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(54) Title: IMMUNOPROTECTIVE BARRIER FOR TRANSPLANTATION OF CELLS

(57) Abstract

Cells for implantation into a patient in need thereof are packaged within an immunoprotective barrier prior to implantation, thereby obviating or minimizing rejection of the cells. The preferred immunoprivileged tissue for forming the barrier is cartilage. The tissue is formed into a layer that is thin enough to allow diffusion of nutrients and gases into the center of the cell mass placed within the immunoprotective barrier, typically less than 300 microns, preferably between 5 and 20 microns. Cells to be implanted, typically dissociated parenchymal cells including hepatocytes, Islets of Langerhans, or other cells having metabolic functions, are then placed on the barrier, and the barrier folded to seal the cells to be implanted within the barrier. In the preferred embodiment, the dissociated cells are first seeded onto a polymeric fiber matrix. The packaged cells are then implanted at a location providing an appropriate blood supply for diffusion of nutrients and gases through the barrier, for example, adjacent the mesentery. The example demonstrates packaging of Islets of Langerhans within a barrier formed by a monolayer of chondrocytes seeded onto polymer fibers.

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IMMUNOPROTECTIVE BARRIER FOR TRANSPLANTATION OF CELLS

Background of the Invention

The present invention generally relates to a method and composition for minimizing rejection of foreign cells implanted into an individual in need thereof, and more specifically is directed to a method using a barrier formed of immunoprivileged cells around the cells to be implanted.

A variety of methods have been used to prevent rejection of foreign cells, either allografts or xenografts, following implantation into an individual having a competent immune system. In most cases of organ transplantation, it is not possible to obtain autografts, so allografts are used in combination with immunosuppression using a drug such as cyclosporin. Cyclosporin is expensive, must be used daily for the rest of the life of the patient, and has side effects which can be serious. Autografts are typically possible only in the case of cells forming cartilage and skin. Other methods that have been used to prevent rejection of foreign cells have typically used synthetic materials such as alginate or polylysine-polyethylene glycol polymers that can be ionically crosslinked to form microcapsules that can be implanted to protect the cells, but still allow diffusion of nutrients and gases into and out of the microcapsules, along with the soluble products of the implanted cells. These materials tend to biodegrade after a period of time, however, and the cells are destroyed. Attempts to overcome this problem using non-biodegradable synthetic polymers such as ethylene vinyl acetate or polymethacrylate have been equally limited in effectiveness due to encapsulation of the implanted material by fibrotic material which "walls off" the foreign material from the rest of the tissues.

For example, Diabetes Mellitus is a common disorder of the glucose metabolism due to a reduction of insulin production or secretion. Six percent of the U. S. population (14 million patients) suffer from this disease; four million are on regular insulin medication. There are 30,000 new cases of Insulin Dependent Diabetes Mellitus (IDDM) every year. The estimated annual health care costs and lost wages are $92 billion. Diabetes Mellitus is the third most common disease and the eighth leading cause of death in the US. The standard therapy for patients with IDDM is a subcutaneous administration of insulin, a polypeptide, in differently intense regimens. Since this method of therapy does not provide the natural glucose/insulin feedback mechanism, frequent blood glucose measurements to adjust the dose of insulin are necessary. Events of glucose imbalance are still threatening and chronic complications will still occur.

Some current experimental therapeutic approaches try to overcome those problems by transplanting Islets of Langerhans. The β-cells of the islets produce and secrete the insulin and control the glucose/insulin feedback. Clinically the Islets of Langerhans are transplanted by transplantation of the entire pancreas although they represent only 1 to 2% of the pancreas mass or by transplantation of isolated islets. The two major problems are the necessity of immunosuppression and the scarcity of donor tissue, as discussed above.

Other methods for addressing the problem with rejection include immunomodulation, where the cell surface is altered so that the immune system can not recognize those cells as foreign, and immunoprotection, where a barrier for immunorecognition of the transplanted cells/tissue is provided, as discussed above. Current attempts utilize gelatinous or membranous inert materials to encapsulate the islets. Major problems of those methods have been that either the passage of nutrients for the islets was not sufficient or the materials attracted a non-specific immunoresponse against the cells.

It is therefore an object of the present invention to provide a method and compositions for implanting allografts and xenografts into a patient which minimizes the need for immunosuppression or subsequent rejection of the cells.

Summary of the Invention

Cells for implantation into a patient in need thereof are packaged within an immunoprotective barrier prior to implantation, thereby obviating or minimizing rejection of the cells. The preferred immunoprivileged tissue for forming the barrier is cartilage, although other tissues include cells forming the blood brain barrier as well as other cell types. The tissue is formed into a layer that is thin enough to allow diffusion of nutrients and gases into the center of the cell mass placed within the immunoprotective barrier, typically less than 300 microns, preferably between 5 and 20 microns. In the preferred embodiment, the barrier is formed by culturing dissociated cells directly on the surface of a culture dish or on a polymeric matrix, for example, formed of polyglycolic acid suture fibers spread on the bottom of a culture dish. Cells are grown to confluence, and in the case of chondrocytes, until matrix has been deposited. Cells to be implanted, typically dissociated parenchymal cells including hepatocytes, Islets of Langerhans, or other cells having metabolic functions, are then placed on the barrier, and the barrier folded to seal the cells to be implanted within the barrier. In the preferred embodiment, the dissociated cells are first seeded onto a polymeric fiber matrix. The packaged cells are then implanted at a location providing an appropriate blood supply for diffusion of nutrients and gases through the barrier, for example, adjacent the mesentery.

The example demonstrates encapsulation of Islets of Langerhans within a barrier formed by a monolayer of chondrocytes seeded onto polymer fibers.

Brief Description of the Drawings

Figure 1 is a schematic of the packaging of dissociated parenchymal cells attached to a polymeric fiber matrix within a monolayer of cartilage formed by chondrocytes proliferated on a fiber matrix.

Figure 2 is a micrograph (50x) of rat Islets of Langerhans on polyglycolic acid (PGA) fibers in culture for two weeks.

Figure 3 is a micrograph (100x) of rat Islets of Langerhans on PGA polymer fibers in culture for nine days, wrapped with a monolayer of bovine chondrocytes for four days, showing the margin of the construct.

Detailed Description of the Invention

A. Immunoprotective Barrier.

Immunoprivileged Tissue

Immunoprivileged tissue refers to tissues that surround a region of the body which is not exposed to a immune response. Examples include cartilage, the interior of the eye containing the vitreous, the vascular endothelium of the brain (the blood brain barrier), the maternal-fetal interface in the placenta, and the region of the testicles isolating the sperm. In the preferred embodiment described herein, cells forming cartilage such as chondrocytes or fibroblasts are used to form an immunoprotective barrier.

Cells are typically obtained by biopsy, most preferably from the patient into which the cells are to be implanted, although they can also be obtained from established cells lines or related individuals. Cells are obtained using standard techniques, for example, by punch biopsy or laproscopic surgery. Cells are dissociated by treatment using collagenase or trypsin, using standard methodology.

Matrix

Although it is not essential to seed the cells onto a matrix for use in forming an immunoprotective barrier, a matrix can be used to provide structural support for the cells to facilitate transfer and packaging of the cells to be implanted. Examples of suitable matrix materials are biodegradable or non-biodegradable polymers including polyhydroxy acids such as polyglycolic acid, polylactic acid, and copolymers thereof, polyanhydrides, polyorthoesters, polymers of synthetic and natural proteins, ethylene vinyl acetate, polyvinyl alcohol and many other polymers suitable for implantation into a person. The polymer is preferably in fibrous form, which can range from a single fiber of the type used as a suture, typically coiled or intertwined to form a support structure on a single plane, to woven or non-woven matrices of fibers, to porous sponge-like matrices.

Method for Manufacture

The dissociated cells of the immunoprivileged tissue are seeded onto the fibrous matrix, or seeded onto the bottom of a culture dish, and grown under standard conditions to confluence. Chondrocytes or fibroblasts are preferably grown until matrix is deposited and the tissue has the histology of cartilage. The cells are preferably in a very thin layer, ranging from a monolayer of cells between 5 and 20 microns in thickness, up to hundreds of microns, depending on the final application. In all cases, the thickness must be sufficient to prevent penetration by immunocompetent cells through the barrier, while allowing sufficient exchange of nutrients and gases and soluble metabolic products for the cells within the barrier to survive and serve their intended purpose.

B. Cells to be Implanted.

Although almost any cell can be implanted, the preferred cells are parenchymal cells having a metabolic rather than structural function. Examples include hepatocytes, Islets of Langerhans, spleen, pancreas, gall bladder, kidney, and other tissues having exocrine function. For ease of reference herein, all cells which are to be implanted within the barrier are referred to as "parenchymal cells". The cells are typically obtained from a donor or from cell culture, using standard biopsy or surgical techniques. The cells can also be genetically engineered to produce a desired molecule. Examples include cells engineered to express an enzyme missing or defective in the recipient or which express a therapeutic agent such as a toxin directed against cancer cells. Although discussed herein primarily with reference to allografts and xenografts, the technique can be used to decrease any encapsulation that may be present following implantation of autografts, particular using polymeric matrices that may elicit an inflammatory reaction or as part of the normal inflammatory process associated with surgery.

Cells are dissociated using standard techniques such as incubation in collagenase or trypsin solutions. A sufficient number of cells to provide the desired function following implantation must be obtained. The number of cells required can be determined based on *in vitro* assays, and known values for certain conditions. For example, many enzymes are measured in the blood stream as indicators of liver function; blood sugar levels are indicative of insulin production. The requisite cell mass for a desired function in a particular patient can also be determined based on comparison with normal organ function.

Packaging within the Barrier

The dissociated parenchymal cells are packaged within the barrier by placing the cells, either directed as obtained from a patient, dissociated, or after cell culture, onto the barrier layer. In the preferred embodiment, the cells are first seeded onto a suitable polymeric matrix, similar to that described above for forming the barrier, having interstitial spacing or pores of between approximately one hundred and 300 microns in diameter, although the structural requirements allow for a more random or thicker three dimensional shape, allowed to attach, and optionally proliferated in cell culture, then placed within the barrier layer. As shown in the following example, the barrier can be folded over the parenchymal cells and allowed to attach to itself, in a manner similar to making of an omelet, to form the final structure for implantation.

C. Implantation

The packaged parenchymal cells are implanted using standard surgical techniques, most preferably immediately adjacent to a highly vascularized tissue such as the mesentery. Hepatocytes are most preferably implanted with a portocaval shunt, to provide the hepatotrophic factors required for optimal survival of implanted hepatocytes. Implantation into the mesentery is particularly preferred.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: ***In vitro*** **culture of a tissue engineered construct of Islets of Langerhans on a polymer scaffold encapsulated with a monolayer of chondrocytes.**

This example utilizes the immunoprotective/immunoprivileged qualities of a chondrocyte matrix. The Islets of Langerhans are encapsulated with chondrocytes of the recipient. The matrix laid down by the chondrocytes protects the islets from immunorecognition of two different kind: first, immunorecognition of the islets as non-autologous (foreign) cells; second, autoimmunorecognition as a part of the disease process (IDDM is thought to be a autoimmune disease). In this way, the immunoprotection not only allows the use of allogeneic (same species) but also xenogeneic (different species) cell, which would solve the problem of donor scarcity.

Methods and Materials:

<u>Islets of Langerhans</u>

The Islets of Langerhans are harvested by injection of a collagenase solution retrograde through the common bile duct into the pancreatic duct. The pancreas is excised, kept on ice and then digested at 37°C. The islets are separated from the rest of the pancreatic tissue using a filter device and a gradient centrifugation. The number of islets is counted under the microscope.

The islets are seeded onto a biodegradable polymer, either poly(glycolic acid) (PGA), or poly(L-lactic acid) and allowed to attach to the polymer.

<u>Chondrocytes:</u>

Cartilage tissue is harvested and digested using a collagenase solution. The cells are filtered and centrifuged to select the viable cells. Next the chondrocytes are plated on a culture dish and kept in culture using the appropriate culture medium until they form a confluent monolayer. The layer of cells is then detached from the bottom of the dish using a cell scraping device.

<u>Covering of the islets on polymer with a monolayer of chondrocytes:</u>

As shown schematically in Figure 1, the detached layer of chondrocytes 10 is spread out as a flat layer. The islet loaded polymer12 is laid onto the chondrocyte layer 10. The islet loaded polymer 12 is then completely wrapped with a chondrocyte layer 10, which is lifted up from the bottom of the dish.

Figure 2 is a micrograph of rat Islets of Langerhans on polyglycolic acid (PGA) fibers in culture for two weeks. Figure 3 is a micrograph of rat Islets of Langerhans on PGA polymer fibers in culture for nine days, wrapped with a monolayer of bovine chondrocytes for four days, showing the margin of the construct. The islets are clumps of hundreds of cells. These are readily apparent in Figure 2 and Figure 3, even through the monolayer of cartilage forming the barrier.

Example 2: Preservation of function of cells in *in vitro* culture of a tissue enginnered construct of Islets of Langerhans on a polymer scaffold encapsulated with a monolayer of chondrocytes.

Purpose: The purpose of this study was to demonstrate the functional survival of Islets of Langerhans within a capsule of chondrocytes, which may serve as an immunoisolation barrier utilizing the immunoprivileged properties of the chondrocyte matrix.

Methods: Islets of Langerhans were isolated from Lewis rats. 1000 islets were seeded on a 1 x 1 x 0.06 cm biodegradable, porous polyglycolic acid (PGA) polymer scaffold (density 40 mg/cc) and encapsulated with a monolayer of bovine chondrocytes grown in culture. Encapsulated constructs and non-encapsulated controls were kept under standard culture conditions with a glucose content of 100 mg/dl. The cultures were exposed to a glucose challenge at a concentration of 400 mg/dl at days 3 and 5. The secretion of insulin into the culture medium was measured using a radio-immuno-assay (RIA). Histological and immunohistochemical studies using a polyclonal anti-insulin antibody were performed on the specimens.

Results: Histology demonstrated viability of the Islets of Langerhans completely encapsulated with several chondrocyte layers after 6 weeks in culture. Immunohistochemistry showed positive staining for insulin within the beta cells of the islets after 6 weeks in culture. Both the encapsulated constructs as well as the non-encapsulated controls showed an increase in insulin secretion into the culture medium after the glucose challenge.

The results are shown in Table 1.

Table 1: Results of in vitro culture of islet cells encapsulated in chondrocyte monolayers on polymers.

Days in culture	Insulin [μIU/ml] non-encapsulated control	Insulin [μIU/ml] encapsulated construct
2	2400	1900
3	800	1125
4 (24 hrs after glucose challenge)	1600	2300
5	800	1600
6 (24 hrs after glucose challenge)	1700	2100

Conclusions: (1) One can tissue engineer an encapsulated construct using autologous chondrocytes to encapsulate xenogeneic Islets of Langerhans. (2) Islets of Langerhans survive within the chondrocyte capsule up to 6 weeks in culture. (3) The glucose/insulin feedback mechanism of the encapsulated islets remains intact. (4) The chondrocyte capsule permits free diffusion of glucose and insulin.

Example 3: *In vivo* culture of a tissue enginnered construct of Islets of Langerhans on a polymer scaffold encapsulated with a monolayer of chondrocytes.

Purpose: The purpose of this study was to demonstrate *in vivo* survival of Islets of Langerhans within a capsule of chondrocytes, which may serve as an immunoisolation barrier utilizing the immunoprivileged properties of the chondrocyte matrix. *In vitro* studies described in examples 1 and 2 showed that the glucose/insulin feedback mechanism of the encapsulated islets remains intact and that the chondrocyte capsule permits diffusion of glucose and insulin.

Methods: Islets of Langerhans were isolated from Lewis rats. One thousand islets were seeded on a 1x1x0.06 cm biodegradable, highly porous polyglycolic acid (PGA) polymer scaffold (density 40 mg/cc) and encapsulated with a monolayer of bovine chondrocytes grown in culture. The encapsulated constructs and non-encapsulated controls were kept under standard culture conditions for three days and then implanted into the subcutaneous space of nude mice for two weeks. Histological and immunohistochemical studies using a polyclonal anti-insulin antibody were performed on the specimens.

Results: Histology demonstrated viability of the Islets of Langerhans completely encapsulated with a chondrocyte layer after 2 weeks *in vivo*. Immunohistochemistry showed positive staining for insulin within the beta cells of the islets.

Conclusion: (1) Capsules of autologous chondrocytes to encapsulate allogeneic or xenogeneic Islets of Langerhans can be tissue engineered. (2) Islets of Langerhans survive and continue to function within a chondrocyte capsule up to 2 weeks *in vivo*.

I claim:

1. A method for immunoprotecting cells for implantation comprising

packaging the cells to be implanted within a barrier formed by cells of immunoprivileged tissue, wherein the barrier is effective to immunoprotect the cells packaged therein and allows sufficient exchange of nutrients and gases to the packaged cells to maintain the viability of the cells.

2. The method of claim 1 wherein the packaged cells are parenchymal cells.

3. The method of claim 1 wherein the immunoprivileged tissue is cartilage and the cells are chondrocytes or fibroblasts.

4. The method of claim 1 wherein the packaged cells are dissociated cells seeded onto a polymeric fibrous matrix.

5. The method of claim 1 wherein the barrier is formed by seeding dissociated cells onto a polymeric fibrous matrix.

6. The method of claim 1 wherein the package or barrier further comprises a matrix formed of a biodegradable material.

7. Cells for implantation comprising

cells to be implanted packaged within a barrier formed by cells of immunoprivileged tissue, wherein the barrier is effective to immunoprotect the cells packaged therein and allows sufficient exchange of nutrients and gases to the packaged cells to maintain the viability of the cells.

8. The cells of claim 7 wherein the packaged cells are parenchymal cells.

9. The cells of claim 7 wherein the immunoprivileged tissue is cartilage and the cells are chondrocytes or fibroblasts.

10. The cells of claim 7 wherein the packaged cells are dissociated cells seeded onto a polymeric fibrous matrix.

11. The cells of claim 7 wherein the barrier is formed by seeding dissociated cells onto a polymeric fibrous matrix.

12. The cells of claim 7 wherein the package or barrier further comprises a matrix formed of a biodegradable material.

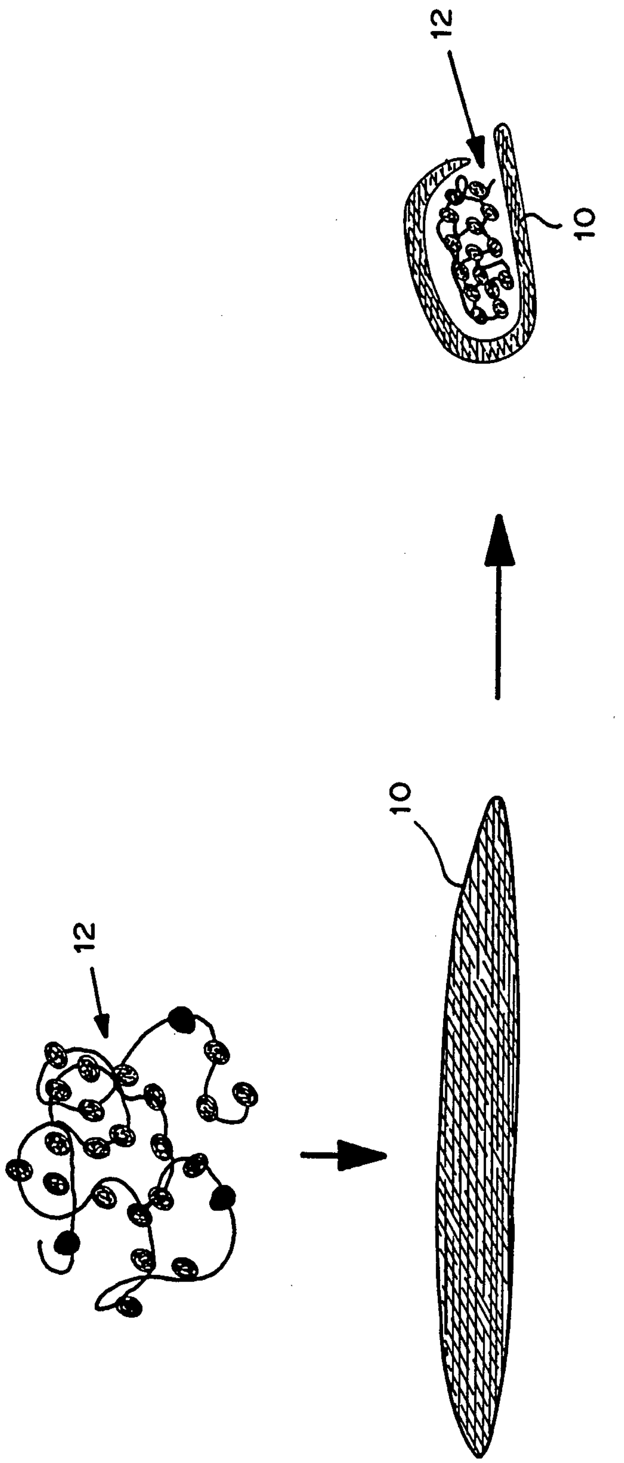

FIG. 1

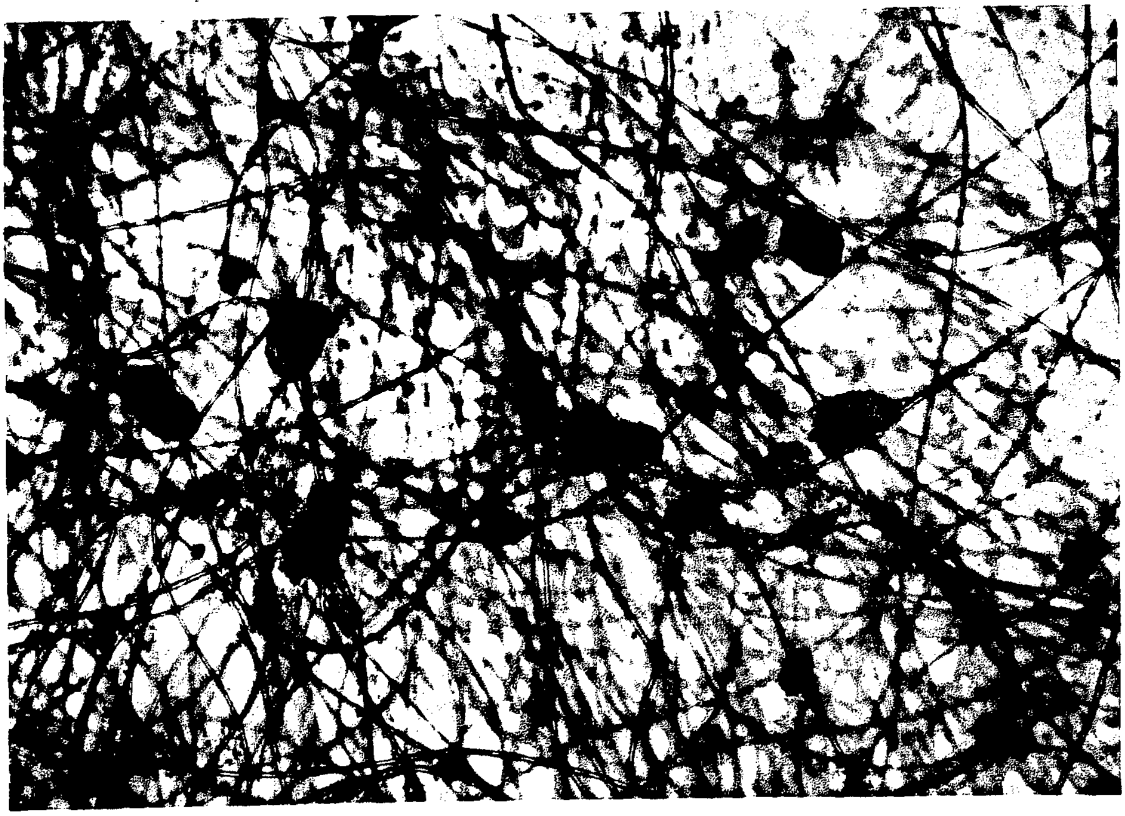

FIG.2

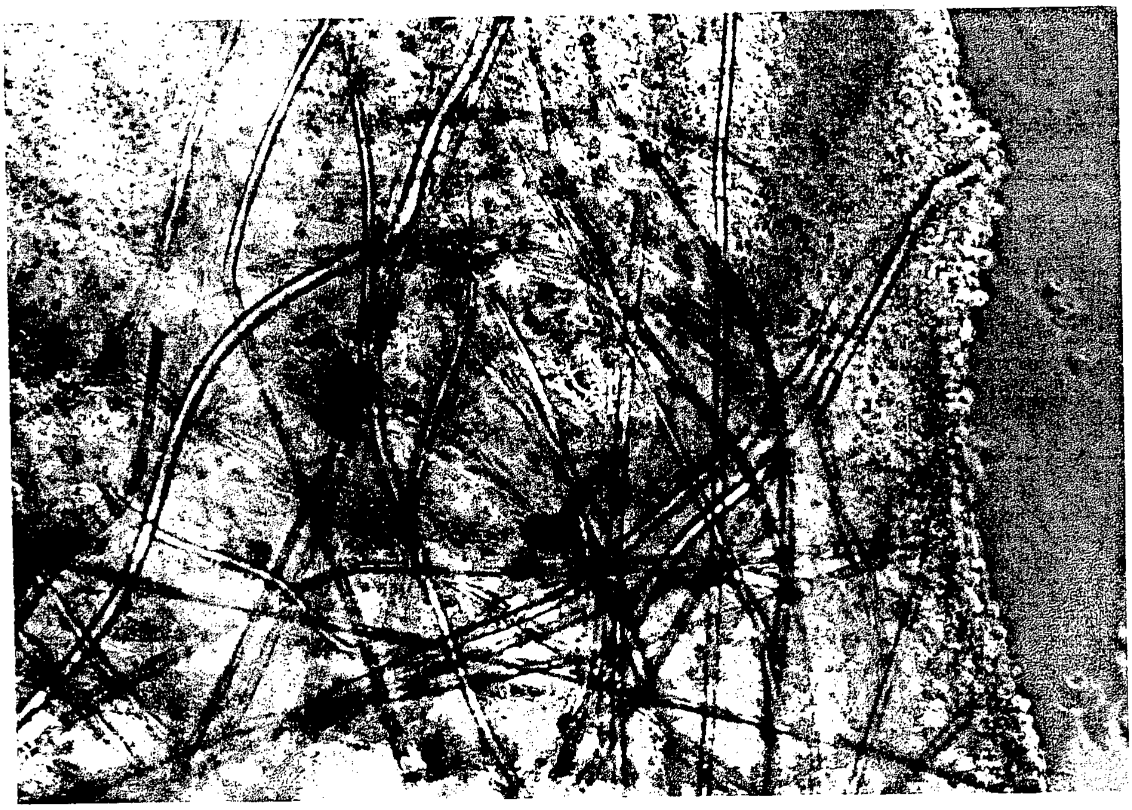

FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/08751

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 11/00, 11/02, 11/10, 11/08, 11/04; A61K 35/12
US CL :435/174, 177, 178, 180, 182; 424/93.7
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. : 435/174, 177, 178, 180, 182; 424/93.7

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)

APS,
search terms: immunoprotecting cells, implanting cells, parenchymal cells, chondrocytes, fibroblasts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/19195 A (BROWN UNIVERSITY RESEARCH FOUNDATION) 12 November 1992, entire document.	1-12
Y	US 5,041,138 A (VACANTI ET AL) 20 August 1991, entire document.	1-12
Y	US 4,505,266 A (YANNAS ET AL) 19 March 1985, entire document.	1-12
Y	US 5,324,518 A (ORTH ET AL) 28 June 1994, entire document.	1-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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