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(71) Demandeur/Applicant:
    GOVERNORS OF THE UNIVERSITY OF ALBERTA, CA

(72) Inventeurs/Inventors:
    KOBAYASHI, TSUNEHIRO, CA;
    RAYAT, GINA R., CA;
    RAJOTTE, RAYMOND V., CA

(74) Agent: BENNETT JONES LLP

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IN VIVO CULTURE OF CELLS AND TISSUES

FIELD

The present invention relates to cell culture methods, and more particularly to methods of tissue culture, preservation and maturation. The invention also relates to transplantation of cells into animals.

BACKGROUND

Conventionally, living cells have been cultured in vitro with media including amino acids and other nutrients inside a 37°C incubator. Such conventional in vitro tissue culture methods require several media changes, and culturing time can be limited due to the associated risk of bacterial and fungal contamination. Moreover, an in vitro environment is not a physiologically ideal or suitable environment for growth and maintenance of living cells.

What is needed is a method of culturing cells that avoids the hazards associated with in vitro cell culturing.

Cryo-preservation is one option for preserving living cells for a long duration. However, a considerable percentage of frozen cells lose viability and/or function in freezing and thawing processes.

What is needed is a method of culturing cells that avoids the loss of cell viability and/or function associated with cell preservation.

There are many diseases and disorders for which transplantation with, for example, normal tissues or cells may provide an attractive solution. For example, islet transplantation has been shown to be a promising alternative treatment for type 1 diabetes (1). However, islet transplantation has its own associated problems, including the requirement that immunosuppressive drugs which have harmful side effects must be used, in order to avoid rejection of the transplanted material by the recipient. Moreover, in the case of humans,
especially, there is generally a shortage of conspecific (i.e., human) donor tissue, and therefore xenotransplantation has been considered as an option in this situation. Insulin independence in type 1 diabetic patients can be achieved when a sufficient islet mass (>11000 islets/kg body weight) is transplanted, however this islet mass often requires more than one donor pancreas (1, 2). Currently, the shortage of human islet donors and the need for continuous administration of immunosuppressive drugs limit the large-scale application of islet transplantation. Therefore, in order for islet transplantation to become a widespread treatment for type 1 diabetes, an alternative source of abundant islets must be developed. Pigs are most likely to provide a solution to the shortage of human islets because they are inexpensive, breed rapidly, have many litters, can be housed in a pathogen-free environment and have many physiological similarities to humans. Pig insulin is structurally similar to human insulin and has been used safely in the treatment of diabetes for decades.

In comparison with adult pig sources for islets, neonatal porcine islets (NPI) are an attractive alternative for clinical transplantation because NPI can be easily isolated, maintained in culture for several days and have enormous growth potential (3). Several groups (3-6) have shown that NPI are capable of correcting diabetes in immune-deficient mice and large animal recipients (7). However transplantation of NPI in mice does not result in immediate reversal of diabetes and normoglycemia is achieved only by 6-8 weeks post-transplantation (3). This delay is possibly due to the fact that the immature β cells that are transplanted must first develop in to mature β cells, before they will produce sufficient insulin to be effective in restoring normal blood glucose levels.

Therefore, what is needed is a means of maturing immature cells before they are transplanted into a recipient host, to thereby avoid the delay in function that is observed when immature cells are transplanted into a recipient.

Embryonic stem (ES) cells have been proposed as a potential source of cell transplantation, since they can differentiate in vitro into several different cell types (19-24). However, in vitro differentiation of ES cells to the functional cells which are available for transplantation are very difficult within a limited time of in vitro culture (25).
Therefore, what is needed is a means of culturing cells for long time, which is effective for cell differentiation.

In immune-competent mice, alginate microcapsulated NPI were rejected, and they failed to reverse hyperglycemia, after transplantation (12). Although the alginate microcapsules provided a physical barrier that separated the islets from the recipients' immune cells, it appeared that shed xenoantigens escaped the capsule, attracting the immune cells to the microencapsulated NPI. In fact, there appeared to be leakage of porcine antigens from the capsule, which triggered a strong humoral immune response (12). Therefore, when transplanting into immune-competent mice, rejection of the cells or tissues occurs and it is necessary to use immunosuppressive drugs to avoid this rejection.

Therefore, what is needed is a means of avoiding the rejection of cells or tissues, when transplanted into a recipient animal.

SUMMARY

Disclosed herein is a method of culturing living cells by covering, surrounding or embedding the cells in a capsule comprised of a semipermeable membrane and implanting the capsule into immune-deficient animals. This method of in vivo cell culture enables living cells to be preserved for an extended period of time in a physiologically suitable environment without significant loss of cell function. Further, the semipermeable membrane of the capsule does not allow passage of cells, and therefore the cells in the capsule can be extracted without contamination by other host cells, by removing the capsule from the animal.

Also disclosed herein is a method of avoiding the rejection of cells or tissues, when transplanted into a recipient animal. The applicants have found that the initial transplantation of microencapsulated NPI into immune-deficient mice, and re-transplantation of the microencapsulated islets into diabetic immune-competent mice, does not require immune suppression (anti-rejection therapy) in the immune-competent mice. It appears that culturing the cells in immune-deficient mice eliminates immunogenic
porcine antigens resulting in immune modulation of NPI in vivo and also allows NPI to mature as well.

This in vivo culturing method may be used to grow undifferentiated cells, in an environment in which they may differentiate and otherwise mature. Another possible use of this long-term culture method is that the immunogenicity of the cells may be reduced prior to transplantation, with the potential to prevent rejection or lessen the need for anti-rejection therapy. Banking and long-distance transport of living cells becomes possible, allowing transplantation with consideration to the matching of donors and recipients, without the restriction of distance. The long-term culture of cell may also contribute to development of tissue engineering using stem cells.

Therefore, in one aspect the invention is a method of in vivo cell culture, comprising:

(a) encapsulating at least one cell in a capsule, said capsule comprising a semipermeable membrane;

(b) implanting the capsule into the body of a host animal;

(c) incubating the capsule in the host for a selected period of time; and

(d) removing the capsule from the body of the host animal.

In one embodiment, the method may further comprise the step of extracting the at least one cell from the capsule.

The period of culture time may be selected to be sufficient to reduce immunogenicity of the at least one cell.

In another embodiment, the method may further comprise the step of implanting said capsule into the body of a recipient animal.
The capsule may be implanted into a body cavity of the host animal, and in one embodiment is implanted into a peritoneal cavity of the host animal.

The host animal may be an immune-deficient animal.

In one embodiment, the at least one cell is a mammalian cell, and in another embodiment the at least one cell is a component of a tissue. The tissue may be an islet, and in one embodiment the tissue is a neonatal porcine islet. In another embodiment the tissue is human renal tissue.

In one embodiment the host animal is a mouse

The semipermeable membrane is selected from the group consisting of: alginate, agarose, cellulose, poly-vinyl alcohol, polyacrylonitrile, aromatic polyamide, polysulfone, hydrogels and ultrafiltration membranes.

In another embodiment, the method may further comprise the step of implanting the capsule into the body of a next host animal.

In another aspect the invention is a living cell cultured in accordance with the methods disclosed herein.

In yet another aspect, the invention is a living tissue cultured in accordance with the methods disclosed herein.

In yet another aspect, the invention is a product made from a cell cultured in accordance with the methods disclosed herein.

In yet another aspect, the invention is a product made from a tissue cultured in accordance with the methods disclosed herein.
BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an embodiment of the tissue preservation method disclosed herein. Living cells are covered with a semipermeable membrane capsule. The capsule is implanted into an immune-deficient animal. The semipermeable membrane of the capsule allows passage of gases and nutrients necessary for cell survival, and removal of waste products, and therefore the cells can be preserved for an extended period of time in this physiologically ideal environment. The capsules may then be collected, and the living cells extracted from the capsule.

FIG. 2 is a schematic of the semipermeable membrane capsule. Any type of semipermeable membrane that allows passage of gases, nutrients and waste products, and does not allow passage of cells may be used. The capsule may be in the form of a sphere, bag, disk, cylinder, and sheet.

FIG 3 shows dithizone staining of non-cultured and in vivo cultured microencapsulated NPI. Intense red staining by dithizone was observed in in vivo cultured NPI (B, D) compared to non-cultured NPI (A, C). Scale bar represents 100 μm.

FIG. 4 shows representative immunohistochemical staining of islet cells in non-cultured and in vivo cultured microencapsulated NPI. Abundant insulin-positive β cells were detected after 100 days of in vivo culture while no significant change in other hormone-secreting cells was observed compared to non-cultured microencapsulated NPI. In contrast, significantly less CK7-positive cells was observed in in vivo cultured microencapsulated NPI. Scale bar represents 100 μm.

FIG. 5 shows in vivo culture of microencapsulated NPI in C.B.-17 SCID BEIGE mice resulted in significant reduction of α-Gal, SLA-II, and von Willebrand factor expression compared to non-cultured NPI. The majority of in vivo cultured NPI examined completely lost the expression of these antigens. Scale bar represents 100 μm.

FIG. 6 compares transplantation of non-cultured and in vivo cultured microencapsulated NPI
into diabetic BALB/c mice and blood glucose levels of recipients post-transplantation. Figure 6A is a schematic showing the procedure used. Streptozotocin-induced diabetic BALB/c mice were transplanted with either non-cultured (Group I) or *in vivo* cultured microencapsulated NPI (Group II) intraperitoneally.

Figure 6B shows that recipients of non-cultured microencapsulated NPI (*n*=10) failed to achieve normoglycemia during the 100 days post-transplantation follow-up period. Dithizone-staining of a representative recovered microencapsulated NPI from these mice at 100 days post-transplantation revealed the presence of non-viable islets and cellular growth around the microcapsules.

Figure 6C shows that all mice that received *in vivo* cultured microencapsulated NPI (*n*=10) immediately returned to normoglycemia and 6/10 mice remained normoglycemic for 100 days post-transplantation. The remaining 4/10 mice rejected the microencapsulated NPI on days 17, 24, 37 and 87 post-transplantation (closed circles). The numbers below the dashed line represent the number of remaining animals at these days after transplantation.

Representative *in vivo* cultured microencapsulated NPI recovered from long-term surviving recipients are viable and free of cellular growth. Scale bar represents 100 μm.

FIG. 7 shows that *in vivo* culture of microencapsulated NPI reduces the levels of mouse anti-porcine antibodies in BALB/c mice. Neonatal porcine spleen cells (1×10⁶) from islet donor pigs were incubated with serum (1:128 dilution) collected from non-transplanted naïve mice (A), mice transplanted with non-cultured microencapsulated NPI (B), mice transplanted with *in vivo* cultured microencapsulated NPI that remained normoglycemic for 100 days post-transplantation (C), and mice transplanted with *in vivo* cultured microencapsulated NPI that rejected the graft at 87 days post-transplantation (D). Binding of mouse anti-porcine IgG was measured and analyzed by flow cytometry. Controls for this experiment consisted of unstained cells and secondary antibody without serum.
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains.

Disclosed herein is a method of long-term storage of living cells (in some embodiments, possibly for more than a year) without loss of function, which is accomplished by culturing the cells *in vivo* in the body of an animal. During this time of culturing, the cells remain viable, and furthermore they may undergo differentiation, maturation, and immune modulation resulting to the cells becoming less immunogenic.

In accordance with the disclosure herein, living cells may be cultured, matured, and/or preserved in an *in vivo* environment provided by a host animal. Such cells may be extracted free of contamination with other cells, from the host animal, and transplanted into recipient animals, or otherwise used for a variety of purposes - *e.g.*, they may be grown as *in vivo* cultures for use in research or industry.

The methods of the invention include encapsulating one or more living cells of interest, or a tissue, in a semipermeable membrane, to form a capsule, and implanting one or more capsules into the body of a host. Various kinds of cells may be cultured at the same time in this method. For example, bone marrow stem cells may be co-cultured with pancreas islet cells, or human stem cells may be co-cultured with mouse cells. Preferably, the capsule will be implanted into a cavity in the animal, such as the peritoneal cavity, retroperitoneal cavity or subcutaneous space. Most preferably, the cells will be cultured in the peritoneal cavity. However, other body sites may also be used for *in vivo* culture according to the methods disclosed herein, including omentum, liver, thyroid, thymus, ovary, testis and mesentery.

"Encapsulating" as used herein means embedding, covering, enclosing or surrounding the cells and/or tissue with the semipermeable membrane. The capsule of the invention includes at least one cell, and in some embodiments may include tissues.
A suitable semipermeable membrane for use in the capsule herein is nontoxic, allows passage of gases, nutrients, wastes and other molecules required for cell growth, and does not allow passage of cells, for the duration of the time that the capsule is in the host animal. The membrane may be comprised of, without limitation, alginates, agarose, cellulose, poly-vinyl alcohol, polyacrylonitrile, aromatic polyamide, polysulfone, hydrogels and ultrafiltration membranes. Although the term "membrane" is used, it is apparent that the semipermeable membrane need not be a sheet-like structure that encloses the cells and/or tissue, but rather may be a matrix-like or other structure in which the cells and/or tissue is embedded. Such semipermeable membranes enable their enclosed living cells to be preserved for a long duration in a physiologically suitable environment. The semipermeable membrane may provide a supporting matrix for the cultured tissue and/or cells and therefore may prevent tissue from cellular disaggregation and consequent loss of function. The semipermeable membrane also blocks intrusion and/or interference from cells outside of the capsule and therefore ultimately allows extraction of pure cultured cells from the capsule, without contamination by other cells from the host animal. The capsule may be in a number of forms including spheres, bags, disks, cylinders, and sheets.

A "host animal" as used herein is an animal that is used as a culture medium to support the growth of the cells and/or tissue that is within the capsule. For greater certainty, a host animal may be any mammalian species. A suitable host animal for the methods of the invention is an immune-deficient individual that can provide a sterile body site suitable to support the viability and growth of the cells and/or tissue in the capsule. Immune-competent animals may also be used as a host animal, provided that the cultured cells and/or tissue are not destroyed by host's immune system. Therefore, the host animal will provide the encapsulated cells and/or tissue with sufficient gases, nutrients, growth factors, hormones, fluids and so forth, as needed for viability and growth, as well as to eliminate harmful waste products produced by the cells and/or tissue in the capsule. In such a host, the temperature is kept consistently at a biologically appropriate temperature (for example, in mouse hosts, at 37 °C). The constant temperature, adequate oxygen and other gases, and abundant nutrients enable long-term cell culture in a physiologically
suitable environment by this *in vivo* tissue culture technique, unlike the conventional *in vitro* tissue culture.

An encapsulated cell or tissue need not be derived from a species that is of the same species as the host animal. Therefore, the cells and/or tissue in the capsule may be from a different mammalian species than the host animal. Moreover, the host may be wild-type (other than in respect of its immune-deficient status), or it may be a mutant or chimera. For example, it may be desirable to select a host that does not produce certain proteins, or alternatively that does produces certain proteins, of interest in respect of the growth, development, and/or differentiation of the encapsulated cells and/or tissue. For example, the host animal may be a transgenic mouse.

Not only mammalian cells but also other types of living cells (e.g., fish, bird or insect cells) can be a material for *in vivo* culture. For example, insulin producing cells may be made from fish islet cells. A vaccine against influenza virus may be made from bird cells. Perfume may be made from pheromone producing insect cells.

The encapsulated cells and/or tissue are cultured for a selected period of time in the host animal. The selected culture period may be indefinite, and may be limited only by the lifespan of the host animal. In this connection, it is to be understood that the capsule may be removed from the host animal and re-implanted into a new host animal, and a series of such removals and re-implantations may be effected to extend the culture period as desired. In some embodiments, the encapsulated cells and/or tissues may be stored, transported, or experimented upon while remaining within the body of the host individual.

Indefinite culturing time may be useful for tissue engineering, as conventional *in vitro* culture methods do not generally achieve complete differentiation from precursor stem cells. Potential cells and/or tissue useful for medical treatment, which may be produced by *in vivo* culture include insulin producing cells for diabetes treatment, hepatocytes for liver cirrhosis, dopamine producing cells for Parkinson's disease, estrogen producing cells for menopausal disorders, thyroid hormone producing cells for hypothyroidism, parathyroid hormone
producing cells for hypoparathyroidism, growth hormone producing cells for pituitary dwarfism and arginine vasopressin producing cells for diabetes insipidus. Further, hormones may also be made from engineered cells and/or tissue developed by \textit{in vivo} culture method.

The culture period may be selected to be at least a minimum time required to reduce the immunogenicity of the cells, particularly if the cells and/or tissue is intended to be used for transplantation. Such minimum time for immune modulation may vary depending on a number of factors, including without limitation cell type, host species, health status of the host individual, properties of the capsule medium (such as degree of permeability), and so forth.

Alternatively, the culture period may be selected to be at least a minimum time required to allow the cells to mature, meaning to differentiate or otherwise mature into the type of cell desired for transplantation. Such minimum time for maturation may vary depending on a number of factors, including without limitation cell type, host species, health status of the host individual, properties of the capsule medium (such as degree of permeability), and so forth.

The processes the encapsulated cells and/or tissue undergo during the time that they are maintained in the host animal may for present purposes be referred to as "maturation". Therefore, "maturation" as used herein is a reference to differentiation, immune modulation and other known and/or unknown processes that occur during the time that the capsule is in the host animal. Persons skilled in the art may determine the maturation period based on the particular materials and circumstances in a given situation.

Ultimately, the cells and/or tissue will be removed from the host animal and/or capsule, and may either be stored (\textit{e.g.,} frozen), used directly for experimentation, cultured \textit{in vitro} or transplanted into a suitable recipient animal. Cells and/or tissue that are cultured \textit{in vitro} may subsequently be used for medical treatment, producing drug, food, cosmetics, and so forth. The \textit{in vivo} culture method may also be used to preserve DNA or virus.
The cells of interest may be extracted from the capsule, after maturation, without contamination by other cells from the host animal. Therefore, the in vivo culture method disclosed herein provides in vitro tissue cultured cells that are free of cellular contaminants from the host animal.

In aspects involving transplantation, the encapsulated cells and/or tissue may be collected from the host, and extracted from the capsule, using methods such as those disclosed below. Alternatively, the capsule itself may be transplanted into a recipient animal. A "recipient animal" as used herein is an animal that is the final recipient of the cells and/or tissue that were grown in the one or more host animals described above. In preferred embodiments, the recipient animal is a transplant recipient. For greater certainty, a recipient animal may be any mammalian species.

In some embodiments the cells and/or tissue in the capsule, the host animal, and recipient animal are all conspecific, while in other embodiments only two of the three are conspecific, while in yet other embodiments, all three belong to different species. In one embodiment the cells and/or tissue in the capsule are selected from an animal or species that produces one or more molecules that are structurally and functionally similar or identical to one or more molecules in which the recipient animal is deficient, and these cells and/or tissue are involved in the production of such molecule or molecules.

In one example, living islet cells are covered with a semipermeable membrane and cultured in vivo. The cells may first be washed with an appropriate fluid, such as Hank's balanced salt solution (HBSS; Sigma, St Louis, MO, USA) supplemented with 10 mmol/l N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES buffer, Sigma, St Louis, MO, USA), then suspended in a nontoxic medium for encapsulation, such as 0.45 ml HBSS and 0.45 ml of 1.5% (w/v) alginate (PRONOVA UP MVG, Pronova Biomedical, Oslo, Norway) dissolved in HBSS medium. The resulting islet/alginate mixture can be mixed to obtain a homogeneous solution, and then transferred into a suitably-sized syringe (for example, a 1 mL syringe). Microcapsules can be formed by passing the cell/medium suspension through
an electrostatic generator followed by collection in a 120 mmol/L CaCl₂, 10 mmol/l HEPES buffer and 0.05% Tween 20 solution (P5927, Sigma, St Louis, MO, USA) for 10 min. The membrane-coated cells may then be washed, such as by gravity sedimentation in supplemented HAM’s F10 medium (Gibco, Burlington, Ontario, Canada).

Then, the coated cells may be implanted into the peritoneal cavity of immune-deficient mice and cultured there. The alginate membrane capsule allows passage of gases, nutrients, wastes, and other molecules involved in biological processes, thereby enabling living cells to be preserved for long durations, provided the host animal remains alive. As the coated cells in this example float in ascites that contains amino acids, proteins, and oxygen, they can secure sufficient nutrients, oxygen, and so forth from the mouse ascites, as well as eliminate wastes. In addition, the temperature is kept consistently at 37°C. In the present example, the peritoneal cavity of a mouse provides a physiologically suitable environment for living cells. The capsule does not allow passage of cells, so it protects living cells from mixture with host cells such as cells of the immune system. Finally, the capsules may be collected, for example by peritoneal lavage using HBSS. The alginate capsules of the present example can be dissolved using Hank’s media without divalent cations, and so permit extraction of the cells of interest without contamination by other cells (such as cells from the host individual).

While the invention has been described in conjunction with the disclosed embodiments, it will be understood that the invention is not intended to be limited to these embodiments. On the contrary, the invention is intended to cover alternatives, modifications and equivalents, which may be included within the spirit and scope of the invention as defined by the appended claims. Various modifications will remain readily apparent to those skilled in the art. Examples provided above and below are not intended to be limited to those examples alone, but are intended only to illustrate and describe the invention rather than limit the claims that follow.
EXAMPLES

RESEARCH DESIGN AND METHODS

Animals. C.B.-17 SCID-BEIGE mice (C.B-Igh-1\textsuperscript{b}GbmsTac-Prkd\textsuperscript{scid}-Lyst\textsuperscript{bg} N7, Taconic, Germantown, NY, USA) and BALB/c mice (H-2\textsuperscript{d}, University of Alberta, Edmonton, Alberta, Canada) were used as transplant recipients. Porcine islets were obtained from 1- to 3-day-old Landrace Yorkshire (1.5-2.0 kg body wt) neonatal pigs of either sex. Recipient mice were rendered diabetic by a single intraperitoneal injection of 225 mg/kg streptozotocin (STZ, Sigma, St Louis, MO, USA) 4 to 5 days before transplantation. Diabetes or hyperglycemia was defined as a minimum of two consecutive ≥ 20 mmol/l blood glucose measurements. Blood from the tail vein of each recipient was collected two times a week between 7:00 and 9:00 A.M. to monitor glucose levels using a One Touch Ultra glucose meter (Lifescan, Inc., Milpitas, CA, USA). All mice were housed and fed under specific pathogen-free conditions and were cared for according to the guidelines of the Canadian Council on Animal Care.

Statistical analysis. Data are expressed as means±SEM of n independent observations. Statistical significance of differences in insulin and DNA content as well as pancreatic hormones and immunological markers between non-cultured and in vivo cultured microencapsulated NPI were calculated by a two-tailed unpaired Student’s t test. Difference in graft survival between transplanted mouse recipients was sought by a log-rank test. SPSS statistical software, version 12.0 for Windows (Chicago, IL, USA) was used in all analyses. A p value of less than 0.05 was considered statistically significant.

Isolation of Neonatal Porcine Islets: Isolation of NPI was performed as previously described (3). Briefly, neonatal pigs were anesthetized with halothane and subjected to laparotomy and exsanguination. Pancreases were removed, cut into small pieces and digested with 2.5 mg/ml of collagenase (Sigma, St Louis, MO, USA). Digested pancreatic tissue was then filtered through a 500 μm nylon screen and cultured for 7 days at 37°C (5% CO\textsubscript{2}, 95% air) in HAM’s F10 medium (Gibco, Burlington, Ontario, Canada) containing 10
mmol/l glucose, 50 μmol/l isobutylmethylxanthine (ICN Biomedicals, Montreal, Ontario, Canada), 0.5% bovine serum albumin (fraction V, radioimmunoassay grade; Sigma), 2 mmol/l L-glutamine, 10 mmol/l nicotinamide (BDH Biochemical, Poole, Dorset, UK), 100 U/ml penicillin and 100 μg/ml streptomycin. The medium was replaced every two days of culture and at 7 days NPI formed spherical shaped aggregates.

Example 1: Long-term culture of neonatal porcine pancreatic islet cells

Microencapsulation of NPI: Islets were washed with Hank’s balanced salt solution (HBSS; Sigma, St Louis, MO, USA) supplemented with 10 mmol/l HEPES buffer (Sigma, St Louis, MO, USA) then suspended in 0.45 ml HBSS and 0.45 ml of 1.5% (w/v) alginate (PRONOVA UP MVG, Pronova Biomedical, Oslo, Norway) dissolved in HBSS medium. The resulting islet/alginate mixture was mixed to obtain a homogeneous solution, and then transferred into a 1 ml syringe. Microcapsules were formed by passing the islet/alginate suspension through an electrostatic generator followed by collection in a 120 mmol/L CaCl2, 10 mmol/l HEPES buffer (Sigma, St Louis, MO, USA) and 0.05% Tween 20 solution (P5927, Sigma, St Louis, MO, USA) for 10 min. Consequently 700-1000 μm of microencapsulated NPI, which includes 4-10 islets, was produced using this method. Microencapsulated NPI were washed by gravity sedimentation in supplemented HAM’s F10 medium (Gibco, Burlington, Ontario, Canada), cultured in the same medium with 10% newborn pig serum at 37°C (5% CO2 and 95% air) for 7 days. These islets are referred to as non-cultured microencapsulated NPI.

These microencapsulated NPI were transplanted into the peritoneal cavity of streptozotocin-induced diabetic C.B.-17 SCID-BEIGE mice and monitored for 100 days. On day 100 post-transplantation, normoglycemic C.B.-17 SCID-BEIGE recipient mice were sacrificed and microcapsules were collected by peritoneal lavage using HBSS medium. At this point, the alginate capsules may be dissolved using Hank’s media without divalent cations. The living cells are thus extracted without contamination by other cells and can be placed in HAM’s F10 medium. These are referred to as in vivo cultured microencapsulated
NPI.

As shown in Figure 3, in vivo cultured NPI were intensely stained positive for dithizone compared to non-cultured microencapsulated NPI.

The function of the recovered porcine islet tissue was evaluated by the following method in order to ascertain whether porcine islet tissue was successfully preserved without any loss of function after 100 days culture. To assess the insulin secretory function of non-cultured and in vivo cultured islets, static incubation assays were performed. Five hundred µl of microencapsulated NPI were washed with HBSS and diluted to a final volume of 10 ml. Then, 300 µl were taken from 10 ml and incubated for 120 min in 1.5 ml of HAM's F10 medium (Gibco, Burlington, Ontario, Canada) supplemented with 2 mmol/l L-glutamine, 0.5% bovine serum albumin and either 2.8 mmol/l (low-concentration) or 20.0 mmol/l (high-concentration) glucose. Islets and culture medium were then separated by centrifugation and assayed for their respective insulin contents as described below for Table 2. Table 1 presents the insulin concentrations in each culture solution. Stimulation indices were calculated by dividing the amount of insulin released after stimulation with 20.0 mmol/l glucose by that released after stimulation with 2.8 mmol/l glucose.

Table 1: Glucose stimulated insulin secretory activity of non-cultured and in vivo cultured neonatal porcine islets.

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mmol/l)</th>
<th>Stimulation Index (20.0/2.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPI</td>
<td>n</td>
<td>2.8</td>
</tr>
<tr>
<td>Non-cultured</td>
<td>11</td>
<td>0.52±0.04</td>
</tr>
<tr>
<td>In vivo cultured</td>
<td>11</td>
<td>11.92±3.81</td>
</tr>
</tbody>
</table>

Data are means±SEM. *p=0.0025, †p=0.0009, ‡p=0.0007 compared with non-cultured NPI.

As shown in Table 1, in vivo cultured neonatal porcine islets using this method showed better insulin-secreting capacity in both low- and high-concentration of glucose stimulation. Thus, not only were neonatal porcine pancreatic islet cells successfully cultured and stored...
for 100 days without loss of function, undifferentiated neonatal cells were successfully differentiated and matured after 100 days culture by this method.

Next, the DNA and insulin content of in vivo cultured islets was measured and compared to that in non-cultured islet. Five hundred μl of non-cultured or in vivo cultured microencapsulated NPI were washed with cation and phenol-red-free HBSS medium to dissolve the alginate. The resulting non-encapsulated NPI were diluted with HBSS medium to a final volume of 10 ml. Then, duplicate aliquots of 200 μl were taken from the diluted sample of islets and homogenized by ultrasonication on ice in distilled water. A sample of the homogenate was analyzed in duplicate for DNA content using a fluorometric method based on diaminobenzoic acid-induced fluorescence (13).

The remaining homogenate was extracted overnight in 70% acid ethanol (0.3 N HCl in absolute ethanol). Insulin was measured from a 1:20 dilution of the extract using a commercial radioimmunoassay kit cross-reacting with porcine and human insulin (Linco, St Charles, MO, USA). Values are presented as μg of DNA or insulin content in 1 ml of microencapsulated NPI or approximately 2,000 islets.

**Table 2:** DNA and insulin content of non-cultured versus in vivo cultured NPI

<table>
<thead>
<tr>
<th>NPI</th>
<th>no.</th>
<th>DNA content (μg)</th>
<th>Insulin content (μg)</th>
<th>Insulin/DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cultured</td>
<td>11</td>
<td>39.8±6.8</td>
<td>6.0±1.4</td>
<td>0.17±0.04</td>
</tr>
<tr>
<td><em>In vivo</em> cultured</td>
<td>11</td>
<td>196.7±90.1†</td>
<td>166.8±18.5‡</td>
<td>2.80±0.57‡</td>
</tr>
</tbody>
</table>

Data are means± SEM. †p=0.0980, ‡p<0.0001, ††p=0.0002 compared with non-cultured NPI.

As shown in Table 2, both DNA and insulin content increased remarkably in the in vivo cultured NPI, and the ratio of insulin content to DNA content in the in vivo cultured NPI was disproportionately greater than in the non-cultured NPI. This suggests that undifferentiated cells were successfully differentiated and matured after 100 days culture by this method.

These islets were also evaluated histologically to further examine whether microencapsulated NPI have matured in vivo. A portion of non-cultured and in vivo
cultured microencapsulated islets were fixed in Bouin's solution for two hours, washed three times with 70% ethanol, and embedded in paraffin. Five μm sections of these samples were stained with guinea pig anti-porcine insulin antibody (1:1000 dilution; Dako Laboratories, Mississauga, Ontario, Canada), guinea pig anti-glucagon antibody (1:5000 dilution, Linco Research, Inc., St. Charles, Missouri, USA), goat anti-human somatostatin antibody (1:1000 dilution, Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), rabbit anti-human pancreatic polypeptide antibody (1:1000 dilution; Dako Laboratories), goat anti-human von Willebrand factor (1:200 dilution; Santa Cruz), and mouse anti-human intracellular adhesion molecule-1 (ICAM-1, CD54, 1/200 dilution; Serotec, Raleigh, NC) for 30 min. Secondary antibodies used were biotinylated goat anti-guinea pig IgG (1:200 dilution; Vector Laboratories, Burlingame, CA, USA), goat anti-rabbit IgG (1:200 dilution; Vector Laboratories), goat anti-mouse IgG (1:200 dilution; Vector Laboratories), or biotinylated horse anti goat IgG (1:200 dilution; Vector Laboratories). The presence of islet ductal precursor cells was determined by the expression of cytokeratin 7 (CK7).

Paraffin-embedded sections were treated with 0.1 M citrate buffer then heated for 1.5 min using a domestic microwave oven. Mouse anti-human CK7 antibody (1:200 dilution; Dako Laboratories) was applied for 30 min followed by the addition of biotinylated goat anti-mouse IgG (1:200 dilution; Vector Laboratories) secondary antibody. The expression of α-Gal xenoantigen was detected using biotinylated α-Gal–specific BS-1 isoelectin B4 from Bandeiraea simplicifolia (1:25 dilution; Sigma). The avidin-biotin complex/horseradish peroxidase (ABC/HP; Vector Laboratories) and 3, 3-diaminobenzidinetetrahydrochloride (DAB; BioGenex, San Ramon, CA, USA) was used to produce a brown color. Sections were counter-stained with Harris' hematoxylin alone or with cosin (H&E).

The expression of SLA-II was examined on frozen sections of non-cultured and in vivo cultured microencapsulated NPI. Five μm sections of tissue were air dried for 10 min then fixed in acetone for 3 min at 4°C. These sections were then washed in PBS and non-specific binding was eliminated by incubating the tissue sections in 2% FBS in PBS for 20 min. In addition, endogenous biotin or biotin-binding proteins present in the sections
were also eliminated using the avidin/avidine blocking kit (Vector Laboratories). Anti-porcine MHC class II (1:300 dilution; VMRD Inc., Pullman, Washington, USA) antibody was applied to designated sections for 30 min at room temperature. Biotinylated goat anti-mouse IgG secondary antibody (1:200 dilution; Vector Laboratories) was added and incubated for 30 min. ABC/HP Reagent (Vector Laboratories) and DAB were applied to produce a brown color. Sections of microencapsulated NPI were then counter-stained with Harris’ hematoxylin.

Five representative islets (corresponding to approximately 811.0±54.4 cells; mean±SEM) in each sample were evaluated. Cells were counted using a Zeiss Axioskop 2 microscope (Carl Zeiss Canada, ON, Canada) and the percentage of positive stained cells was calculated. Values are presented as a percentage of positively stained cells per total number of islet cells counted.

Table 3. Cellular composition of non-cultured and in vivo cultured microencapsulated NPI.

<table>
<thead>
<tr>
<th>NPI</th>
<th>Insulin</th>
<th>GLU</th>
<th>SS</th>
<th>PP</th>
<th>CK7</th>
<th>α-Gal</th>
<th>SLA</th>
<th>vWF</th>
<th>ICAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cultured</td>
<td>9.5±</td>
<td>27±</td>
<td>8.2±</td>
<td>8.7±</td>
<td>60.5±</td>
<td>43.2±</td>
<td>19.7±</td>
<td>57.4±</td>
<td>0.3±</td>
</tr>
<tr>
<td>In vivo cultured</td>
<td>2.2</td>
<td>3.6</td>
<td>1.4</td>
<td>1.3</td>
<td>2.7</td>
<td>3.0</td>
<td>4.3</td>
<td>5.7</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>71.5±</td>
<td>17±</td>
<td>5.2±</td>
<td>4.5±</td>
<td>3.4±</td>
<td>0.3±</td>
<td>3.8±</td>
<td>0.4±</td>
<td>0.2±</td>
</tr>
<tr>
<td>cultured</td>
<td>4.9</td>
<td>2.4</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
<td>0.3†</td>
<td>1.0‡</td>
<td>0.3‖</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Data are means ± SEM for five samples. *p=0.001, †p=0.040, ‡p=0.005, ‖p=0.006, †‖p=0.001 compared with non-cultured NPI. GLU: glucagon; SS: somatostatin; PP: pancreatic polypeptide; CK: cytokeratin; SLA: swine leukocyte antigen II; vWF: von Willebrand factor; ICAM: intercellular adhesion molecule I.

The results demonstrated that in vivo cultured neonatal porcine islets comprise substantially no other type of cell, suggesting that this method allows extraction of pure porcine islet tissue without contamination of mouse cells. Immunohistochemical comparison of non-cultured and in vivo cultured neonatal porcine islets revealed an increase in insulin positive cells, suggesting successful differentiation and maturation after 100 days culture.
Immunohistochemical analysis of the islet hormones reveals more insulin-positive β cells in the in vivo cultured compared to non-cultured cells. This increase possibly occurs by differentiation of precursor cells found within the non-cultured NPI, as identified by CK7-positive cells, which are believed to be the precursors of β cells (6, 14). A slight decrease in glucagon-, somatostatin-, and PP-secreting cells in the in vivo cultured compared to non-cultured microencapsulated NPI was observed (Fig. 2 and Table 3). It is interesting to note that only the proportion of insulin-positive β cells was significantly increased after transplantation of microencapsulated NPI suggesting a biased differentiation of islet precursor cells to β cells.

The expression of α-Gal, SLA-II and von Willebrand factor in non-cultured microencapsulated NPI were significantly higher compared to in vivo cultured microencapsulated NPI. ICAM-1 expression was not detected in either non-cultured or in vivo cultured NPI (Table 3, Figure 3). These data suggest that in vivo culture of microencapsulated NPI alters the expression of α-Gal, SLA-II and von Willebrand factor which may possibly reduce NPI immunogenicity in vivo.

Non-cultured and in vivo cultured microencapsulated NPI were transplanted into diabetic BALB/c mice. Figure 6B shows that mice transplanted with non-cultured microencapsulated NPI were not able to achieve normoglycemia. In contrast, all of the mice that received in vivo cultured microencapsulated NPI achieved normoglycemia within two days post transplantation (see Figure 6C). Four of these mice rejected their grafts and returned to hyperglycemia on days 17, 24, 37 and 87 post-transplantation. However, the remaining 6 mice maintained normoglycemia for 100 days post-transplantation indicating that islet xenograft survival was significantly prolonged in mice transplanted with in vivo cultured NPI compared with mice that received non-cultured NPI (p<0.0001, log-rank test).

Recovered non-cultured microencapsulated NPI were surrounded with layers of immune cells forming a fibrotic cellular overgrowth and contained non-viable islets (Fig. 6B). In contrast, in vivo cultured microencapsulated NPI recovered from normoglycemic mice with long-term graft survival, were free of fibrotic cellular overgrowth and contained healthy
islets (Fig. 6C).

To determine if a humoral immune response to non-cultured and in vivo cultured microencapsulated NPI occurs after transplantation, serum samples from BALB/c recipients were collected and analysed by flow cytometry. Spleen cells from the same islet donor pigs were isolated and 1x10^6 cells were incubated with 100 µl of 1:128 dilution of the mouse serum for 1 hour at 37°C, 95% air and 5% CO₂. The cells were washed with PBS by centrifugation at 1500 rpm for 5 min and mouse anti-porcine IgG antibodies were detected by adding 100 µl of FITC-conjugated goat anti-mouse IgG antibody (1:100 dilution, Southern Biotechnology Associates, Inc., Birmingham, AL, USA) to the cell pellet and incubating at 4°C for 1 hour. The cells were washed with PBS, then suspended in 500 µl of PBS with 2% FBS. The levels of mouse anti-porcine IgG antibodies were determined by flow cytometry using single-parameter fluorescence histograms on a BD FACS Calibur machine (BD Biosciences, Ontario, Canada) after gating on viable cells. Sera from non-transplanted naïve B6 mice, unstained pig spleen cells, and pig spleen cells stained with secondary antibody alone without mouse serum were included as controls.

The levels of mouse anti-porcine IgG antibodies in long-term normoglycemic mice transplanted with in vivo cultured microencapsulated NPI were greatly reduced (Fig. 7C) and comparable to the levels found in naïve control (non-transplanted) BALB/c mice (Fig. 7A). Sera from BALB/c mice transplanted with in vivo cultured microencapsulated NPI that achieved normoglycemia but eventually rejected the islet xenografts, demonstrated almost similar levels of anti-porcine IgG antibodies observed in mice transplanted with non-cultured microencapsulated NPI (Figure 7D and 7B, respectively). These data indicate that in vivo culture of microencapsulated NPI resulted in the elimination of immunogenic porcine antigens after transplantation into immune-deficient C.B.-17 SCID-BEIGE mice.

The above experiments demonstrate that microencapsulated NPI may be successfully cultured for 100 days in vivo without loss of function. Differentiation and maturation of NPI was demonstrated by an increase in insulin and DNA content as well as an increase in
the number of β cells after 100 days transplantation into C.B.-17 SCID-BEIGE mice. These results suggest that in vivo culture of NPI results in the biased differentiation of porcine islet precursor cells to β cells possibly influenced by the diabetic state of the recipients.

Transplantation of in vivo cultured NPI into diabetic BALB/c mice resulted in immediate reversal of hyperglycemia within 2 days post-transplantation and 6 of 10 recipient mice maintained normoglycemia for 100 days post-transplantation without anti-rejection therapy. In contrast, non-cultured microencapsulated NPI failed to reverse hyperglycemia suggesting rejection of the islets. The failure of non-cultured NPI to reverse hyperglycemia could not be attributed to the immature nature of NPI since the same batch of non-cultured microencapsulated NPI reversed hyperglycemia in immune-deficient C.B.-17 SCID-BEIGE mice within 4 weeks post-transplantation (data not shown). In vivo culture appears to eliminate or reduce porcine antigens that may be immunogenic to BALB/c mouse recipients. These antigens may either be lost or shed during the maturation process of NPI. It is possible that porcine antigens may have traversed the microcapsules and been taken up by host APCs in C.B.-17 SCID-BEIGE mice, but due to the lack of functional T and B cells in these mice, no immune response against porcine islets was produced. This speculation was partly confirmed by immunostaining for α-Gal, SLA-II and von Willebrand factor antigen expression, which were remarkably decreased or lost after 100 days transplantation. In our model, it is highly unlikely that elimination of α-Gal may contribute to the prolonged graft survival in BALB/c mice, since recipient mice also express α-Gal and therefore, do not elicit an immune response to this antigen. However, the observation of reduced α-Gal expression may be important in the clinical application of this study. Reduction of SLA-II expression may contribute to the reduction of immunogenicity since it has been shown that MHC class II expression is important in xenograft rejection (15). Von Willebrand factor, a marker of endothelial cells, has completely disappeared after in vivo culture. This is an interesting finding because endothelial cells are believed to be very immunogenic for transplantation (16, 17). Expression of ICAM-1 on both non-cultured and in vivo cultured NPI was not detected. However, ICAM-1 is rarely expressed in normal tissue, and its
expression can be induced at the site of inflammation by various cytokines such as TNF-a, IL-1, and IFN-. (18).

Protection of *in vivo* cultured microencapsulated NPI was associated with a remarkable reduction of cellular overgrowth surrounding the microcapsules. Moreover, the levels of mouse anti-porcine antibodies in BALB/c recipients with long-term surviving *in vivo* cultured microencapsulated NPI were reduced compared to recipients of non-cultured microencapsulated NPI. These data suggest that porcine antigens responsible for the sensitization of mouse immune cells were eliminated by *in vivo* culture of NPI and resulted in long-term survival of these islets in immune-competent BALB/c mice without anti-rejection therapy. At this time the possibility that immune modulation of NPI may have also resulted from the loss of other unidentified porcine antigens expressed or secreted by live or dead islet cells cannot be ruled out.

**Example 2: Reduction of immunogenicity of pancreatic islet cells by long-term culture**

Pancreatic islets were isolated from C57BL6 mice by collagenase digestion, as described above. Islets were washed with Hank's balanced salt solution (HBSS; Sigma) supplemented with 10 mmol/l HEPES then suspended in 0.45 ml HBSS and 0.45 ml of 1.5% (w/v) alginate (PRONOVA UP MVG, Pronova Biomedical, Oslo, Norway) dissolved in HBSS medium. The resulting islet/alginate mixture was mixed to obtain a homogeneous solution, and then transferred into a 1 ml syringe. Microcapsules were formed by passing the islet/alginate suspension through an electrostatic generator followed by collection in a solution containing 120 mmol/l CaCl₂, 10 mmol/l HEPES and 0.05% Tween 20 for 10 min. Microencapsulated islets were washed by gravity sedimentation in supplemented HAM's F10 medium (Gibco, Burlington, Ontario, Canada).

Microencapsulated islets of C57BL6 mice were implanted into the peritoneal cavity of immune-deficient mice and stored 100 days. The capsules were removed from the peritoneal cavity after 100 days, and the islets were extracted by dissolving the capsules with
Hank's media without divalent cations.

The aforementioned in vivo cultured pancreatic islet of a C57BL6 mouse was transplanted under the kidney capsule of a diabetic BALB/c mouse (blood glucose levels are more than 400mg/dl). No anti-rejection therapy was administered to the mice.

All of the mice (6/6) transplanted aforementioned in vivo cultured islets decreased blood glucose levels to the normal range within two days after transplantation. This result shows that these islets maintained their function even after 100 days storage. All of these 6 mice maintained normoglycemia more than 100 days without administration of immune suppressive drugs. Removal of graft bearing kidney in these mice results in recurrence of diabetes, suggesting that these islet graft was responsible for normoglycemia. Histological examination of islet bearing kidney revealed that these islets were not rejected by recipients.

In contrast, all of the 5 mice transplanted with non-cultured islets recurred diabetes within 40 days, indicating graft rejection. Table 4 presents the graft survival of the transplanted islet in each group. These results indicate that the immunogenicity of the pancreatic islet cells was reduced by 100 days culturing using this method, and that immune response against these islets were no longer induced.

**Table 4:** Graft survival of C57BL6 mouse islets in BALB/c mice.

<table>
<thead>
<tr>
<th>Transplanted islets</th>
<th>N</th>
<th>Graft survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-cultured islets</td>
<td>5</td>
<td>5, 6, 11, 12, 38</td>
</tr>
<tr>
<td><em>In vivo</em> cultured islets</td>
<td>6</td>
<td>&gt;100, &gt;100, &gt;100, &gt;100, &gt;100, &gt;100</td>
</tr>
</tbody>
</table>

Example 3: Long-term storage of cells by means of another material as a semipermeable membrane capsule.

Pancreatic islets were isolated from BALB/c mice by collagenase digestion, as described above. An agarose powder (Agarose-LGT, Nacalai Tesque Inc, Kyoto, Japan) was diluted
to a 5% concentration by Hank's balanced salt solution. The agarose solution was completely solubilized by 120°C autoclaving for 30 minutes. The solubilized agarose solution was cooled in a 37°C water bath, and the mouse pancreatic islet tissue was admixed. Liquid paraffin was added to the 37°C solubilized agarose solution containing mouse pancreatic islet tissue, and the material was agitated in ice. The agarose was gelled by cooling, and the pancreatic islet was enclosed in agarose capsules.

The aforementioned agarose-encapsulated pancreatic islet was implanted into the peritoneal cavity of immune-deficient mice and cultured. The capsules were recovered after 100, 200, 300 and 400 days after implantation, and the pancreatic islet tissue was investigated histologically. Hematoxylin-eosin staining, immunohistochemical insulin staining, and aldehyde-fuchsin staining indicated that these islets secrete sufficient insulin for the reversal of diabetes in recipient animal. (data not shown) Thus, long-term storage of cells is possible even when an agarose is used as the material of a semipermeable capsule.

Example 4: Long-term storage of cells by means of another form and material as a semipermeable membrane capsule.

Pancreatic islet was isolated from BALB/c mice by collagenase digestion, as described above. Cellulose acetate tube (Daicel Chemical Industries, Ltd. Osaka, Japan) was connected to one ml syringe. Islet cells were aspirated into a cellulose acetate tube and both ends of the tube were closed by clips. Islet cells covered with cellulose acetate tubes are implanted into immune-deficient mice and stored.

The cellulose tubes were recovered after 50 days after implantation, and the pancreatic islet tissue was evaluated histologically. Hematoxylin-eosin staining, immunohistochemical insulin staining, and aldehyde-fuchsin staining indicated that these islets secrete sufficient insulin for the reversal of diabetes in recipient animal. (data not shown) This result shows that storage of cells is possible even when a cellulose membrane is used as the material of a semipermeable capsule, and also shows that storage of cells is possible even when other shape of capsule is used.
Example 5: Long term storage of other types of living cells.

An agarose powder (Agarose –LGT, Nacalai Tesque Inc, Kyoto, Japan) was diluted to a 5% concentration by Hank’s balanced salt solution. The agarose solution was completely solubilized by 120°C autoclaving for 30 minutes. The solubilized agarose solution was cooled in a 37°C water bath, and the human embryonic kidney 293 cells were admixed. Liquid paraffin was added to the 37°C solubilized agarose solution containing 293 cells, and the material was agitated in ice. The agarose was gelled by cooling, and the pancreatic islet was enclosed in agarose capsules.

The aforementioned agarose-encapsulated 293 cells were transplanted into the peritoneal cavity of immune-deficient mice and stored. The capsules were recovered after 50 days, and the cells were investigated histologically.

Hematoxylin-eosin staining indicated that these cells are viable even after in vivo culture. (data not shown) Thus, the present invention provides effective methods for storage of living cells other than neonatal porcine islet cells.

REFERENCES

The following references are cited in the application at the relevant portion of the application. Each of these references is incorporated herein by reference.


710-719, 2001


CLAIMS

What is claimed is:

1. A method of *in vivo* cell culture, comprising:
   a. encapsulating at least one cell in a capsule, said capsule comprising a semipermeable membrane;
   b. implanting the capsule into the body of a host animal;
   c. incubating the capsule in the host for a selected period of time; and
   d. removing the capsule from the body of the host animal.

2. The method of claim 1, further comprising the step of extracting said at least one cell from the capsule.

3. The method of claim 1 wherein the period of culture time is selected to be sufficient to reduce immunogenicity of the at least one cell.

4. The method of claim 1 or 3, further comprising the step of implanting said capsule into the body of a recipient animal.

5. The method of claim 1, 2, 3 or 4, wherein the capsule is implanted into a body cavity of the host animal.

6. The method of claim 1, 2, 3 or 4 wherein the capsule is implanted into a peritoneal cavity of the host animal.

7. The method of claim 1, 2, 3, 4, 5 or 6, wherein the host animal is an immune-deficient animal.

8. The method of claim 1, wherein the at least one cell is a mammalian cell.

9. The method of claim 1, wherein the at least one cell is a component of a tissue.
10. The method of claim 9, wherein the tissue is an islet.

11. The method of claim 10, wherein the tissue is a neonatal porcine islet.

12. The method of claim 9, wherein the tissue is human renal tissue.

13. The method of claim 1, wherein the host animal is a mouse.

14. The method of claim 1, wherein the semipermeable membrane is selected from the group consisting of: alginate, agarose, cellulose, poly-vinyl alcohol, polyacrylonitrile, aromatic polyamide, polysulfone, hydrogel and ultrafiltration membrane.

15. The method of claim 1, further comprising the step of implanting said capsule into the body of a next host animal.

16. A living cell cultured in accordance with the method of claim 1.

17. A living tissue cultured in accordance with the method of claim 1.

18. A product made from a cell cultured in accordance with the method of claim 1.

19. A product made from a tissue cultured in accordance with the method of claim 1.
Application number: numéro de demande: 02527176

Figures: 1-5, ab-c

Pages:

DRW-IP

Unscannable items received with this application
(Request original documents in File Prep. Section on the 10th Floor)

Documents reçus avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au 10ième étage)
Figure 6A

A

Group I

Microencapsulated NPI

Transplantation

Diabetic BALB/c Recipient

Group II

Microencapsulated NPI

Transplantation

In Vivo Maturation (100 days)

Diabetic SCID Mouse

Re-transplantation

In Vivo Matured Microencapsulated NPI
Figure 7

Panel A: 
- Unstained
- 2° Ab (Control)
- Naive

Panel B: 
- Unstained
- 2° Ab (Control)
- Non Matured

Panel C: 
- Unstained
- 2° Ab (Control)
- In Vivo Matured (100 d)

Panel D: 
- Unstained
- 2° Ab (Control)
- In Vivo Matured (87 d)