An implantable extravascular therapeutic device comprising an immunoisolating semipermeable membrane and a source chamber is described. The semipermeable membrane has a critically smooth exterior surface. The source chamber is separated from the extravascular tissue of the implant recipient by the semipermeable membrane and is capable of containing a source of a diffusible product, for example, as produced by one or more cells. A method of making the device from the membrane and a source chamber, as well as a method of using the device in an extravascular site, are also disclosed.
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IMPLANTABLE IMMUNOISOLATED THERAPEUTIC DEVICES

Background of the Invention

The invention relates to implanting immunoisolated therapeutic diffusion devices in an animal, e.g., a human, and more particularly to the implantation of cell-based devices.

A wide range of diseases require enzyme or hormone replacement therapy. For example, a host of well-characterized disorders caused by the loss or malfunction of specific cells in the body are amenable to replacement therapy. These disorders include diabetes mellitus, which is treated within insulin, hypothyroidism, which is treated with thyroid hormone, and hemophilia, which is treated with Factor VIII. Implantation of living cells which produce these substances in which the body is deficient should serve to correct these disorders.

Implantation of specific cells, tissues or organs could also serve to detoxify or to remove deleterious substances from the circulation. For example, the implantation of appropriate living cells could restore normal physiologic function by providing replacement for the diseased cells, tissues, or organs, in hepatic encephalopathy (produced by liver disease) or uremia (produced by kidney failure). In addition, implanted cells modified by recombinant methods may be employed to treat degenerative disorders such as Alzheimer's disease, by producing nerve growth factor (NGF) and other neurotropic factors which normally act to support the viability and function of cholinergic neurons.

Unfortunately, the implantation of living cells (or tissues or organs) between humans, and particularly between animals and humans, is blocked by immunologic barriers which result in recognition and destruction of the implanted material within days or weeks.
Immunologic rejection of implanted material can be delayed if the cellular implanted material is immunoinsolated, i.e., physically isolated from attack by the recipient's immune system. Immunoinsolation can be achieved by enveloping the cells in a material which prevents direct contact of the implant with the immune system of the host.

Diabetes mellitus is a widespread disease which involves absolute or relative deficiency of the hormone insulin. Much effort has gone into developing methods of treating diabetic patients by transplantation or implantation of immunoinsolated insulin-producing tissue. Because the number of potential pancreatic tissue transplant recipients far outstrips the supply of human donor material, any widespread transplant therapy would probably involve the use of xenographic implant pancreatic tissue, i.e., the use of non-human, e.g., porcine, tissue. The immunoinsolation methods used must be capable of withstanding the exceptionally vigorous immune response which is directed at xenographic implants.

Immunoinsolation devices used in the implantation of pancreatic tissue are generally of two types, intravascular and extravascular. Intravascular devices are typically implanted into the circulatory system and function essentially as a shunt within a blood vessel or between two vessels. These devices can be internal or external, and generally consist of one or more tubes made of a semipermeable membrane. Blood flows through the lumen of the membrane(s) and the insulin-producing cells are located on the other (exterior) side of the wall of the membrane. Examples of intravascular devices are described in, e.g., Matsumura, U.S. Patent 3,827,556, which describes an artificial pancreas device which includes a semipermeable membrane on one side and one-
dispersed live pancreatic islets of Langerhans on the other (islets of Langerhans are the pancreatic structures which contain insulin producing cells); Sun et al., U.S. Patent 4,323,457, which describes a similar artificial pancreas device; and Chick et al., U.S. Patents 4,242,459, and 4,242,460, which describe cell culture devices which can be used as artificial pancreases.

Extravascular devices have taken a number of forms including planar diffusion chambers, filled hollow fibers, and microcapsules. Planar diffusion chambers are typified by devices which consist essentially of two flat membranes glued to a plastic support ring, as described, e.g., in Scharp et al., 1984, World J. Surg. 8:221.

Extensive efforts have been directed toward the use of small diameter hollow fibers as implantable chambers. In most of these studies, islets were placed in tubular hollow fibers and the ends of the fiber sealed. Typically the fibers were fabricated from a polyvinyl chloride/polyacrylonitrile copolymer with a molecular weight cutoff of about 50,000-80,000 daltons. As described in Altman et al., 1986, Diabetes 35:625, the membrane most often used was asymmetric in cross section with a smooth thin skin on the luminal (interior) side, an open trabecular structure in the wall, and a honeycombed surface on the outer side.

As opposed to the approaches described above, in which relatively large numbers of islets are encapsulated in a relatively large preformed mechanical device, microencapsulation involves the in situ encapsulation of one or a relatively small number of islets in a bead of gel. Microcapsules possess the advantage of significant reduction in diffusion time lags but, because many thousands of beads must be injected into an animal, retrieval of the beads is difficult.
Summary of the Invention

In general, the invention features an implantable extravascular diffusion device, such as an implantable artificial organ, which includes an immunoisolating semipermeable membrane with a critically smooth exterior surface and a source chamber. The source chamber is completely enclosed either by the semipermeable membrane or by a combination of the semipermeable membrane and a second material which is less permeable than the semipermeable membrane. The source chamber is separated from the extravascular tissue of the recipient by the semipermeable membrane or by the semipermeable membrane and the second material, and is capable of containing a source of a diffusible product, e.g., one or more cells capable of producing the diffusible product. For example, the source chamber may contain one or more islets of Langerhans, which are capable of producing insulin.

In one aspect, the invention features an implantable extravascular diffusion device which includes an envelope of a semipermeable immunoisolating membrane. The envelope defines and/or encloses a source chamber capable of containing a source of a diffusible product. The exterior of the device is critically smooth. The device can be formed from a tubular membrane which is sealed at both ends.

In another aspect, the invention features an implantable microcapsule, suitable for implantation into the body of an animal including: a core including one or more living cells and preferably a gel matrix; and a semipermeable coating surrounding the core, the semipermeable membrane having a critically smooth surface.

In another aspect, the invention features a method of inhibiting the deposition of fibrotic tissue on a
semipermeable membrane of an implanted device (the implanted device being exposed to extravascular tissue of a recipient animal) including providing the membrane with a critically smooth exterior surface.

In another aspect, the invention features a method for making an implantable extravascular device. The device includes a semipermeable polymer membrane and a source chamber capable of containing a source of a diffusible product. The method includes: (a) selecting a semipermeable polymer membrane characterized in having at least one critically smooth surface and immunoisolating characteristics; (b) fashioning the membrane such that its exterior surface is a critically smooth surface and its interior surface defines the source chamber; and (c) contacting and sealing the opposing edges of the membrane in the configuration of step (b) to immunoisolate and the source chamber within said membrane.

In another aspect, the invention features a method for supplying a diffusible product to an animal in need of the diffusible product. The method includes: (a) implanting in an extravascular site in the animal a device comprising an immunoisolating semipermeable membrane having a critically smooth exterior surface and an internal source chamber containing a source of a diffusible product; and (b) allowing the diffusible product to diffuse from the device into the extravascular site. In preferred embodiments the source chamber contains one or more pancreatic islets and the diffusible product is insulin.

In another aspect, the invention features an implantable extravascular device made by the methods described herein.

Implantable diffusion devices of the invention allow long term implantation of xenografts by minimizing
extravascular fibrotic growth on the semipermeable surface of the implanted device. Extravascular fibrotic growth prevents diffusion of vital substances into and out of the implant. It thus contributes to implant-cell death, e.g., by reducing the diffusion of oxygen into the implant. Fibrotic growth also reduces the magnitude and rate of cellular response by hindering the diffusion into and out of the implant of substances important to the desired response, e.g., glucose and insulin in the case of an implanted artificial pancreas.

The invention provides extravascular implants which do not require vascular surgery or shunt connection with the attendant risks of thrombosis, calcification, and infection. This is especially advantageous in that the problems associated with the use of devices connected to the circulatory system are compounded in diabetic patients by the fact that there is significant vascular disease associated with diabetes mellitus.

The invention also provides a variety of other features which enhance implant function and longevity, including positively sealed chambers, cells support matrices, and for islet cells, optimum cell densities. Other features and advantages of the invention will be apparent from the following description of the preferred embodiments and from the claims.

**Description of the Drawings**

FIG. 1 is a graphic representation of the insulin secretory response, to a 300 mg per dl dose of glucose, by perfused macroencapsulated (top) and by nonencapsulated (bottom) canine islets *in vitro*.

FIG. 2 is a graphic representation of the fasting plasma glucose levels in ten diabetic rats that received intraperitoneal implants of macroencapsulated or nonencapsulated canine islets.
FIG. 3 is a graphic representation of the fasting plasma glucose levels in six diabetic rats that received intraperitoneal implants of macroencapsulated or nonencapsulated bovine islets.

FIG. 4 is a graphic representation of the fasting plasma glucose levels in five diabetic rats that received intraperitoneal implants of macroencapsulated or nonencapsulated porcine islets.

FIG. 5 is a graphic representation of plasma glucose levels in four diabetic rats that received intraperitoneal implants of macroencapsulated canine islets.

FIG. 6 is a graphic representation of plasma glucose levels of two diabetic BB rats implanted with macroencapsulated canine islets.

FIG. 7 is a graphic representation of the plasma glucose levels (7A) and the body weights (7B) of two BB rats implanted with macroencapsulated canine islets.

FIG. 8 is a graphic representation of the fasting plasma glucose levels of 4 spontaneously diabetic BB rats that received intraperitoneal transplants of nonencapsulated canine islets.

FIG. 9 is a graphic representation of the fasting plasma glucose levels in response to i.v. glucose (0.5 g/kg) in untreated diabetic BB rats (triangles), normal control rats (squares) and implanted rats (circles).

DETAILED DESCRIPTION OF THE INVENTION

The following defined terms are used herein to describe and claim the invention:

A "diffusible product," as used herein, is a product which can diffuse through the semipermeable membrane of an implantable device.

"Extravascular," as used herein, means outside or exterior to the lumen of the circulatory system of the recipient, that is, not within or connected to one or
more arteries, veins, or capillaries. Thus, an extravascular source chamber is not continuous with the lumen of the circulatory system of the recipient. The membrane surface (of an extravascular source chamber) facing or contacting the recipient's tissue is extravascular, that is, it is not exposed to the lumen of the recipient's circulatory system but is rather outside that lumen.

"Macropencapsulation," as used herein, refers to the immunoinosulation of the source material to be implanted by placing the material in a previously formed enclosure. Macropencapsulation is exemplified by enclosing islets in tubular membranes or planar diffusion devices.

"Micropencapsulation," as used herein, refers to the immunoinosulation of the source material to be implanted by forming the immunoinosulating enclosure on or about the surface of the material to be implanted. Micropencapsulation is exemplified by the formation, in situ, of a layer of semipermeable polymer on or about one or a small number of islets.

"Euglycemia," as used herein, refers to a condition wherein the blood glucose concentration is within the normal range.

"Critically smooth," as used herein, refers to any of the following:

(1) the smoothness of a surface, which when characterized as an essentially planar surface is invested with pits or pores having either or both of the following characteristics: (i) a mode, mean, or average diameter of approximately 0.1 micron or less, or (ii) a mode, mean, or average depth of approximately 0.1 - 0.05 micron or less;

(2) the smoothness of the smoother of the two surfaces on a polyvinyl chloride/polyacrylonitrile
tubular semipermeable membrane produced as described below; or

(3) a smoothness sufficient to prevent fibrotic inactivation of an implanted therapeutic device.

"Fibrotic inactivation," as used herein, refers to the inactivation of an implantable extravascular diffusion device by the deposition of a layer of fibrotic tissue on the surface of the device which substantially interferes with the diffusion of substances into or out of the device. More specifically, fibrotic inactivation is indicated by any of the following:

(1) Coverage of more than a predetermined portion, e.g., more than 25%, 50%, or 75%, of the exterior surface of the semipermeable portion of the implantable device with fibrotic growth after being exposed to fibrotic growth in the peritoneal cavity of the implant recipient, e.g., a dog, mouse, or rat, for a predetermined period. The predetermined period can be defined as a period of days, e.g., 30 days, 60 days, 90 days, 120 days, 150 days, or 180 days, or some fraction e.g., 1/4, 1/2, or 3/4 of the expected life of the cells in question when grown in culture. The extent of coverage can be determined by histological and microscopic methods known to those skilled in the art.

(2) Coverage of the implanted device with an amount of fibrotic growth which results in a reduction in the diffusion in or out of the device by a predetermined amount, e.g., by more than 10%, 25%, 50%, or 75%, of any critical substance, i.e., a substance required by the implanted cell which diffuses into the chamber (e.g., oxygen), or a substance made by the implanted cells and required by the recipient, (e.g., insulin). The comparison can be made in any of a number of methods known to those skilled in the art. For example, a pancreatic device (previously implanted and removed)
exposed to fibrotic growth can be compared with an otherwise identical device (which has not been implanted) by filling both with a substance needed by the cells for function, e.g., glucose, or a substance produced by cells normally held within the device, e.g., insulin, and measuring the rate at which the substance diffuses out. Similarly, the rate of such a substance diffusing into the devices could be measured. Alternatively, the diffusion rate of a test substance which does not normally diffuse in or out of the device in when in use can also be used if the test substance diffuses through the membrane of the device with essentially the same kinetics as a substance which diffuses in or out of the device in normal (implanted) use. For example, in the case of an artificial pancreas, the diffusion rate could be measured with any convenient test molecule which diffuses through the type of membrane in question with similar kinetics to those of insulin or glucose. The comparison between the device exposed to fibrotic growth and the control (non-exposed) device is made after a predetermined period of exposure to fibrotic growth in a predetermined organism (as described in (1) above).

(3) Coverage of a device containing islet cells, which device has been implanted in the peritoneal cavity of an animal, for a predetermined period of time as described in (1) above, with sufficient fibrotic growth such that the ability of the device to respond to glucose in culture under the conditions described below, is reduced by a predetermined amount, e.g., by more than 10%, 25%, 50%, or 75%, in terms of insulin response time, total amount of insulin secreted, the maximum rate at which insulin is secreted, or the amount of glucose needed to produce a given level of response. The ability to respond in culture can be measured by methods known to those skilled in the art, e.g., by placing the devices
exposed to fibrotic growth in medium containing glucose and measuring the amount of insulin secreted into the medium. The comparison can be made between the devices taken from the test animals and similar devices (that have not been implanted) containing freshly isolated cells (isolated in the same way as the implanted cells). Alternatively, the device which has been exposed to fibrotic growth can be opened, refilled with freshly isolated cells, and compared to a device which is identical except for not having been exposed to fibrotic growth.

(4) Coverage of an implanted device with sufficient fibrotic growth to result in the inability of the device, after a predetermine amount of time (as described in (1) above), to maintain fasting euglycemia in a predetermine percentage, e.g., in at least 25%, 50%, or 75%, of mice, rats, or dogs implanted with such devices when said device contains an amount of canine, porcine, or bovine islets which would result in fasting euglycemia when the device is not subject to fibrotic inactivation.

(5) Coverage of the device with fibrotic growth which results in a reduction in the viability of cells contained in the device by a predetermine amount, e.g., by more than 10%, 25%, 50% or 75%. The comparison can be made in any of a number of methods known to those skilled in the art, e.g., the number of viable cells remaining in the device can be determined and compared to the number of viable cells the device contained at the time of implantation. Alternatively, a device which as been exposed to fibrotic growth for a predetermine amount of time as described in (1) above could be compared with an otherwise identical device (which has not been implanted) by filling both with cells and placing them in a culture medium that will support such cells. The number of
viable cells surviving at some specified period after being placed in the devices is measured. A level of viability, determined, e.g., by the number of living cells as a fraction of total added cells, in the device exposed to fibrotic growth that is less than a predetermined level, e.g., less than 50%, 75%, or 90% of the control device indicates inactivation. The cells tested may be the cells used in the implant or any convenient test cells.

"Molecular weight cutoff," as used herein, refers to, the size of the largest particle that is not substantially retained by a semipermeable membrane. Particles with molecular weight above the molecular weight cutoff are substantially retained.

**Macroencapsulated Devices**

Macroencapsulated implantable extravascular devices of the invention include an immunoisolating semipermeable membrane having a critically smooth exterior surface and a source chamber capable of containing a source of a diffusible product. The configuration of the device is such that the source chamber is completely enclosed either by the semipermeable membrane or by a combination of the semipermeable membrane and a second material which is less permeable than the semipermeable membrane. In preparing these macroencapsulated devices, the semipermeable membrane and enclosed source chamber are preformed, and the source of diffusible product is added subsequently.

The implantable extravascular device may include a semisolid matrix capable of holding a source of diffusible product in suspension. In a preferred embodiment the implantable extravascular device includes a source of insulin, e.g., pancreatic islets. The preferred implantable extravascular device further
contains islets present at a density of between approximately 10 and approximately 60 islets per mm$^3$, more preferably at a density of between approximately 30 and approximately 45 islets per mm$^3$.

Semipermeable Membranes

The semipermeable membranes suitable for use in the implantable devices of this invention must have sufficient mechanical strength to maintain their integrity in the in vivo environment. They must be nontoxic and inert and must be capable of being positively and reliably sealed or closed. The membranes must be free of faults such as breaks, tears, and holes, and must be resistant to tearing. This can be checked by methods known to those skilled in the art, e.g., by checking the ability of a membrane to contain a liquid dye.

In terms of permeability, the semipermeable membrane must allow passage of nutrients, oxygen, and other substances which are required for viability and functioning of the implanted cells. The membrane must also allow diffusion of desired cellular products as well as waste material from the implanted cells. Substances which regulate the desired cellular function must be able to pass through the membrane. The products of the encapsulated cells needed by the implant recipient must also be able to diffuse through the membrane. For example, where the cells are islets, the semipermeable membranes must allow diffusion of glucose into the extravascular chamber containing the islets, and must allow diffusion of insulin from the chamber.

At the same time, the semipermeable membranes must isolate the implanted cellular source of diffusible product from the immune system of the implant recipient, that is, the membranes must have immunoisolating characteristics. First, the membranes must prevent the
passage of immune cells. The maximum size of continuous passages which will prevent the entry of immune cells is about 0.1μm. In addition, the non-cellular products of the immune system, e.g., immunoglobulins, must be excluded. Membranes suitable for use in the invention have a molecular weight cutoff of between approximately 50,000 and approximately 100,000 and preferably between approximately 50,000 and approximately 80,000 daltons. A membrane with a molecular weight cutoff of approximately 50,000 daltons or greater is most preferred.

The exterior surface of the membrane, i.e., the surface which is in contact with the recipient organism, must be such that when implanted it remains substantially free of fibrotic growth. Fibrotic growth on the surface of the implant can block the diffusion of essential substances in and out of the chamber. The smoothness of the exterior surface is an essential factor inhibiting or retarding fibrotic growth. A critically smooth membrane surface minimizes, e.g., resists or inhibits, fibrotic growth. A surface, which is not critically smooth does not generally minimize fibrotic growth.

The exterior surface should be sufficiently smooth to result in an acceptable level of fibrotic growth over the expected life of the device. For example, if the life of a device is intended to be 18 months, the surface should resist fibrotic growth that would substantially impair the function of the device over that time period. Generally, it is preferred that the entire exterior surface of the membrane should be critically smooth.

A polyvinyl chloride/polyacrylonitrile copolymer has been found to be a particularly suitable material for the fabrication of semipermeable membranes suitable for use in this invention, although membranes fabricated from other materials may be suitable as long as they minimize fibrotic growth and meet the permeability requirements.
discussed above. Polyvinyl chloride/polyacrylonitrile copolymer resin is available from the Kaneka Corp. (Japan).

Semipermeable membranes suitable for use in the invention can be manufactured by methods known to those skilled in the art for the preparation of skinned semipermeable polymer membranes. The skinned membranes useful in this invention are anisotropic, i.e., one side has a smooth surface, or skin, and the other side has a rough or trabecular surface. Tubular, or hollow fiber, membranes having an outer skin or both an inner and outer skin will be suitable. In membranes of the latter type, the walls of the membrane have three distinct layers consisting of two smooth skins separated by an inner trabecular, or spongy, layer. Flat sheet membranes having at least one skinned surface may be used. References herein to skinned surfaces are intended to mean that those surfaces are critically smooth.

Preparation of hollow fiber and flat sheet membranes having one or two skinned surfaces is within the knowledge of those practicing this art. For example, see U.S. Patents 3,615,024 and 3,691,068, which describe similar procedures. It is within the ability of those of ordinary skill to make the necessary modifications for formation of an exterior skinned surface.

In general, the membranes are formed by phase inversion in a liquid-liquid precipitation scheme. This process involves the conversion of a liquid homogeneous polymer solution (casting solution) into a solid, but flexible porous film or fiber. The casting solution contains the polymer, one or more solvents for the polymer and one or more non-solvents for the polymer.

The casting solution is extruded into a liquid precipitation medium, where formation of the membrane occurs by phase inversion, that is, by precipitation of
the polymeric component from the casting solution. This is referred to as liquid-liquid phase separation or, for hollow fibers, as wet or solution spinning. During the initial contact with the precipitation solution, solvent is drawn from the casting solution, increasing the concentration of non-solvent and polymer until the increased polymer concentration causes precipitation of the polymer from the casting solution. The membrane continues to form as both solvent and non-solvent are leached out and the polymer precipitates completely.

Tubular membranes of polyvinylchloride/polyacrylonitrile or other polymers can, as mentioned above, be made by wet spinning. In this procedure, tubular membranes are formed on the surface of a stream of precipitation solution. The solution is emitted from a nozzle. Casting solution is emitted from a ring shaped nozzle which surrounds the precipitation solution nozzle. The central solution (precipitation solution) stream extracts components from the surrounding tubular stream of casting solution, causing it to solidify and thus form a tubular membrane. The general methods described above can be modified, by means known to those skilled in the art, to make tubular membranes with smooth outer skins (single-skinned membranes) and tubular membranes with smooth inner and outer skins (double-skinned membranes).

Preferred embodiments of this invention use polyvinylchloride/polyacrylonitrile single-skinned tubular membranes with internal diameters (I.D.) of approximately 0.5-7 mm, preferably 1-6 mm, and wall thickness of approximately 60-200 μm, preferably 60-125 μm. Hydraulic permeability of the membranes used in the experiments reported herein varied between approximately 2 and 50, preferably between 20-50, ml/min/m²/mmHg. BSA rejection was approximately 15-98%, and preferably approximately 30-75%. The tubular membranes with
internal diameters of less than 4.7 mm were single-skinned and those with diameters of more than 4.7 mm were double-skinned.

The membranes preferably are soaked in phosphate buffered saline prior to use to insure that they are free of glycerine and other substances used in the production or storage of the membranes. Other suitable methods may be utilized for this purpose.

Isolation of Cellular Source Material

In a preferred embodiment of this invention, islets are the cellular source material used in the device. However, other desired cells or tissues may be used. Isolation will be by procedures known to the art. Preferred embodiments include those in which: the source of diffusible product includes one or more cells; the source of diffusible product is pancreatic islets; and the islets are present at a density of between approximately 10 and approximately 60 islets per mm³, more preferably at a density of between approximately 20 and approximately 45 islets per mm³.

Islets maybe prepared from adult mongrel dogs, pigs, or bovine calves (0-2 weeks old) by methods known to those skilled in the art, e.g., by a modification of the method of Warnock et al. 1988, Diabetes 37:467.

Pancreatic tissue may, for example, be dissociated using a collagenase digestion procedure and the islets separated from exocrine tissue on a discontinuous Ficoll density gradient (Pharmacia). Other procedures may be used.

Isolated islets then may be cultured in a suitable medium. For example, M199/Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum, 20 mM HEPES, 100 mg per dl glucose, and 400 IU/ml penicillin (canine) is a preferred medium, as is α-MEM plus 10% heat-inactivated horse serum (bovine and porcine). The islets may be
cultured a humidified atmosphere of 5% CO₂/95% air at 37°C, or under other suitable conditions. Ischemic deterioration is minimized by using tissue fragments of a suitable size, e.g., islet fragments should be approximately 85 microns in diameter.

Viability, growth, longevity and/or function of the islet cells can be enhanced by coculturing, i.e., by mixing other cell types with the suspension prior to seeding the device. Useful cell types include cells which secrete growth hormone, e.g., GH-3 cells, or cells which secrete connective tissue and/or extracellular matrix components, e.g., fibroblasts, and endothelial cells. Islets can be cocultured with red blood cells to enhance oxygen availability. Hemoglobin may also be added to the islet cell suspension in order to enhance oxygen availability.

**Immobilization**

In some embodiments of this invention, cellular function and viability can be significantly enhanced by the use of a supporting material, such as a semisolid matrix, to immobilize the tissue or cells within the extravascular source chamber. The supporting material can be any of a variety of substances which are capable of maintaining cellular viability and physically supporting the tissue or cells in suspension. For example, in one embodiment, a semisolid matrix may be formed by adding islets to solution of nutrient medium and liquified alginate to form a suspension. A crosslinking agent, e.g., calcium chloride (1.5% of the alginate mixture), may be used to polymerize the alginate. Alternatively, liquified agar can be used as the supporting material, as can agarose. Carrageenan (0.5%) or Kelcosol™ beads (Merck) (1.0%) can also be used as the supporting material.
Where a supporting material is used the matrix should be sufficiently viscous to maintain the source of diffusible product in a dispersed state. For example, it is preferred for the matrix to have a viscosity of 900 and 1000 centipoises at 37°C.

The immobilizing or supporting material, as well as all other elements of the implantable device, should be the purest grade available in order to minimize the chance of the inclusion of a biologically incompatible contaminant.

In the case of diffusion devices containing islets, agar can be added up to about 3%, preferably to about 1-2%, and the solution cooled to form a semisolid support in which the islets are disposed. Viscosities produced by 3% (or higher) solutions of agarose or alginate are less preferred, as they are too viscous and often contain bubbles.

The supporting material lends increased rigidity to the extravascular source chamber. This has the advantage of making it less susceptible to breakage once the device is implanted in the recipient.

Device Preparation

Implantable extravascular diffusion devices which include a semipermeable polymer membrane and a source chamber capable of containing a source of a diffusible product can be made by the method described below. The method includes: (a) selecting a semipermeable polymer membrane characterized in having at least one critically smooth surface and immunisolating characteristics; (b) fashioning the membrane such that its exterior surface is a critically smooth surface and its interior surface defines the source chamber; and (c) contacting and sealing the opposing edges of the membrane in the configuration of step (b) to immunolate and the source chamber within said membrane.
The shape or configuration of the membrane and source chamber may be varied. In one preferred embodiment, the semipermeable membrane of step (a) is tubular in form, and the opposing edges at each end of the tube are sealed. In an alternative device, the semipermeable membrane of step (a) is a flat sheet membrane which is fashioned into an envelope defining the source chamber and all opposing edges are sealed to immunoisolate the source chamber. Other preferred embodiments include those in which the semipermeable membrane includes a seal joining two or more edges of the membrane to enclose the source chamber. Numerous configurations are possible.

Positive and reliable sealing of the extravascular source chambers is an extremely critical aspect of the fabrication of these devices to ensure that the source material remains immunoisolated. Failure of the membrane or seal results in the invasion of the device by elements of the recipient's immune system, leading to rapid destruction of the implanted cells.

One method of sealing invokes a two-step procedure in which the membranes are closed by the sequential application of first heat and pressure, and then a sealing compound. In this method, the cut ends of tubular or hollow fiber membranes may be picked up individually with forceps or a similar device. A second pair of sterile forceps may be heated and used to pinch-seal each cut end of the membrane. After pinch-sealing, the membrane may be dipped in a casting solution of the polymer from which the membrane was formed, to form a small cap at the end of the device. The capped ends of the membrane may be solidified by briefly exposing them to an aqueous solution. They may be redipped in the polymer solution. This dipping procedure involves only the tips or edges of the membrane and forms a cap
extending approximately 1/8" onto the surface of the device, which strengthens the seal formed in the first (pinch-seal) step. A similar two-step heat-dipping procedure may be used for sealing implants of other geometries, including flat disks, as well as disks with plexiglass support. Alternatively, the pinch-seal, or fused junction, can be formed by ultrasound, the application of an adhesive or by other sealing methods or other forms of joining known to those skilled in the art.

The most effective seal between a membrane and the endcap just described is obtained when the membrane is free of any substances which prevent adherence of the cap to the membrane. In the case of polyvinyl chloride/polyacrylonitrile membranes, the amount of water in the membrane should be minimized. Thus, for the most effective seal, the seal preferably should be made prior to exposure of the membrane to water, e.g., before washing of the membrane.

Alternatively, the devices can be closed with mechanical closures. For example, clamps or ball and collar devices (wherein the collar surrounds the outside of the membrane and the ball is inside the membrane and the ball can be locked into a depression on the inner surface of the collar) may be used.

The source of diffusible product can be loaded prior to sealing both ends of a tubular membrane, or the opposing edges of another configuration. Alternatively, a small port may be made in a the fully sealed membrane to allow introduction of tissue or cells and (optionally) the semisolid supporting matrix described above. The port can be closed with epoxy or by other methods known to those skilled in the art. A coating of the polymer solution can subsequently be applied to the closed port. The fully sealed extravascular semipermeable source
chambers are then washed extensively in sterile aqueous buffer.

The implantable extravascular source chamber can also include a housing of any acceptable biocompatible material as long as the source chamber comprises a critically smooth window of polyvinyl chloride/-polyacrylonitrile or similar polymeric material of suitable permeability to allow diffusion of the desired material. In such an embodiment, the housing material must be less permeable than the critically smooth window. That is, if the housing material is porous, the porosity should be such that the molecular weight cutoff is less than about 100,000 daltons, preferably less than about 80,000 daltons.

Microencapsulation

Microcapsules suitable for implantation into the body of an animal include: a core including one or more living cells as a source of a diffusible product, and, preferably, a gel matrix; and a semipermeable coating surrounding the core, the semipermeable membrane having a critically smooth surface.

In a preferred embodiment, the semipermeable coating includes or consists essentially of a polyvinyl chloride/-polyacrylonitrile copolymer. The semipermeable coating of the microcapsule of the invention has a molecular weight cutoff of between approximately 50,000 and approximately 100,000 daltons, preferably between approximately 50,000 and approximately 80,000 daltons. The source of diffusible product is a cell or a tissue structure including more than one cell, e.g., a pancreatic islet including insulin-producing cells.

The polymer coating can be applied in any of the following ways to form the microcapsule in situ. In one method, unsolidified polyvinyl chloride/polyacrylonitrile
copolymer is sprayed with an air brush-type atomizer onto the surface of alginate droplets which contain one or more cells. Immediately thereafter they are dropped into a tissue culture media or other aqueous solution to solidify the polyvinyl chloride/polyacrylonitrile solution and thus form a critically smooth skin on the outside surface. Another method for forming the polyvinyl chloride/- polyacrylonitrile semipermeable skin on the outside surface of the alginate/cell droplets involves immersing the droplets in a rapidly vibrating mixture of polyvinyl chloride/polyacrylonitrile copolymer solution in alcohol. This mixture is then rapidly diluted with a large volume of tissue culture medium or other aqueous solution, resulting in solidification of the polymer. Still another method for microencapsulation uses a triple nozzle in which the polymer is extruded from a larger-diameter outer needle which surrounds an inner needle from which an alginate/cell mixture is extruded. Compressed gas is forced from a third concentric needle in order to form droplets. Agar or other supporting materials may be used instead of alginate.

Use of the Implantable Devices

A diffusible product can be supplied to an animal in need of the diffusible product by: (a) implanting in an extravascular site in the animal a device comprising an immunosolating semipermeable membrane having a critically smooth exterior surface and an internal source chamber containing a source of a diffusible product, and (b) allowing the diffusible product to diffuse from the device into the extravascular site.

The number of devices implanted and the site of implantation will vary with factors known to those skilled in the art, e.g., the design and capacity of the device used, the type and quantity of tissue implanted,
the condition of the recipient, and the desired
therapeutic effect. The devices may be used for
peritoneal implantation. Alternatively, they may be
implanted in any location where they will be in contact
with vascularized tissue, e.g., subcutaneously, in an
organ, e.g., the brain or liver, or intramuscularly.

Implantable extravascular diffusion chambers
(macrocapsules) or microcapsules of the invention can be
used to implant a variety of tissues or cells, e.g.,
pancreatic, neural or liver tissue. The tissue or cells
may be genetically engineered. In one preferred
embodiment, the source chamber contains one or more
pancreatic islets and the diffusible product is insulin.

The tissue or cells may be obtained directly from
an organism, e.g., a human or an animal, or may be
derived from cultured tissue or cells. With respect to
properties of cell proliferation, cultured tissue or
cells may be normal or abnormal, e.g., transformed.
Tissue or cells derived directly from animals or humans
may be of normal or neoplastic origin (benign or
malignant). The implantable devices of the invention can
contain a source of diffusible product or can contain an
agent or a source of an agent which modifies (e.g.,
detoxifies, breaks down, inactivates, or sequesters) a
substance which diffuses into the device from the
recipient. The selection of diffusible product and the
appropriate source for its production is within the
knowledge and skill of the practitioner.

The examples which follow are given for
illustrative purposes and are not meant to limit the
invention described herein. The following abbreviations
have been used throughout in describing the invention:
cm, centimeter; μC; microCurie; dl, decaliter; g, gram;
I.D., inside diameter; i.v., intravenous; kg, kilogram;
mg, milligram; ml, milliliter; mm³, cubic millimeter;
min, minute; mm, millimeter; μl, microliter; μm, micrometer; ng, nanogram; %, percent; SEM, standard error of the mean; wt, weight; vol, volume; μU, microunit; wk, week.

Example 1 (Macroencapsulation)

The islets were prepared by collagenase digestion of pancreatic tissue and separation of islets from exocrine tissue on a discontinuous density gradient. Islets were maintained under sterile conditions prior to use. After culturing overnight, the islets were encapsulated in semipermeable polyvinyl chloride polyacrylonitrile double-skinned tubular membranes of various dimension, e.g., in tubular membranes 2-3 cm long, 1.77 mm ID, 69 μm wall thickness, with a nominal molecular weight cut-off of 50,000 daltons.

The islets were suspended in 1.2% (wt/vol) sodium alginate (Protan, Drammen, Norway) at a concentration of 30-45 islets per mm³. The islets/alginate mixture was then gently injected into the ends of 12 inch lengths of the tubular membrane.

The tubular membranes containing islet suspension dipped for 1-5 minutes in a solution of 1.5% calcium chloride (CaCl₂) to solidify the alginate, and then washed in culture medium. The seeded segments of membrane were cut into lengths of one to one-and-one-half inches. The cut ends of the membranes were heat sealed and then dipped in polyvinylchloride/polyacrylonitrile casting solution to form a polymer cap over the heat seal. After dipping in casting solution, the ends of the tubular membranes were dipped in medium (L-MEM in the case of bovine or porcine islets, M199/Eagles in the case of canine) for 1-2 seconds to solidify the polymer cap.

Example 2 (Immobilization Matrix)

In vitro experiments were used to optimize the choice of immobilization matrix. Canine islets in
culture for 3-4 weeks secrete an average of 0.2 to 0.8 units of insulin per day. Canine islets were loaded into tubular polyvinyl/polyacrylonitrile membranes (1.5 mm I.D., 110 μm wall thickness) with and without a supporting or immobilizing matrix, as described in Example 1. Two different supporting materials were used: 1.2% alginate or 1.0% agar. The tubular devices were maintained in tissue culture medium at 37°C. Insulin secretion was tested by radioimmunoassay. It was found that unsupported islets retained 70-80% of their insulin secretion capacity. Islets supported in alginate retained about 100% and islets supported in agar retained 75-90%.

Histological examination also indicated that the viability of the immobilized islets was increased as compared with islets that were not immobilized. The immobilized islets were morphologically intact with well-granulated β-cells.

Example 3 (The Effect of Islet Density on Insulin Secretion)

To examine the effect of islet density, polyvinylchloride/polyacrylonitrile tubular membranes (1.77 mm I.D., 69 mm wall thickness) were seeded at 50-200 islets per mm³ (isolated as described above) in a matrix of 1.2% alginate and maintained in culture for more than 10 weeks. Insulin secretion (units of insulin per islet per day) was significantly higher at the lower islet densities. The insulin secretory response of the 50 islets per mm³ devices was approximately fivefold greater than that of the 200 islets per mm³ devices. Furthermore, the higher density devices exhibited poor viability as determined by with hemotoxin and eosin, by methods known to those skilled in the art. The islets were in poor condition and overall, were estimated to be no more than 41% viable. In contrast, perhaps 70% of the
islets seeded at a density of 50 islets per mm³ had survived. The viable β-cells were well-granulated with stored insulin.

The density effect was also observed in vivo, with tubular membranes of various diameters. In one set of experiments, tubular membranes of 1.7 mm I.D. were seeded at 30, 45 and 60 islets per mm³ and implanted into the peritoneum of streptozotocin-induced diabetic rats. The 30 islets per mm³ and 45 islets per mm³ devices reversed the diabetic state of the recipients within 24 hours and maintained euglycemic levels for longer than 1 month. At 60 islets per mm³, however, the islets exhibited only partial function, and failed altogether at 2-3 weeks postimplantation. In other experiments, tubular membranes of 4.7 mm I.D. seeded at 25 or 35 islets per mm³ reversed diabetes. When seeded at 45 islets per mm³ these devices showed only partial or delayed function.

Example 4 (In Vitro Secretory Capacity)

In vitro perfusion was carried out to test the insulin secretory activity from the islets seeded within the membrane, and to evaluate the kinetic performance of this kind of diffusion-based biohybrid artificial pancreas as an insulin delivery device.

The in vitro insulin secretory response of macroencapsulated and nonencapsulated islets is shown in FIG. 1. (Data are presented herein as mean ±SEM, and compared by using the unpaired Student’s t test or one-way analysis of variance (ANOVA). In conjunction with ANOVA, multiple comparison methods of either Tukey or Scheffe were used, with a significance level of 0.05. Correlation was analyzed by univariate linear regression with the StatView II program (Abacus Concepts, Inc., Berkeley, CA).) The insulin secretory response (filled circles) to 300 mg per dl glucose (open circles) by perfused macroencapsulated and nonencapsulated canine
islets is shown in FIG. 1A and 1B, respectively. The increase in insulin release from macroencapsulated islets was only slightly delayed in comparison to nonencapsulated islets. An approximately fourfold average increase from the basal insulin secretion was observed in both groups. The secretory response of the macroencapsulated islets was sustained for 60 minutes of glucose stimulation (300 mg per dl) and returned to basal levels after perfusion with the low glucose solution (100 mg per dl). The lagtime of the increase in insulin release in response to glucose was 5 minutes in comparison to the nonencapsulated islets. Eight separate experiments in which this protocol was used indicated a average delay of 6 ± 1 minute between the addition of glucose and the appearance of an increase in insulin concentration in the perfusate. This finding indicates that the islets encapsulated by this procedure were functionally intact, and could respond rapidly to an increase in glucose concentration.

In vitro glucose-insulin kinetics were determined as follows. Islets were isolated (without coculture as described above and sealed at 30 islets per mm³ in 1.2% alginate) and sealed in polyvinyl chloride/polyacrylonitrile 1.7 mm I.D. and 69 μm wall thickness tubular membranes. Encapsulated and nonencapsulated islets (381±48 equivalents) were perfused with glucose concentrations of 100, 300, and 100 mg/ml for 60 minutes at each concentration as previously described, Scharp et al., 1987, Surgery 102:869. The flow rate was 0.5 ml per minute and the perfusate was collected with a microfraction collector (Gilson Model 203, Middleton, WI). The samples were frozen for subsequent insulin assay, which was performed by a standard radioimmunoassay protocol, Swope et al., 1984, Proc. Natl. Acad. Sci. USA 81:1822, in which the binding
was allowed to go to equilibrium during a 16-hour incubation at 4°C. $^{125}$I-labeled porcine insulin with a specific activity of 80-120μCi per mg. Guinea pig antiserum raised against monocomponent porcine insulin was used at a final dilution of 1/80,000. Free antigen was separated from antigen-antibody complex by precipitation with Staphylococcus aureus protein A (The Enzyme Center, Malden, MA). The limit of assay sensitivity was 25 μU (1 ng) per ml and data reduction was done by a lof-logit transformation of the standard curve by Probe gamma counter (Packard Instruments, Downers Grove, IL).

Example 5 (The Artificial Pancreas In Vivo)

Adult male Lewis rats (Charles River, Wilmington, MA) weighing 250-300 g were used as artificial pancreas implant recipients. Diabetes was induced by a single injection of streptozotocin 42 mg per kg into the tail vein of each rat ten to fourteen days prior to surgery. Only rats with high persisting plasma glucose levels were used in these studies. Fasting plasma glucose concentrations (mg per dl) were measured by the glucose oxidase method (Beckman Glucose Analyzer 2, Fullerton, CA) thrice weekly for 30 days, and then weekly for the duration of follow-up. Failure was considered to have occurred when glucose concentrations exceeded 200 mg per dl on two consecutive testings.

Intravenous glucose tolerance tests were performed at 1 month after implantation, and compared with normal (n=4) and untreated diabetic (n=4) rats. Fifty percent (wt/vol) glucose (0.5 g per kg body weight) was injected into the tail vein, and plasma glucose concentrations measured at 0, 1, 5, 10, 20, 30, 40, 50, and 60 minutes after the glucose injection. K-values (decline in glucose levels, % per minute) were calculated according
Endocrinol 24:145.

Islets were isolated, as described above in
Example 1, and seeded into tubular membranes 1.7 mm I.D.
and 69 μm wall thickness at a density of 30-45 islets per
mm$^3$ in a 1.2% alginate matrix. Recipient rats were
anesthetized with ether inhalation. Free or
macroencapsulated islets ($1-2 \times 10^4$ islet equivalents (an
islet equivalent is a volume of islets equal to the
volume of an average islet (150 μm in diameter)) were
introduced into the peritoneal cavity through a small (1-
2 cm) midline incision. Two-layer closure of the wound
was done with 4-0 silk suture. In the experimental group
of 27 rats, 6 received islets implants from bovine
calves, 7 from pigs and the remaining 14 were from canine
donors. In a control group of 10 diabetic rats, 4
received nonencapsulated canine islets, 3 received
nonencapsulated bovine islets, and the remaining 3 were
given nonencapsulated porcine islets.

As shown in FIGS. 2-4, these intraperitoneal
implants of encapsulated islets reversed the diabetic
state of all 27 recipient rats within 24 hours. FIG. 2
shows fasting plasma glucose levels (x ±SEM) in ten
diabetic rats that received intraperitoneal implants of
encapsulated canine islets. The insert shows fasting
plasma glucose levels in rats implanted with
nonencapsulated canine islets (n=4). The remaining four
rats failed (one each at 54, 94, 130, and 142 days
postimplantation) and were explanted. The failed
implants were free of fibrotic growth. Two rats with
functioning implants were sacrificed on each of day 102
and 130.

FIG. 3 shows average fasting plasma glucose levels
(x ±SEM) in the six diabetic rats that received
intraperitoneal implants of encapsulated bovine islets.
The insert shows fasting plasma glucose levels in rats implanted with nonencapsulated bovine islets (n=3). One animal with a functioning implant was sacrificed on day 88.

FIG. 4 shows average fasting plasma glucose levels (x ±SEM) in the 7 diabetic rats that received intraperitoneal implants of encapsulated porcine islets. The insert shows fasting plasma glucose levels in rats implanted with nonencapsulated porcine islets (n=3).

Three animals with functioning implants were sacrificed on day 82.

Mean ±SEM fasting plasma glucose levels dropped from a preimplantation level of 487±36 mg/dl to 84±2 mg/dl (canine), 81±4 mg/dl (bovine) and 81±3 mg/dl (porcine) during the first week for the encapsulated implants. All of the animals sustained this euglycemic state for at least 1 month. Four rats which received encapsulated canine islet xenointplants reverted to diabetes after implant removal on day 30. One other rat subsequently reverted to diabetes at 54 days postimplantation. Twenty-two rats maintained fasting euglycemia for more than 10 weeks. These animals all showed normal gain in body weight. However, those that had their implants removed lost weight during the follow-up period of observation. Normal urine output and absence of any evidence of cataract development were also observed in the membrane-protected islet implant groups. In contrast, nonencapsulated islet implants only partially reversed the diabetic state of the recipients, with hyperglycemia returning to preimplantation levels after 6-7 postoperative days (FIGS. 2-4, inserts).

The long-term survival of the rats was as follows. On day 88, 6 of 6 animals which had received encapsulated bovine islets were euglycemic. One of these animals (with a functioning implant) was sacrificed on day 88.
The 5 remaining animals were euglycemic on day 150. On day 82, 7 of 7 animals with encapsulated porcine islet implants were euglycemic. Three of them (with functioning implants) were sacrificed on day 82. The remaining 4 were euglycemic on day 100. On each of days 54 (referred to above) and 94 a canine implant failed. The remaining 8 animals which had received encapsulated canine implants were euglycemic on day 100. The majority of the devices from sacrificed animals and from animals in which the implants failed were completely or almost completely free of fibrotic growth.

The results of intravenous glucose tolerance tests on normal control (non-implanted, diabetic) and implanted rats are shown in Table 1.

Table 1. Fasting plasma glucose concentrations (mean ±SEM) and IVGTT K-value at one month in implanted, normal and untreated diabetic rats.

<table>
<thead>
<tr>
<th>Implant</th>
<th>Canine</th>
<th>Bovine</th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPG (mg per dl)</td>
<td>82±7</td>
<td>101±1</td>
<td>94±1</td>
<td>347±52</td>
</tr>
<tr>
<td>K-value (%/min)</td>
<td>3.5±0.1</td>
<td>3.3±0.1</td>
<td>3.3±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Number of animals</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Glucose clearance (K) rates of the animals implanted with encapsulated islets were not statistically different from those of normal rats at 1, 5, 10, 20, 30, and 40 minutes after administration of glucose (P=0.63). Plasma glucose declined promptly, reaching preinjection levels after 60 minutes and resulting in K-values of 3.5±0.30 (canine islets), 3.3±0.08 (bovine islets) and 3.32±0.09 (normal). By contrast, the untreated
streptozotocin-induced diabetic rats remained
significantly hyperglycemic after glucose challenge, with
K-values impaired at 0.91±0.11 (P<0.0001).

The euglycemic state of implant recipients was
confirmed by histological analysis. Pancreata from the
experimental and control groups were routinely fixed and
processed. The artificial pancreata (with encapsulated
canine islets) recovered from a normal control animal and
a streptozotocin-induced diabetic animal sacrificed 93
days after implantation were examined histologically.
The islets of the implant exhibited signs of
streptozotocin toxicity with loss of normal islet
architecture. Virtually no granulated β-cells were seen.
Histology of the implant retrieved from this animal
showed well-preserved islets, interspersed with solid,
basophilic alginate, much as in freshly seeded chambers.
Overall, perhaps 75-80% of the seeded endocrine tissue
remained viable. Glucagon and somatostatin-containing
cells were found among the β-cells in most section planes
intacting that alpha and delta cells also remained
viable. Thus, the islet cell community as a whole was
intact.

Implant and pancreas biopsies were fixed in
Bouin’s solution, and then dehydrated and embedding
paraffin by routine histologic methods. The tissue was
sectioned serially (5-μm sections) and stained with
hematoxylin-eosin; the presence of insulin, glucagon and
somatostatin in donor islets was determined using
immunoperoxidase histochemistry, Warnke et al., (1980) J.
Histochem. Cytochem. 28:771.

To further confirm that the euglycemic state of
the implant recipients resulted from the implantation of
macroencapsulated islets, membranes containing
encapsulated canine islets were removed 30 days after
implantation in 4 rats. Hyperglycemia was observed
Immediately in all the animals, with plasma glucose levels rising to >600 mg per dl within 3 days, as shown in FIG. 5. The implanted membranes were generally free floating and free of cellular overgrowth. The external surfaces were free of fibrosis and host cell adherence. On microscopic examination, the membranes contained mostly (85-90%) viable islets. Immunohistochemical staining of the islets revealed hormone-producing cells, with varying degrees of alpha, beta and delta granulation. These results demonstrate that inclusion of islet tissue inside tubular membranes provided long term protection of islet xenoimplants from rejection in the absence of any immunosuppressive therapy.

The advantages of the devices of this invention were also demonstrated in BB rats. BB rats are a widely used animal model for human Type I diabetes. BB rats are spontaneously diabetic and exhibit an autoimmune etiology similar to that of human Type I (insulin-dependent) diabetes. To demonstrate that the tubular membranes can protect xenoimplanted islets from both implant rejection and autoimmune destruction, canine islets in 1.2% alginate were seeded into membranes (2-3 cm in length, 1.6-2.2 mm I.D., 69-85 \( \mu \)m wall thickness) by the procedure of Example 1 and implanted in the peritoneal cavity of 10 diabetic BB rats. All the recipient animals promptly reached fasting euglycemia, with plasma glucose levels (FPG) dropping from a preimplantation level of 459±30 mg/dl to 104±12 mg/dl during the first month, see FIGS. 6 and 7. The inset in FIG. 6 shows the effect of removal of the implants on FPG. FIG. 7 shows data for 2 rats, one indicated by filled circles and the other by open circles. (FIG. 7A shows plasma glucose levels for both rats and FIG. 7B shows body weight for the rats). The implant was removed from the rat indicated by filled circles on day 60. Removal of the implants from three
rats led to hyperglycemia, with FPG levels rising from 100±3 mg/dl to 510±43 mg/dl within 1 day. Normal body weight and urine output were maintained during the euglycemic period. In contrast, control rats which received nonencapsulated islets became hyperglycemic in < 1 wk, as shown in FIG. 8. IVGII K-values at 10 days were 2.3±0.4 compared with 0.6±1 (P<0.005) and 3.1±0.1 (P<0.02) for untreated (n=4) and normal control (n=4) groups, see FIG. 9. FIG. 9 shows the fasting plasma glucose levels in response to i.v. glucose (0.5 g/kg) in untreated diabetic BB rats (triangles), normal control rats (squares) and implanted rats (circles).

Histology and electron microscopy of long term functioning implants of this invention presented well preserved islets, with hormone-producing alpha, beta and delta cells. The membranes were essentially free of fibrotic overgrowth. These results clearly demonstrate the protective capability of the tubular membranes, and the potential of the invention as a clinical treatment of diabetes mellitus.

Example 6 (The Artificial Pancreas In Vivo)

Canine islets were isolated and seeded into polyvinylchloride/polyacrylonitrile tubular membranes (1.0-1.3 mm I.D., 100-200 μm wall thickness in a matrix of 1.2% alginate by the procedures of Example 1. The devices were implanted into the peritoneal cavity of 26 nonimmunosuppressed streptozotocin-induced diabetic rats. Plasma glucose was promptly lowered in 18 recipients, dropping from a mean preimplant level of 522 mg/dl to 219 mg/dl (P<0.001). One rat died the day of implantation. A second animal was implanted with 45,000 islets, which was too few to effect a drop in plasma glucose. In addition, a third rat was implanted with membranes that were seeded at a very high density. These devices also failed to lower the plasma glucose levels.
Five of the remaining 23 rats received implants that were found to be contaminated with bacteria. These implants failed to reverse the diabetic state of the recipients. The 18 successful implants were explanted 22 days after implantation. Hyperglycemia was observed immediately, with plasma glucose levels rising to a mean of 523 mg/dl. Intravenous glucose tolerance (IVGTT) K-values before and after implant removal were 3.2±0.5 and 1.3±0.2, respectively (compared with 3.8±0.5 for normal control rats), confirming that the devices of the membrane can serve as an artificial pancreatic diffusion device, and can respond appropriately to changes in peripheral plasma glucose concentration. On histological examination, the explanted devices were found to contain evenly dispersed islets which exhibited excellent viability. The β-cells were mostly well-granulated with stored insulin. The membranes were essentially free of fibrotic growth.

Example 7 (Devices of this Invention Do Not Stimulate Fibrosis) Polyvinylchloride/polyacrylonitrile tubular membranes (ranging from 1.5-5.7 mm I.D., 60-200 μm wall thickness) were seeded with canine islets in a matrix of 1.2% alginate and a density of 30-45 islets per mm³ and implanted into the peritoneal cavity of diabetic and nondiabetic Lewis, Wistar-Furth, and BB rats. The devices were removed at various time intervals and examined for cellular overgrowth by phase microscopy. The membranes of the explanted devices were found to be free of host cell adherence after being implanted for up to 170 days. Viable islets were found throughout the luminal space (source chamber) of the membranes. Immunohistochemical staining further revealed hormone-producing cells, with varying degrees of alpha, beta and delta granulation.
In addition, tubular membrane devices were also implanted in the intraperitoneal cavity of dogs. In one experiment, 270 polyvinyl chloride/polyacrylonitrile tubular membranes (1.7 mm, 2.2 mm, and 4.8 mm I.D., 3 cm in length) containing canine islets (100,000-125,000 islet equivalents) at a density of about 25 islets per m³ in a matrix of 1.5% alginate were implanted in the intraperitoneal cavity of a pancreatectomized dog. The implants were removed on day 47. The implants were essentially free of fibrotic growth.

In another experiment, empty tubular membranes were implanted in the intraperitoneal cavity of five normoglycemic dogs and similar tubular membranes (3 cm long, 1.7 mm I.D., 69 μm wall thickness) containing canine islets (100,000-125,000 islet equivalents) were implanted in three diabetic dogs. The implants were removed 3-4 weeks after implantation. In 6 of the 8 animals, the membranes were substantially free of host cell adherence. By contrast, the membranes removed from two dogs were found to be encapsulated within a thick mass of fibrofatty tissue. Many of the membranes from these 2 dogs were distorted and bent.

Example 7 (Microencapsulation of Islets)

Islets isolated as described in Example 1 (above) were suspended uniformly in a solution of 1.6% sodium alginate at a concentration of approximately 1 x 10⁵ islets/ml. Alginate droplets were formed by syringe extrusion, using an 18-30 gauge needle, and collected in a solution of CaCl₂ (1.5%) to form spherical islet-containing calcium alginate beads ranging from 600 μm to 3000 μm in diameter. Each bead contained approximately 1-25 islets. The beads were washed twice with an aqueous culture medium, and then coated with a layer of polyvinyl chloride/ polyacrylonitrile polymer.
The resulting microcapsules were cultured in vitro for up to four weeks, and the insulin secretion compared to free islets containing islets and alginate as prepared in Example 1. The insulin secretory response of the microcapsules was approximately 50-80% of that of the free islets. Histological examination at four weeks revealed viable endocrine tissue within the microcapsules. The islets were morphologically intact, and contained well granulated β-cells.

The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since these are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art without departing from the spirit of the invention.
Claims

1. An implantable extravascular device comprising an immunoisolating semipermeable membrane having a critically smooth exterior surface and a source chamber capable of containing a source of a diffusible product, the configuration of said device being such that said source chamber is completely enclosed either by said semipermeable membrane or by a combination of said semipermeable membrane and a second material which is less permeable than said semipermeable membrane.

2. The implantable extravascular device of Claim 1 wherein said semipermeable membrane comprises a polyvinyl chloride/polyacrylonitrile copolymer.

3. The implantable extravascular device of Claim 1 wherein said semipermeable membrane is a polyvinyl chloride/polyacrylonitrile copolymer.

4. The implantable extravascular device of Claim 1 wherein said membrane has a molecular weight cutoff of between approximately 50,000 and approximately 100,000 daltons.

5. The implantable extravascular device of Claim 3 wherein said membrane has a molecular weight cutoff of between approximately 50,000 and approximately 80,000 daltons.

6. The implantable extravascular device of Claim 1 wherein said membrane has a hydraulic permeability of between approximately 2 and approximately 50 milliliters per minute per square meter per millimeter of mercury.
7. The implantable extravascular device of Claim 1 wherein said membrane has a bovine serum albumin rejection of between approximately 15 and approximately 98 percent.

8. The implantable extravascular device of Claim 1 in which said semipermeable membrane is in the form of an envelope containing said source chamber.

9. The implantable extravascular device of Claim 8 further comprising a seal joining the edges of said envelope to enclose said source chamber.

10. The implantable extravascular device of Claim 9 wherein said seal comprises a fused junction and a cap.

11. The implantable extravascular device of Claim 10 wherein said cap comprises a polymer coating.

12. The implantable extravascular device of Claim 11 wherein said polymer is a polyvinyl chloride/-polyacrylonitrile copolymer.

13. The implantable extravascular device of Claim 10 wherein said fused junction is a heat-formed junction.

14. The implantable extravascular device of Claim 1 which further comprises a supporting material disposed within said source chamber, said supporting material capable of maintaining said source of a diffusible product in suspension.

15. The implantable extravascular device of Claim 14 wherein said supporting material has a viscosity of between approximately 900 and 1000 centipoises.
16. The implantable extravascular device of Claim 14 wherein said supporting material is alginate, agar, agarose, or carrageenan.

17. The implantable extravascular device of Claim 15 wherein said source comprises one or more cells.

18. The implantable extravascular device of Claim 16 wherein said source is pancreatic islets.

19. The implantable extravascular device of Claim 17 wherein said islets are present at a density of between approximately 10 and approximately 60 islets per cubic millimeter.

20. The implantable extravascular device of Claim 18 wherein said islets are present at a density of between approximately 20 and approximately 45 islets per cubic millimeter.

21. An implantable microcapsule, suitable for implantation into the body of an animal, comprising a core comprising one or several living cells, and a semipermeable coating surrounding said core, said semipermeable membrane comprising a critically smooth exterior surface.

22. The microcapsule of Claim 21 wherein said semipermeable coating comprises a polyvinyl chloride/polyacrylonitrile copolymer.

23. The microcapsule of Claim 21 wherein said semipermeable coating is a polyvinyl chloride/-polyacrylonitrile copolymer.
24. The microcapsule of Claim 21 wherein said semipermeable coating has a molecular weight cutoff of between approximately 50,000 and approximately 100,000 daltons.

25. The microcapsule of Claim 21 wherein said core comprises a pancreatic islet.

26. The microcapsule of Claim 21 wherein said core further comprises a supporting material.

27. A method for making an implantable extravascular device comprising a semipermeable polymer membrane and a source chamber capable of containing a source of a diffusible product, comprising

(a) selecting a semipermeable polymer membrane characterized in having at least one critically smooth surface and immunoisolating characteristics,

(b) fashioning said membrane such that its exterior surface is a critically smooth surface and its interior surface defines said source chamber, and

(c) contacting and sealing the opposing edges of said membrane in the configuration of step (b) to immunoisolate said source chamber within said membrane.

28. The method of Claim 27 wherein the semipermeable membrane of step (a) is tubular in form, and wherein said opposing edges at a first end of the tube are brought together and sealed, and said opposing edges at the second end of the tube are brought together and sealed.
29. The method of Claim 27 wherein the semipermeable membrane of step (a) is a flat sheet membrane which is fashioned into an envelope defining said source chamber.

30. The method of Claim 27 wherein said sealing comprises contacting said opposing edges with a solution comprising the polymer of which the membrane is formed.

31. The method of Claim 27 wherein said sealing forms a polymer cap on the sealed edges.

32. The method of Claim 27 wherein said sealing is by applying heat, ultrasound, or an adhesive composition.

33. The method of Claim 27 wherein said sealing comprises a first step of fusing said edges and a second step of forming a polymer cap on said fused edges.

34. The method of Claim 27 which further comprises making a port in the sealed membrane, of sufficient size to permit introduction of a source of a diffusible product into the source chamber.

35. The method of Claim 34 which further comprises sealing said port.

36. The method of Claim 27 wherein said source of a diffusible product comprises one or more cells.

37. A method for supplying a diffusible product to an animal in need of said diffusible product, comprising
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(a) implanting in an extravascular site in said animal a device comprising an immunoisolating semipermeable membrane having a critically smooth exterior surface and an internal source chamber containing a source of a diffusible product, and

(b) allowing said diffusible product to diffuse from said device into the extravascular site.

38. The method of Claim 37 in which said source chamber contains one or more pancreatic islets and said diffusible product is insulin.


40. The implantable extravascular device of Claim 39 further comprising a semisolid matrix capable of holding a source of diffusible product in suspension.

41. The implantable extravascular device of Claim 40 further comprising a source of insulin.

42. The implantable extravascular device of Claim 41 wherein said source is pancreatic islets.

43. The implantable extravascular device of Claim 42 wherein said islets are present at a density of between approximately 10 and approximately 60 islets per cubic millimeter.

45. The implantable extravascular device of Claim 39 wherein said islets are present at a density of between approximately 30 and approximately 45 islets per cubic millimeter.
FIG 3

FIG 4
FIG 5

FIG 6
FIG. 7a

PLASMA GLUCOSE (mg/dl)

FIG. 7b

BODY WEIGHT (g)
FIG 8

FIG 9
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**
- IPC(5) :B29B 9/10
- US CL :264/4

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
- U.S. : 264/5,7,9,10; 435/1; 604/892.1; 210/638,500.43,500.42,500.23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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Date of the actual completion of the international search: 30 OCTOBER 1992

Date of mailing of the international search report: 07 DEC 1992

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