow diffusion therethrough of solutes having a molecular weight up to about 150,000 (Mr).

The permeability of the polymeric coating can be varied by controlling the viscosity of the polymeric solution, such that upon coagulation, the coating will form with a network of microchannels to provide diffusion pathways. In one embodiment, this can be achieved by employing a water-miscible solvent as a component of the polymeric solution and maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during extrusion. As the tubular extrudate forms, water from the aqueous cell suspension infiltrates into the coagulating polymer to replace the solvent as the solvent is driven outward by the pressure difference. Upon coagulation, the water which has infiltrated into the polymeric coating provides a network of pores. The optimal pressure and viscosity will, of course, vary with the solvent and polymer employed but can be readily ascertained for any particular polymer/solvent combination by those skilled in the art without undue experimentation.

In another aspect of the invention, systems are disclosed for encapsulating cells to produce the tubular extrudate and multi-compartment cell capsule products described above. This system can include an extrusion head assembly (e.g., a spinneret or the like) having a first inner bore and a second, concentric, outer bore, as well as a cell suspension supply means for supplying the aqueous cell suspension to the inner bore of the extrusion head assembly, and a polymeric solution supply means for supplying the polymeric solution to the outer pore of the extrusion head assembly. As the cell suspension and polymeric solution are co-extruded, they form a tubular extrudate having a polymeric outer coating which encapsulates the cell suspension.

Such systems may be embodied according to a further aspect of the invention in apparatus for encapsulating viable cells, comprising:

an extrusion head assembly having at least a first inner bore and a second, concentric, outer bore;

cell suspension supply means for supplying an aqueous cell suspension to the inner bore of said extrusion head assembly;

polymeric solution supply means for supplying a polymeric solution to the outer bore of the extrusion head assembly, and pressure differential means to provide a positive pressure differential between the cell suspension and the polymeric solution in the course of extrusion such that said cell suspension and said polymeric solution on co-extrusion from said head assembly form a tubular extrudate having a coagulated polymeric outer coating which encapsulates said cell suspension, said positive pressure differential in use driving polymer solvent outwardly away from the cell sus-

pension during coagulation.

The tubular extrudate can be sealed at intervals by any one of a number of mechanisms. In one illustrated embodiment, two wheels with occluding elements on their periphery cooperate in rotation to periodically pinch the tubular extrudate and thereby seal it. This mechanical compression system can be replaced by a variety of other mechanical or pneumatic compression systems to seal the tubular extrudate at intervals.

Alternatively, the system can include a flow control means for varying the pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion. For example, each of the components supply means can include an infusion pump which is operated by a computer or other control element. In the normal operation, the infusion pumps are controlled to maintain a pressure differential between the aqueous cell suspension and the polymeric solution, such that the polymer solvent is driven outward during coagulation. By periodically varying the pressure, the tubular extrudate can be collapsed at intervals to define individual cell compartments. This can be accomplished, for example, by reducing the aqueous solution pressure. In some instances, it may be preferable to terminate the flow of the aqueous solution entirely and create a vacuum to ensure a complete seal between compartments.

Various other techniques can likewise be employed to interrupt the flow of the aqueous solution at intervals and thereby cause the tubular extrudate to collapse and form multiple compartments. For example, a retraction mechanism can be incorporated into the extrusion head assembly for moving the inner bore relative to the outer bore, such that the flow of the aqueous solution is interrupted to define separate cell compartments at intervals.

The systems disclosed herein can further include a quenchant bath for coagulating the polymeric solution following extrusion, and various mechanisms for drying the tubular extrudate as it emerges from the extrusion head, including blowers, or evacuation chambers. The extrusion head assembly can incorporate additional bores to provide multiple coatings or to deliver a quenchant fluid about the tubular extrudate. The system can also include a sedimentation chamber for the cell suspension, or an equivalent cell packing mechanism, to increase the cell density within the aqueous cell suspension.

The invention will next be described in connection with certain illustrated embodiments; however, it should be clear that various additions, subtractions or modifications can be made by those skilled in the art without departing from the spirit or scope of the invention.

Brief Description of the Drawings

FIG. 1 is an overall schematic diagram of a system for encapsulating viable cells according to the invention;

FIG. 2 is a more detailed schematic diagram of an extrusion head assembly for use in the system of FIG. 1;

FIG. 3 is a schematic diagram of an alternative extrusion head assembly for use in the system of FIG. 1;

FIG. 4 is a schematic diagram of a mechanism for periodically sealing a tubular extrudate according to the invention to form a multi-compartment cell culturing vehicle;

FIG. 5 is a schematic diagram of a mechanism for forming tethered cell capsules;

FIG. 6 is a graph showing dopamine release versus time for capsules containing dopamine secreting cells produced according to the present invention with three different solvent systems;

FIG. 7 is graph showing dopamine release by PC12 cells under normal and potassium-stimulated conditions at various times following encapsulation according to the invention;

FIG. 8A is a graph showing the release of catecholamines from encapsulated PC12 cells; and

FIG. 8B is a graph showing the release of catecholamines from encapsulated chromaffin cells.

Detailed Description

In FIG. 1, a system 10 is shown for producing a tubular extrudate 12 according to the present invention, including an extrusion head 14 having a first (innermost) bore 16, a second outer bore 18 and, optionally, a third (outermost) bore 20. The system 10 further includes a cell suspension supply 22 and an associated pump 24, a polymer solution supply 26 and an associated pump 28 and, optionally, a flush solution supply 30 with a pump 32. Additionally, the system can also, optionally, include a outer flowing quenchant supply 34 with an associated pump 36. All of the pump elements can be controlled manually or, preferably, by an automated controller (e.g., a microprocessor) 38. The system 10 can also include a quenchant bath 40, which would normally be disposed directly below the extrusion head 14 during operation. Alternatively, the system can include a blower 41 or the system can be employed within an evacuated or other reduced pressure chamber to aid in solvent removal.

When the system 10 is employed to shape the tubular extrudate into a multi-compartment cell

capsule string, a sealing means can be employed. One such sealing element 42 is shown in FIG. 1, including two motorized wheels 44A and 44B which have a series of protuberances 46 which cooperate during rotation to periodically pinch and seal the tubular extrudate as it passes between the wheels 44A and 44B.

Alternatively, a retraction means 48 can be employed to periodically retract the inner bore so as to interrupt the flow of the cell suspension. The effect of these retractions is to periodically seal the tubular extrudate and again form multiple compartments. In yet another alternative approach, the controller 38 can vary the pressure applied by pump 24 (and/or pump 28) to create periodic interruptions in the flow of the cell suspension.

In FIG. 2, the extrusion head 14 is shown in more detail, including an inner bore 16 for delivery of a cell suspension and an outer bore 18 for delivery of a polymeric solution. As the cell suspension and the polymeric solution are extruded through the common extrusion pore 19, the polymeric solution coagulates to form an outer coating about the cell suspension.

In FIG. 3, an alternative extrusion head 14A is shown in more detail comprising an inner bore 16 for the delivery of the cell suspension, a second bore 18 (surrounding the inner bore) for delivery of the polymeric solution, and an outer most bore 20 for delivery of a flowing quenchant fluid, such as saline. In this embodiment, a smooth coating can be obtained by simultaneously extruding the cell suspension and polymeric solution through common pore 19 while applying a flowing quenchant fluid during the extrusion (e.g., from the outer most bore 20 in the extrusion head assembly 14A.)

In FIG. 4, the sealing element 42 of FIG. 1 is shown in more detail. Motorized wheels 44A and 44B are mounted on opposite sides of the tubular extrudate 12, such that upon rotation protuberances 46 on the wheels periodically come in contact with the extrudate 12 to pinch and seal the extrudate 12 as it exits the extrusion head 14. The wheels 44A and 44B can be mechanically linked and operated by a conventional motor under the control of a controller, such as shown in FIG. 1. The result of the periodic sealing of the extrudate 12 is a multi-compartment macrocapsule strand 50 having a polymeric membrane 52 surrounding an encapsulated cell solution 54 with individual cells 56 disposed therein. The individual cell capsules are joined to each other by connective filaments 58 where the protuberances 46 of the sealing means 42 have pinched the extrudate 12.

Various polymers can be used to form the membrane coatings of the present invention, including polymers derived from solutions which would otherwise be incompatible with the propaga-

tion of living cells. Because of the unique extrusion process disclosed in the present invention, solvents which would otherwise be toxic are quickly driven away from the aqueous cell suspension during the membrane formation process, thereby permitting the use of many new and potentially useful polymeric materials. For example, polymeric membranes can be formed from polyacrylates (including acrylic copolymers), polyvinylidenes, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones, polyacrylonitriles, as well as derivatives, copolymers, and mixtures thereof.

The solvent for the polymer solution will depend upon the particular polymer chosen for the membrane material. Suitable solvents include a wide variety of organic solvents, such as alcohols and ketones, generally, as well as dimethylsulfoxide (DMSO), dimethylacetamide (DMA) and dimethylformimide (DMF), in particular. In general, water-miscible organic solvents are preferred.

The polymeric solution or "dope" can also include various additives, including surfactants to enhance the formation of porous channels, as well as antioxidants to sequester oxides that are formed during the coagulation process. Moreover, when the cell capsules of the present invention are designed for implantation, materials, such as anti-inflammatory agents and cell growth factors, can also be incorporated into the polymeric membrane to reduce immune response or stimulate the cell culture, respectively. Alternatively, these materials can be added to the multi-compartment cell capsule strands after formation by a post-coating or spraying process. For example, the strands can be immersed in a solution which contains an anti-inflammatory agent, such as a corticoid, an angiogenic factor, or a growth factor following extrusion to post-coat the cell capsules.

Post coating procedures can also be used to provide a protective barrier against immunogens and the like. For example, after formation, the cell capsule strands can be coated (e.g., by immersion, spraying or applying a flowing fluid during extrusion) with a surface protecting material, such as polyethylene oxide or polypropylene oxide (e.g., having a molecular weight of about 10,000 Daltons or greater), to inhibit protein interactions with the capsules.

Various techniques can also be employed to control the smoothness or roughness of the outer surface of the polymeric coating. In some instances, a very smooth outer coating can be preferable to reduce scar tissue attachment and other immunoreactions during implantation. Such a smooth coating can be obtained by simultaneously immersing the tubular extrudate in a quenchant, such as a bath of physiological saline, or by apply-

ing a flowing, quenchant fluid during the extrusion (e.g., from a third, concentric, outermost bore in an extrusion head assembly). Alternatively, in some applications a rough outer surface with larger pores may be desired, for example, in instances where capillary ingrowth during implantation is desired, and such a rougher outer surface can be obtained by coagulation in air.

Various cell lines can be encapsulated according to the present invention. As noted above, the multi-compartment cell culture strings are particularly useful for the constitutive delivery of neurotransmitters, such as dopamine, which is secreted by cells of the adrenal medulla, embryonic ventral mesencephalic tissue and neuroblastic cell lines. PC12 cells (an immortalized cell line derived from a rat pheocromocytoma) are particularly preferred in some applications because of their ability to secrete large amounts of dopamine over long periods of time. Other neurotransmitters include gamma aminobutyric acid (GABA), serotonin, acetylcholine, noradrenaline and other compounds necessary for normal nerve functions. A number of cell lines are known or can be isolated which secrete these neurotransmitters. Cells can also be employed which synthesize and secrete agonists, analogs, derivatives or fragments of neurotransmitters which are active, including, for example, cells which secrete bromocriptine, a dopamine agonist, and cells which secrete L-dopa, a dopamine precursor.

In other embodiments of the invention, the encapsulated cells can be chosen for their secretion of hormones, cytokines, nerve growth factors, angiogenesis factors, antibodies, blood coagulation factors, lymphokines, enzymes, and other therapeutic agents.

The aqueous cell suspensions can further include various additives to protect the cells during the extrusion process or to stimulate their growth subsequently. Such additives can include, for example a nutrient medium or growth factors which are incorporated into the aqueous suspension, as well as an anchorage substrate material to enhance cell attachment. The anchorage substrate material can be a proteinaceous material, such as collagen, laminin, or polyamino acids. Alternatively, the cell suspension or the polymeric solution (or both) can include a foaming agent or a blowing agent which can distort the inner surface of the polymeric coating to increase the anchorage surface area of the tubular interior.

The products of the present invention can take various forms, including simple tubular extrudates as well as multi-compartment cell capsule strings. The shape of the multi-compartment strings can be tubular, resembling sausages, or nearly spherical, resembling strings of pearls. The maximum outer

diameter of the strand will typically range from about 0.1 to about 1.0 millimeters. The membrane wall thickness will typically range from about 10 to about 100 micrometers. The strand length of the strands will vary depending upon the particular application.

The products can also take the form of "tethered" cell capsules, that is, one or more individual cell compartments are connected to a long polymeric tube or string. In FIG. 5, such a tethered cell capsule 51 is shown having a polymeric membrane 52 surrounding an encapsulated cell solution 54 with individual cells 56 disposed therein. The cell capsule 51 further includes a long polymeric filament 59 which can be formed by the same apparatus as described above in connection with FIG. 4 by interrupting the flow of the cell solution and constraining the polymeric solution to form a solid tether. The tether also can be post coated with a material (e.g., a polyurethane or the like) which imparts additional strength to the filament. Such tether cell capsules can find a variety of applications, particularly when implanted in a subject for constitutive delivery of active factors. In use, the cell capsule can be located as close to the target region (e.g., in the brain, peritoneal cavity or elsewhere) as desired while the other end of the tether can be fixed at a convenient anchor point or disposal in a readily accessible location for retrieval.

The invention will next be described in connection with certain illustrative, non-limiting examples:

EXAMPLES

An extrusion system similar to that illustrated in FIG. 1 was used, consisting of three electronically controlled programmable infusion pumps, a jet spinneret, two motor-controlled, coaxial wheel systems on the perimeter of which occluding polytetrafluoroethylene tubes were mounted, and an uptake system.

The macrocapsules were formed by injection of a polymeric solution into the outer tube of the spinneret. A coagulant, typically the encapsulated cells in their culture medium, was simultaneously injected in the spinneret inner tube. The encapsulating membrane was formed by a dry-jet, wet spinning process, i.e., the fast stabilization of the polymer solution emerging from the spinneret nozzle by the internal quench medium coupled with further stabilization in a quench bath. The closure of the fiber was performed by mechanically squeezing the forming hollow fiber with the coaxial wheel system prior to immersion in the quench bath. Near the spinneret head, the solvent concentration was sufficiently high to allow proper fusion of the fiber wall. Following each round of encapsulation, pure solvent was flushed automati-

cally through the lumen of the spinneret to avoid clogging of the nozzle.

PC12 cells, an immortalized cell line derived from a rat pheochromocytoma which secretes large amounts of dopamine, were cultivated on collagen-coated tissue culture dishes in RPMI 1640 medium supplemented with 10% heat inactivated horse serum and 5% fetal calf serum. Dissociated bovine adrenal medullary cells, a non-dividing cell type which secretes dopamine, were maintained in DMEM medium supplemented with 5% fetal calf serum. Prior to encapsulation, the cells were harvested and loaded at a concentration of 1×10^5 cells/ml in a 3 ml syringe. A 15 percent vinylchloride-acrylonitrile copolymer solution in either dimethylsulfoxide (DMSO), dimethylformamide (DMF), or dimethylacetamide (DMA) was loaded into a 5 ml glass syringe. Both solutions were then coextruded through the spinneret, and the capsules were collected in a physiologic saline solution. The capsules were rinsed and placed in individual wells containing the appropriate culture media.

Basal and potassium-evoked release of catecholamines was quantified under static incubation conditions by ion-pair reverse-phase high performance liquid chromatography (HPLC) equipped with electrochemical detection at 2 and 4 weeks. Morphological analysis, including light, scanning, and transmission electron microscopy, was performed on representative samples for each time period.

All cell-loaded capsules released dopamine into the medium under basal conditions at all time periods. High potassium treatment increased dopamine release from both PC12 and adrenal medullary cells. Dopamine output by PC12 cells, but not adrenal medullary cells, increased with time. The increase in dopamine release by the PC12 cell-loaded capsules over time is believed to be related to cell proliferation within the polymer capsule. No significant difference in dopamine release could be observed from PC12-loaded capsules extruded with the three different solvent systems (DMSO, DMF, DMA), which suggests that the encapsulation technique of the present invention may prevent cell damage inflicted by solvents (FIG. 6). Due to the higher pressure of the inner bore system, the solvent was quickly driven toward the outside of the polymer capsule which prevented extended cell-solvent contact.

Morphological analysis revealed the presence of small clusters of PC12 cells randomly dispersed throughout the lumen of the capsule. At the electron microscope level, well-preserved PC12 cells, with their typical electron-dense secretory granules, could be observed. Cell division within the capsule space was suggested by the presence of numerous mitotic figures. Although initially coextruded as

a cell suspension, adrenal chromaffin cells formed packed aggregates one week after encapsulation.

FIG. 7 shows the results of an *in vitro* assay in which PC12 cells were encapsulated according to the present invention and monitored for release of dopamine at two and four weeks following encapsulation. Dopamine levels were measured under both normal (controlled) conditions and also under a high potassium stimulation, which is known to induce depolarization of the cells and, consequently, to increase the secretion of dopamine in viable cells. As can be seen from the graph, there was little activity at two weeks; however, at four weeks the encapsulated cells exhibited dopamine secretions not only under normal conditions but also exhibited a strong response to the potassium stimulation, indicating that the cells were indeed viable in their encapsulated state.

FIG. 8A and 8B show the results of further *in vitro* assays in which the secretions of PC12 cells and chromaffin cells, respectively, were monitored four weeks after encapsulation according to the present invention. Again, the cells were stimulated by high potassium concentrations and the medium while the PC12 cells released only dopamine, the chromaffin cells released a variety of catecholamines. The graph shows the levels of noradrenaline (NE), epinephrine (EPI), and dopamine (DA).

Due to their fluid dynamics, the macrocapsules extruded in accordance with the present invention will allow the use of a wider range of polymer/solvent systems and can constitute a more efficient encapsulation technique. The results show that immortalized and differentiated dopamine-secreting cells will survive in macroencapsulation. The ability of these capsules to spontaneously release dopamine over time suggests that polymer encapsulation can provide an alternative to the transplantation of non-encapsulated or microencapsulated dopamine-secreting cells in the treatment of Parkinson's disease.

Claims

1. A method of encapsulating viable cells, the method comprising co-extruding an aqueous cell suspension and a polymeric solution through a common extrusion port having inner and outer bores from which the suspension and polymeric solution respectively issue, said solution on co-extrusion through the port coagulating to form a tubular extrudate having a semipermeable polymeric outer coating which encapsulates said cell suspension.
2. The method of claim 1, which further includes sealing the tubular extrudate at intervals to

define separate cell compartments connected by polymeric links.

3. The method of claim 2, wherein the step of sealing the extrudate comprises compressing the extrudate at intervals to define the separate cell compartments.
4. The method of claim 2, wherein the step of sealing the extrudate comprises modifying the pressure under which the cell suspension or the polymeric solution is extruded, thereby collapsing the tubular extrudate at intervals to define the separate cell compartments.
5. The method of claim 2, wherein the step of sealing the extrudate comprises impeding the flow of the cell suspension at intervals to collapse the tubular extrudate and thereby define separate cell compartments.
6. The method of claim 1, wherein the polymeric coating is coagulated in air.
7. The method of claim 1, wherein the polymeric coating is coagulated in a reduced pressure chamber.
8. The method of claim 1, wherein the polymeric coating is coagulated in a quenchant bath.
9. The method of claim 1, which comprises extruding an aqueous suspension containing cells that secrete a biologically-active factor such as a neurotransmitter.
10. The method of claim 1, wherein the extruded aqueous cell suspension contains a nutrient medium.
11. The method of claim 1, wherein the extruded aqueous cell suspension contains an anchorage substrate.
12. The method of claim 1, wherein the polymeric solution comprises a water-miscible solvent component.
13. The method of claim 1, wherein the polymeric solution further comprises a surfactant and/or an anti-inflammatory agent and/or an antioxidant.
14. The method of claim 1, which further comprises controlling the viscosity of said polymeric solution.

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FIG. 8A and 8B shows the results of further *in vitro* assays in which the secretions of PC12 cells and chromaffin cells, respectively, were monitored four weeks after encapsulation according to the present invention. Again, the cells were stimulated by high potassium concentrations and the medium while the PC12 cells released only dopamine, the chromaffin cells released a variety of catecholamines. The graph shows the levels of noradrenaline (NE), epinephrine (EPI), and dopamine (DA).

Due to their fluid dynamics, the macrocapsules extruded in accordance with the present invention will allow the use of a wider range of polymer/solvent systems and can constitute a more efficient encapsulation technique. The results show that immortalized and differentiated dopamine-secreting cells will survive in macroencapsulation. The ability of these capsules to spontaneously release dopamine over time suggests that polymer encapsulation can provide an alternative to the transplantation of non-encapsulated or microencapsulated dopamine-secreting cells in the treatment of Parkinson's disease.

Claims

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11. The method of claim 1, wherein the extruded aqueous cell suspension contains an anchorage substrate.
12. The method of claim 1, wherein the polymeric solution comprises a water-miscible solvent component.
13. The method of claim 1, wherein the polymeric solution further comprises a surfactant and/or an anti-inflammatory agent and/or an antioxidant.
14. The method of claim 1, which further comprises controlling the viscosity of said polymeric solution.



15. The method of claim 1, which further comprises maintaining a pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion to impede solvent diffusion from said polymeric solution into said cell suspension.
16. The method of claim 1, which further comprises applying a protective barrier material to the outside of the polymeric coating.
17. A cell culturing vehicle (50, 51) comprising a tubular, semipermeable, polymeric membrane (52) encasing a cell culture (54), said vehicle having been formed by co-extruding the cell culture and a polymeric solution through a common co-extrusion port having inner and outer bores to which the culture and polymeric solution are respectively supplied, said culture and polymeric solution having been selected such that the solution coagulates, forming said membrane, on co-extrusion with the culture through the common port.
18. The vehicle of claim 17, wherein the membrane (52) is permeable to molecules having a molecular weight of about 150,000 or less.
19. The vehicle of claim 17, wherein the maximum outer diameter of the cell culture vehicle ranges from 0.1 to 1.0 millimeters.
20. The vehicle of claim 17, wherein the membrane (52) has a wall thickness ranging from 10 to 100 μm .
21. The vehicle of claim 17, wherein the polymeric membrane (52) comprises a polyacrylate material.
22. The vehicle of claim 17, wherein the polymeric membrane (52) comprises a surfactant, and/or an anti-inflammatory agent and/or an anti-oxidant.
23. The vehicle of claim 17, wherein the extruded tubular membrane (52) is sealed at intervals (at 58) to define separate cell compartments (56) connected by polymeric links (58).
24. The vehicle of claim 17, wherein the tubular membrane is sealed to define at least one cell compartment (56) connected to a tethering filament (59).
25. The vehicle of claim 17, wherein the cell culture further comprises an aqueous cell suspension containing cells that secrete a biologically-active factor, e.g. a therapeutic factor.
26. The vehicle of claim 17, wherein the aqueous cell suspension further comprises a nutrient medium.
27. The vehicle of claim 17, wherein the cell culture further comprises an anchorage substrate material.
28. The vehicle of claim 27, wherein the substrate material is selected from a collagen material, a laminin material and a polyamino acid.
29. The vehicle of claim 17, which further comprises a protective barrier material coating at least a portion of the outer surface of the polymeric membrane (52).
30. The vehicle of claim 29, wherein the protective barrier material is an inhibitor of protein interactions.
31. The vehicle of claim 29, wherein the protective barrier material is selected from polyethylene oxides, polypropylene oxides, derivatives and mixtures thereof.
32. Apparatus for encapsulating viable cells, comprising:
 an extrusion head assembly (14) having at least a first inner bore (16) and a second, concentric, outer bore (18);
 cell suspension supply means (22) for supplying an aqueous cell suspension to the inner bore (16) of said extrusion head assembly (14);
 polymeric solution supply means (26) for supplying a polymeric solution to the outer bore (18) of the extrusion head assembly, and pressure differential means to provide a positive pressure differential between the cell suspension and the polymeric solution in the course of extrusion such that said cell suspension and said polymeric solution on co-extrusion from said head assembly (14) form a tubular extrudate having a coagulated polymeric outer coating which encapsulates said cell suspension, said positive pressure differential in use driving polymer solvent outwardly away from the cell suspension during coagulation.
33. The apparatus of claim 32, wherein the pressure differential means comprises flow control means for maintaining the pressure differential between the aqueous cell suspension and the polymeric solution during co-extrusion.



34. The apparatus of claim 33, wherein the flow control means comprises a computer (38) adapted to control a first infusion pump (24) in said cell suspension supply means (22) and a second infusion pump (28) in said polymeric solution supply means (26).

35. The apparatus of claim 32, which further comprises a quenchant bath (40) for coagulating said polymeric solution following extrusion.

36. The apparatus of claim 32, which comprises sealing means for sealing the tubular extrudate at intervals to define separate cell compartments (54) connected by polymeric links (58).

37. The apparatus of claim 36, wherein the sealing means (42) comprises means (44a, 44b, 46) for compressing the extrudate at intervals during extrusion to define the separate cell compartments.

38. The apparatus of claim 36, wherein the sealing means is operable to modify the pressure under which the cell suspension or the polymeric solution is extruded, thereby collapsing the tubular extrudate at intervals to define the separate cell compartments.

39. The apparatus of claim 36, wherein the sealing means comprises means (48) for impeding the flow of the cell suspension at intervals to collapse the tubular extrudate and define the separate cell compartments.

40. The apparatus of claim 36, wherein the sealing means comprises a retraction means (48) for moving the inner bore (16) of said extrusion head assembly (14) relative to the outer bore (18) to interrupt the flow of the tubular extrudate at intervals to define the separate cell compartments.

41. The apparatus of claim 32, wherein the extrusion head assembly (14) includes a third, concentric, outermost bore (20) for delivery of a quenchant fluid to coagulate said polymeric solution.

Patentansprüche

1. Verfahren zur Einkapselung lebender Zellen, wobei das Verfahren aufweist: gemeinsames Ausstoßen einer wäßrigen Zellsuspension und einer Polymerlösung durch eine gemeinsame Ausstoßöffnung mit inneren und äußeren Bohrungen, von denen jeweils die Suspension und die Polymerlösung austreten, wobei die Lö-

sung nach gemeinsamem Ausstoßen durch die Ausstoßöffnung gerinnt, um ein röhrenförmiges Ausstoßprodukt mit einer semipermeablen Außenhülle zu bilden, die die Zellsuspension einkapselt.

2. Verfahren nach Anspruch 1, das weiter in Abständen das Verschließen des röhrenförmigen Ausstoßprodukts einschließt, um durch Polymerverbindungen verbundene getrennte Zellkompartimente zu begrenzen.

3. Verfahren nach Anspruch 2, bei dem der Schritt Verschließen des Ausstoßprodukts das in Abständen Zusammenquetschen des Ausstoßprodukts einschließt, um die getrennten Zellkompartimente zu begrenzen.

4. Verfahren nach Anspruch 2, bei dem der Schritt Verschließen des Ausstoßprodukts einschließt: Verändern des Drucks, unter dem die Zellsuspension oder Polymerlösung ausgestoßen wird, und dadurch Zusammenknicken des röhrenförmigen Ausstoßprodukts in Abständen, um die getrennten Zellkompartimente zu begrenzen.

5. Verfahren nach Anspruch 2, bei dem der Schritt Verschließen des Ausstoßprodukts einschließt: Verzögerung des Flusses der Zellsuspension in Abständen, um das röhrenförmige Ausstoßprodukt zusammenzuknicken und dadurch getrennte Zellkompartimente zu begrenzen.

6. Verfahren nach Anspruch 1, wobei die Polymerumhüllung in Luft geronnen wird.

7. Verfahren nach Anspruch 1, wobei die Polymerumhüllung in einer Niederdruckkammer geronnen wird.

8. Verfahren nach Anspruch 1, wobei die Polymerumhüllung in einem Ablöschbad geronnen wird.

9. Verfahren nach Anspruch 1, das Ausstoßen einer wäßrigen Suspension umfaßt, die Zellen enthält, die einen biologisch aktiven Faktor, wie etwa einen Neurotransmitter, absondern.

10. Verfahren nach Anspruch 1, wobei die ausgestossene wäßrige Zellsuspension ein Nährstoffmedium enthält.

11. Verfahren nach Anspruch 1, wobei die ausgestossene wäßrige Zellsuspension ein Verankerungssubstrat enthält.



12. Verfahren nach Anspruch 1, wobei die Polymerlösung eine mit Wasser mischbare Lösungsmittelkomponente umfaßt.
13. Das Verfahren nach Anspruch 1, wobei die Polymerlösung weiter ein grenzflächenaktives und/oder entzündungshemmendes Mittel und/oder ein Antioxidationsmittel umfaßt. 5
14. Verfahren nach Anspruch 1, das weiter die Steuerung der Viskosität der Polymerlösung umfaßt. 10
15. Verfahren nach Anspruch 1, das weiter Aufrechterhalten eines Druckunterschieds zwischen der wäßrigen Zellsuspension und der Polymerlösung während dem gemeinsamen Ausstoßen aufweist, um Lösungsmitteldiffusion aus der Polymerlösung in die Zellsuspension zu verhindern. 15 20
16. Verfahren nach Anspruch 1, das weiter Auftragen eines Schutzbarrierenmaterials auf die Außenseite der Polymerumhüllung umfaßt. 25
17. Zellkulturträger (50, 51), der eine röhrenförmige, semipermeable Polymermembran (52) aufweist, die eine Zellkultur (54) einkapselt, wobei der genannte Träger durch gemeinsames Ausstoßen der Zellkultur und einer Polymerlösung durch eine gemeinsame Ausstoßöffnung mit inneren und äußeren Bohrungen, denen die Kultur- und Polymerlösung jeweils zugeführt werden, gebildet wird, wobei die Kultur- und Polymerlösung derart ausgewählt werden, daß die Lösung nach gemeinsamem Ausstoßen mit der Kultur durch die gemeinsame Ausstoßöffnung gerinnt und die Membran bildet. 30 35
18. Träger nach Anspruch 17, wobei die Membran (52) für Moleküle mit einem Molekulargewicht von etwa 150000 oder weniger permeabel ist. 40
19. Träger nach Anspruch 17, wobei der maximale Aussendurchmesser des Zellkulturträgers im Bereich von 0,1 bis 1,0 Millimeter liegt. 45
20. Träger nach Anspruch 17, wobei die Membran (52) eine Wanddicke im Bereich von 10 bis 100 µm hat. 50
21. Träger nach Anspruch 17, wobei die Polymermembran (52) ein Polyacrylatmaterial aufweist. 55
22. Träger nach Anspruch 17, wobei die Polymermembran (52) ein grenzflächenaktives und/oder ein entzündungshemmendes Mittel und/oder ein Antioxidationsmittel umfaßt.
23. Träger nach Anspruch 17, wobei die ausgestoßene röhrenförmige Membran (52) in Abständen (bei 58) abgeschlossen wird, um getrennte durch Polymerverbindungen (58) verbundene Zellkompartimente (56) abzugrenzen.
24. Träger nach Anspruch 17, wobei die röhrenförmige Membran abgeschlossen wird, um mindestens ein mit einem zusammengehängten Faden verbundenes Zellkompartiment zu begrenzen.
25. Träger nach Anspruch 17, wobei die Zellkultur weiter eine wäßrige Zellsuspension umfaßt, die Zellen enthält, die einen biologisch aktiven Faktor, z.B. einen therapeutischen Faktor, absondern.
26. Träger nach Anspruch 17, wobei die wäßrige Zellsuspension weiter ein Nährstoffmedium umfaßt.
27. Träger nach Anspruch 17, wobei die Zellkultur weiter ein Verankerungssubstratmaterial umfaßt.
28. Träger nach Anspruch 27, wobei das Substratmaterial aus einem Collagenmaterial, einem Lamininmaterial oder einer Polyaminosäure ausgewählt ist.
29. Träger nach Anspruch 17, der weiter ein Schutzbarrierenmaterial umfaßt, das zumindest einen Teil der äußeren Oberfläche der Polymermembran (52) umhüllt.
30. Träger nach Anspruch 29, wobei das Schutzbarrierenmaterial ein Hemmstoff für Proteinwechselwirkungen ist.
31. Träger nach Anspruch 29, wobei das Schutzbarrierenmaterial aus Polyethylenoxiden, Polypropylenoxiden, Derivaten und Mischungen daraus ausgewählt ist.
32. Vorrichtung zum Einkapseln lebender Zellen, die aufweist:
eine Spritzkopfeinheit (14) mit mindestens einer ersten inneren Bohrung (16) und einer zweiten konzentrischen äußeren Bohrung (18);
eine Zellsuspensionszufuhreinrichtung (22) zum Liefern einer wäßrigen Zellsuspension an die innere Bohrung (16) der Spritzkopfeinheit (14);
eine Polymerlösungszufuhreinrichtung (26) zum Liefern einer Polymerlösung an die äußere Bohrung (18) der Spritzkopfeinheit und eine Druckunterschiedseinrichtung, um im Verlauf



des Ausstoßens einen positiven Druckunterschied zwischen der Zellsuspension und der Polymerlösung zu liefern, so daß die Zellsuspension und die Polymerlösung nach gemeinsamem Ausstoßen aus der Spritzkopfeinheit (14) ein röhrenförmiges Ausstoßprodukt mit einer geronnenen Polymer-Außenhülle bilden, welche die Zellsuspension einkapselt, wobei der verwendete positive Druckunterschied das Polymerlösungsmittel während der Gerinnung nach außen weg von der Zellsuspension treibt.

33. Vorrichtung nach Anspruch 32, wobei die Druckunterschiedseinrichtung eine Flußsteuerungseinrichtung zum Aufrechterhalten des Druckunterschieds zwischen der wäßrigen Zellsuspension und der Polymerlösung während des gemeinsamen Ausstoßens aufweist.

34. Vorrichtung nach Anspruch 33, wobei die Flußsteuerungseinrichtung einen Computer (38) aufweist, der für die Steuerung einer ersten Infusionspumpe (24) in der Zellsuspensionszufuhreinrichtung (22) und einer zweiten Infusionspumpe (28) in der Polymerlösungszufuhreinrichtung (26) geeignet ist.

35. Vorrichtung nach Anspruch 32, die weiter ein Ablöschbad (40) zum Gerinnen der Polymerlösung im Anschluß an das Ausstoßen aufweist.

36. Vorrichtung nach Anspruch 32, die eine Verschießeinrichtungen zum Verschießen des röhrenförmigen Ausstoßprodukts in Abständen aufweist, um getrennte durch Polymerverbindungen (58) verbundene Zellkompartimente (54) zu begrenzen.

37. Vorrichtung nach Anspruch 36, wobei die Verschießeinrichtung (42) Einrichtungen (44a, 44b, 46) zum in Abständen Zusammenquetschen des Ausstoßprodukts während dem Ausstoßen aufweist, um die getrennten Zellkompartimente zu begrenzen.

38. Vorrichtung nach Anspruch 36, wobei die Verschießeinrichtung betreibbar ist, um den Druck zu verändern, unter dem die Zellsuspension oder die Polymerlösung ausgestoßen wird, und dadurch das röhrenförmige Ausstoßprodukt in Abständen zusammenzuknicken, um getrennte Zellkompartimente zu begrenzen.

39. Vorrichtung nach Anspruch 36, wobei die Verschießeinrichtung Einrichtungen (48) zum Verzögern des Flusses der Zellsuspension in Abständen aufweist, um das röhrenförmige Aus-

stoßprodukt zusammenzuknicken und die getrennten Zellkompartimente zu begrenzen.

40. Vorrichtung nach Anspruch 36, wobei die Verschießeinrichtung eine Rückzugeinrichtung (48) zum Bewegen der inneren Bohrung (16) der Spritzkopfeinheit (14) relativ zur äußeren Bohrung (18) aufweist, um den Fluß des röhrenförmigen Ausstoßprodukts in Abständen zu unterbrechen und die getrennten Zellkompartimente zu begrenzen.

41. Vorrichtung nach Anspruch 32, wobei die Spritzkopfeinheit (14) eine dritte konzentrische äußerste Bohrung (20) zum Liefern eines Ablöschfluids aufweist, um die Polymerlösung zu gerinnen.

Revendications

1. Méthode d'encapsulation de cellules viables, la méthode comprenant la co-extrusion d'une suspension aqueuse de cellules, et d'une solution polymère à travers un orifice d'extrusion commun ayant des percages interne et externe, à partir desquels la suspension et la solution polymère sortent respectivement ladite solution lors de la co-extrusion à travers l'orifice coagulant, pour former un extrudât tubulaire ayant un revêtement externe polymère semi-perméable qui encapsule ladite suspension de cellules.

2. Méthode selon la revendication 1, qui comprend en outre le bouchage de l'extrudât tubulaire à différents intervalles pour définir des compartiments de cellules séparés connectés par des liaisons polymères.

3. Méthode selon la revendication 2, caractérisée en ce que l'étape de bouchage de l'extrudât comprend le fait de comprimer l'extrudât à différents intervalles pour définir les compartiments séparés des cellules.

4. Méthode selon la revendication 2, caractérisée en ce que l'étape de bouchage de l'extrudât comprend le fait de modifier la pression sous laquelle la suspension de cellules ou la solution polymère est extrudée en faisant s'aplatir l'extrudât tubulaire à des intervalles différents pour définir les compartiments séparés de cellules.

5. Méthode selon la revendication 2, caractérisée en ce que l'étape de bouchage de l'extrudât comprend le fait d'empêcher le flux de suspension de cellules à différents intervalles pour



faire aplatis l'extrudât tubulaire et par là définir des compartiments séparés de cellules.

6. Méthode selon la revendication 1, caractérisée en ce que le revêtement polymère est coagulé à l'air. 5
7. Méthode selon la revendication 1, caractérisée en ce que le revêtement polymère est coagulé dans une chambre à pression réduite. 10
8. Méthode selon la revendication 1, caractérisée en ce que le revêtement polymère est coagulé dans un bain d'étanchement. 15
9. Méthode selon la revendication 1 qui comprend le fait d'extruder une suspension aqueuse contenant des cellules qui sécrètent un facteur biologiquement actif tel qu'un neuro-transmetteur. 20
10. Méthode selon la revendication 1, caractérisée en ce que la suspension aqueuse de cellules contient un milieu nutritif. 25
11. Méthode selon la revendication 1, caractérisée en ce que la suspension aqueuse de cellules extrudée contient un substrat d'ancrage.
12. Méthode selon la revendication 1, caractérisée en ce que la solution polymère contient un composant solvant miscible à l'eau. 30
13. Méthode selon la revendication 1, caractérisée en ce que la solution comprend en outre un agent tensioactif et/ou un agent anti-inflammatoire et/ou un antioxydant. 35
14. Méthode selon la revendication 1 qui comprend le fait de contrôler la viscosité de ladite solution polymère. 40
15. Méthode selon la revendication 1 qui comprend en outre le fait de maintenir un différentiel de pression entre la suspension aqueuse de cellules et la solution polymère pendant la co-extrusion pour empêcher la diffusion du solvant de ladite solution polymère dans ladite suspension de cellules. 45
16. Méthode selon la revendication 1 qui comprend en outre le fait d'appliquer un matériau de barrière protecteur à la partie extérieure du revêtement polymère. 50
17. Véhicule de culture pour cellules (50, 51) qui comprend une membrane polymère, semi-perméable, tubulaire (52) enfermant une culture

de cellules (54), ledit véhicule ayant été formé en co-extrudant la culture de cellules et une solution polymère à travers un orifice commun de co-extrusion ayant des perçages internes et externes par lesquels la culture de la solution polymère sont respectivement alimentés, ladite culture et ladite solution polymère ayant été choisies de sorte que la solution coagule formant ladite membrane par co-extrusion avec la culture à travers l'orifice commun.

18. Véhicule selon la revendication 17, caractérisé en ce que la membrane (52) est perméable à des molécules ayant un poids moléculaire d'environ 150.000 au moins.
19. Véhicule selon la revendication 17, caractérisé en ce que le diamètre extérieur maximal du véhicule de culture de cellules s'échelonne de 0,1 à 1,0 millimètre.
20. Véhicule selon la revendication 17, caractérisé en ce que la membrane (52) a une épaisseur de paroi s'échelonnant de 10 à 100 μm (10 à $100 \times 10^{-6}\text{m}$).
21. Véhicule selon la revendication 17, caractérisé en ce que la membrane polymère (52) contient un matériau polyacrylique.
22. Véhicule selon la revendication 17, caractérisé en ce que la membrane polymère (52) contient un agent tensioactif et/ou un agent anti-inflammatoire et/ou un antioxydant.
23. Véhicule selon la revendication 17, caractérisé en ce que la membrane tubulaire extrudée (52) est bouchée à différents intervalles (à 58) pour définir des compartiments séparés de cellules (56) reliés par des liaisons polymères (58).
24. Véhicule selon la revendication 17, caractérisé en ce que la membrane tubulaire est bouchée pour définir au moins un compartiment de cellules (56) relié à un filament qui lui est attaché (59).
25. Véhicule selon la revendication 17, caractérisé en ce que la culture de cellules contient en outre une suspension aqueuse de cellules contenant des cellules qui sécrètent un facteur biologiquement actif par exemple un facteur thérapeutique.
26. Véhicule selon la revendication 17, caractérisé en ce que la suspension aqueuse de cellules contient en outre un milieu nutritif.



27. Véhicule selon la revendication 17, caractérisé en ce que la culture de cellules contient en outre un matériau de substrat d'ancrage.

28. Véhicule selon la revendication 27, caractérisé en ce que le matériau substrat est choisi parmi le matériau collagène, le matériau de laminine, et un polyaminoacide.

29. Véhicule selon la revendication 17 qui comprend en outre un matériau de barrière protecteur revêtant au moins une fraction de la surface externe de la membrane polymère (52).

30. Véhicule selon la revendication 29, caractérisé en ce que le matériau de barrière protecteur est un inhibiteur des interactions de protéines.

31. Véhicule selon la revendication 29, caractérisé en ce que le matériau de barrière protecteur est choisi parmi les oxydes de polyéthylène, les oxydes de polypropylène, les dérivés et les mélanges de ceux-ci.

32. Appareil pour l'encapsulation de cellules viables qui comprend:

- un assemblage de tête d'extrusion (14) ayant au moins un premier perçage interne (16) et un second perçage extérieur concentrique (18),
- un moyen d'alimentation en suspension de cellules (22) pour fournir une suspension aqueuse de cellule au perçage interne (16) dudit assemblage de tête d'extrusion (14),
- un moyen d'alimentation de solution polymère (26) pour fournir une solution polymère au perçage externe (18) de l'assemblage de tête d'extrusion et,
- un moyen différentiel de pression pour fournir un différentiel positif de pression entre la suspension de cellules et la solution polymère au cours de l'extrusion, de telle sorte que ladite suspension de cellules, et ladite solution polymère lors de la co-extrusion à partir de ladite tête d'assemblage forment un extrudât tubulaire ayant un revêtement extérieur polymère qui coagule, qui encapsule ladite suspension de cellules, ledit différentiel de pression étant employé pour chasser vers l'extérieur le solvant du polymère en s'éloignant de la suspension de cellules pendant la coagulation.

33. Appareil selon la revendication 32, caractérisé en ce que le différentiel de pression comprend un moyen de contrôle du flux pour maintenir le

différentiel de pression entre la suspension aqueuse de cellules et la solution polymère pendant la co-extrusion.

34. Appareil selon la revendication 33, caractérisé en ce que le moyen de contrôle du flux comprend un ordinateur (38) adapté pour contrôler une première pompe de perfusion (24) dans ledit moyen d'alimentation en suspension de cellules (22) et une seconde pompe de perfusion (28) dans ledit moyen d'alimentation en solution polymère (26).

35. Appareil selon la revendication 32, qui comprend encore un bain d'étanchement (40) pour la coagulation de ladite solution polymère après l'extrusion.

36. Appareil selon la revendication 32 qui comprend un moyen d'obturation pour boucher l'extrudât tubulaire à différents intervalles pour définir des compartiments séparés de cellules (54) connectés par des liaisons polymères (58).

37. Appareil selon la revendication 36, caractérisé en ce que le moyen d'obturation (42) comprend des moyens (44A, 44B, 46) pour comprimer l'extrudât à différents intervalles pendant l'extrusion pour définir les compartiments séparés de cellules.

38. Appareil selon la revendication 36, caractérisé en ce que le moyen d'obturation peut être actionné pour modifier la pression sous laquelle la suspension de cellules ou la solution polymère est extrudée, en mettant à plat l'extrudât tubulaire à différents intervalles pour définir les compartiments séparés de cellules.

39. Appareil selon la revendication 36, caractérisé en ce que le moyen d'obturation comprend un moyen (48) pour empêcher le flux de suspension de cellules à différents intervalles pour mettre à plat l'extrudât tubulaire et pour définir les compartiments séparés de cellules.

40. Appareil selon la revendication 36, caractérisé en ce que le moyen d'obturation comprend un moyen de rétraction (48) pour déplacer le perçage interne (16) dudit assemblage de tête d'extrusion (14) par rapport au perçage externe pour interrompre le flux d'extrudât tubulaire à différents intervalles pour définir les compartiments séparés de cellules.

41. Appareil selon la revendication 32, caractérisé en ce que l'assemblage de tête d'extrusion



(14) comprend un troisième perçage (20) tout à fait extérieur, concentrique pour délivrer un fluide d'étanchement pour coaguler ladite solution polymère.

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