A method for repairing a retinal system of an eye, using bucky paper on which a plurality of retina pigment epithelial cells and/or iris pigment epithelial cells and/or stem cells is deposited, either randomly or in a selected cell pattern. The cell-covered bucky paper is positioned in a sub-retinal space to transfer cells to this space and thereby restore the retina to its normal functioning, where retinal damage or degeneration, such as macular degeneration, has occurred.

7 Claims, 3 Drawing Sheets
BUCKY PAPER AS A SUPPORT MEMBRANE IN RETINAL CELL TRANSPLANTATION

FIELD OF THE INVENTION

This invention relates to use of an artificial substance as a support membrane for transplant of retinal and iris pigment epithelial cells and stem cells that can transform into these cells.

BACKGROUND OF THE INVENTION

Transplantation of retinal pigment epithelial (RPE) cells and iris pigment epithelial (IPE) cells, as a means to rescue or restore diseased photoreceptors in a sub-retinal space, is a leading experimental therapy for treating age-related macular degeneration (AMD), the most common form of blindness for persons over age 65 in Western nations. A sub-retinal space is a space adjacent to or underneath the retina, where the eye’s photoreceptors are located.

The pathogenesis of AMD involves death of RPE cells at the posterior of the eye, underneath the retina in the sub-retinal space. The RPE membrane (Bruch’s membrane) is also damaged in AMD because of new blood vessel growth and other factors related to normal aging. Death of the photoreceptor cells, and eventual blindness, follows death of the RPE cells.

One current theory suggests that replacement of dying RPE cells in the sub-retinal space may rescue or restore the function(s) of the photoreceptor cells. First attempts at RPE cell transplantation involved injecting a suspension of RPE cells into a patient's sub-retinal space. This approach was supplanted by transplant of intact sheets of RPE cells. Each of these techniques was plagued with problems arising from disorientation of the transplanted cells and from destruction of the Bruch’s membrane in the AMD process.

One form of treatment of AMD is growth of RPE cells and/or IPE cells on a suitable support material and transplants of cells and support material into the sub-retinal space of the eye. The RPE and IPE cells have been shown to survive after injection into the sub-retinal space as single cell suspensions, as patches of cells, and as sheets of confluent cells. However, the inability of these transplanted cells to spontaneously form an organized monolayer and perform phenotypic functions of native RPE cells may be a cause of ineffectiveness of this type of treatment. It has been suggested that transplanted RPE cells perform poorly in the pathological sub-retinal space because such cells attach poorly to a damaged Bruch’s membrane of eyes affected by AMD. The Bruch’s membrane can become damaged because of growth of new vessels. Transplantation of cell suspensions, or even of patches or confluent sheets of such cells, may be ineffective for AMD treatment.

One possible approach is to grow RPE and/or IPE on a suitable support material and to transplant both the cells and the support material into the sub-retinal space. Growth properties and related characteristics of pigment epithelial cells are greatly influenced by the surface properties of the growth substrate. Several groups have studies different materials, such as anterior lens capsule and Descemet’s membrane, for transplantation of RPE cells and IPE cells into the sub-retinal space. These attempts have been unsuccessful because of the handling properties of the support materials used by these experimenters. Although cells have been grown on lens capsule, it is difficult to implant lens capsule into the sub-retinal space, due to its tendency to curl on itself, especially in an aqueous environment. It is an even greater challenge to maintain lens capsule material flat when the material is implanted into the sub-retinal space. Additionally, use of a 10–15 μm thick lens capsule structure to replace a 2-μm thick layer of Bruch’s membrane may pose some diffusion problems for the transplanted RPE and IPE cells and for the remaining retina. It is not yet known how porous lens capsule material and Descemet’s membrane material are and whether these materials will allow for proper diffusion of nutrients, waste, oxygen and carbon dioxide.

What is needed is a support material that (1) is biocompatible, (2) will serve as a surface for growing selected cells or sheets of cells, (3) is moderately strong, (4) has a controllable range of porosity, and (5) will not spontaneously roll up or form creases.

SUMMARY OF THE INVENTION

These needs are met by a support material referred to as “bucky paper.” Bucky paper is a mesh of carbon nanotubes (CNTs) whose thickness, density and/or porosity can be controlled in the manufacturing process. Bucky paper is made entirely of carbon and is biocompatible and capable of supporting growth of some biological substances. Because it is a mesh of CNTs, bucky paper is very porous and will allow nutrients, waste, oxygen and carbon dioxide to diffuse relatively easily through the CNT mesh, irrespective of thickness. Bucky paper can be made rigid, but can still be able to conform to the shape of the inner retina with appropriate fabrication. Bucky paper will allow for great precision during surgical handling and will remain relatively flat against the choroid when the combination of bucky paper and RPE cells or IPE cells or stem cells are transplanted into the sub-retinal space. When properly prepared, bucky paper will serve simultaneously as a substrate for cell growth and as a barrier for selectively preventing growth of unwanted biological tissues, such as blood vessels.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photographic image of a reattached retina of a White Rabbit’s eye after the experimental procedure described herein.

FIG. 2 is an SEM image of human RPE cells cultured onto a bucky paper surface.

FIG. 3 is a scanning electron micrograph (SEM) image of a bucky paper scrap after fabrication.

FIG. 4 is a photomicrograph of a typical CNT pattern used on bucky paper.

DESCRIPTION OF BEST MODES OF THE INVENTION

The invention provides rescue or restoration of the diseased photoreceptor cell layer of the retina, using transplantation of RPE cells, IPE cells or stem cells on bucky paper to the sub-retinal space of the eye. The bucky paper serves...
Cell Culture. Human RPE cells for transplantation were
treated first with 0.05% trypsin-EDTA and were cultured
weekly at a 1:10 ratio. A concentration of 10^6 cells/mL was
cultured onto sterilized bucky paper. Stem cells that can
transform or differentiate into RPE cells can also be trans-
planted here.

Animal IPE cells were harvested and isolated from New
Zealand White rabbits, using an enzyme-assisted microdis-
section procedure described by Hu and McCormick, in Arch.
tures of IPE had a thickness in the range of 50-100 µm and an
area density in the range of 700-1500 µg/cm². In one approach,
bucky paper is prepared by immobilizing 1 cm x 0.5 cm
bucky paper after mounting, was itself sterilized by immer-
sion in potassium hydroxide solution (pH=10), then
washed twice by centrifugation and re-suspension. The
purified CNTs were washed twice in distilled water, using
centrifugation and re-suspension. The purified CNTs are
re-suspended in distilled water, then mechanically formed
into bucky paper by removal of water by vacuum filtration
over a cellulose filter or similar filter. Portions of the CNTs
incorporated in the bucky paper produced here may be
"bundled", or partially or fully aligned, due to liquid flow
through the mesh of CNTs, which may provide a higher than
normal density of CNTs in an array.

This bucky paper used in the experiments discussed here
had a thickness in the range of 50-100 µm and an area
density in the range of 700-1500 µg/cm². In one approach,
bucky paper is prepared by immobilizing 1 cm x 0.5 cm
bucky paper on sterilized wax strips, using copper pins. The
bucky paper, after mounting, was itself sterilized by immer-
sion in 70 percent EtOH for 300 sec and subsequent exposure
to ultraviolet light for about 3 hours. FIG. 3 is a scanning electron micrograph (SEM) image of a bucky
paper scrap after fabrication. Separate procedures are
optionally provided for generating and controlling patterns
or densities of growth of an array of single wall nanotubes
or multi-wall nanotubes, with a carbon nanotube (CNT)
length that depends upon the structure involved. A CNT can
be grown with a length between about 25 µm and 200 µm,
or longer if desired. However, control of the length of the
CNTs may not be important for bucky paper applications.

The desired support material (bucky paper) will preferably
form a mesh or mat. The mesh thickness h(mesh) and mesh
density partly determine the bucky paper porosity. A mesh
density range of 4 x 10^6 - 6 x 10^7/cm², corresponding to a
range d = 40 nm - 5 µm for average nearest neighbor center-
to-center separation distance is produced where a substrate
is not used for CNT growth. Use of a higher bucky paper
average thickness h may require use of a higher separation
distance d, to preserve similar bucky paper behavior.

Cell Patterning on Bucky Paper. Enhancement of RPE
cell and/or IPE cell and/or stem cell attachment to bucky
paper can be accomplished by chemical modification of the
bucky paper surface (addition of hydrogen, nitrogen and/or
oxygen molecules) or by adsorption of specific growth factors and/or cytokines and/or antibodies and/or extracellular matrix proteins (such as CNTF, polyl-
sine, collagen, fibronectin, laminin, brain-derived neuro-
rophic factor, ciliary neurotrophic factor, nerve growth factor, forskolin, inhibitors of myelin-associated glycoprotein and inhibitors of NOGO). If necessary, the adsorption of specific growth factors and/or cytokines and/or antibodies and/or extracellular matrix proteins to the bucky paper can be stabilized by partial or complete cross-linking of these
specific growth factors and/or cytokines and/or antibodies and/or extracellular matrix proteins to one another, rather than by direct binding to the CNT elements of the bucky
paper.

In addition these specific growth factors and/or cytokines
and/or antibodies and/or extracellular matrix proteins can be
applied to the bucky paper surface in a specific pattern, such as a grid or geometric arrangement, in situations where a
pattern of RPE cell and/or IPE cell and/or stem cell attachment to the bucky paper may be advantageous, as compared to homogeneous, unpatterned attachment of cells. Patterning of specific growth factors and/or cytokines and/or antibodies and/or extracellular matrix proteins may also facilitate attachment of combinations of RPE cells, IPE cells and/or stem cells to the bucky paper surface, as compared to single populations of cells; transplanation of combinations of cell types may achieve superior results in restoring retinal function a compared to transplantation of only one cell type.

One attractive pattern is a grid-like hexagonal pattern, illustrated in a photomicrograph shown in FIG. 4, where a side of the polygonal array has a length in the range of 50-100 µm. In this approach, one challenge is to deposit the RPE and/or IPE cells and/or stem cells with precision, allowing little or no spillover of the cells into the interstitial filter paper and through at least one filter aperture;

The vacuum filtration device includes a solid support surface, with apertures therein for vacuum filtering, and a filter paper positioned to prevent loss of the CNTs through the apertures. The vacuum filtration process used to fabricate the bucky paper often leaves a pattern of dimples, representing the filter aperture pattern, on the bucky paper. This pattern can be used to provide an array of sites for the RPE, IPE and/or stem cells to be deposited on the paper, by providing a surface pattern that matches the desired pattern of oxygen surfaces. The filter aperture pattern need not be uniform and may have regions where no apertures are present and other regions where a relatively high density of apertures is present. The solid support material of the filtration device is usually a rigid material so that a filter with the desired aperture pattern can be fabricated and will hold the aperture pattern for repeated use, if desired. The filter paper, placed over the solid support material and cut into pieces, is chosen to be flexible, in order to conform to and replicate the aperture pattern on the bucky paper as the paper is formed.

What is claimed is:

1. A method for preparing a retinal system of an eye for repair, the method comprising:
   providing a support material, comprising bucky paper having a selected thickness and a selected porosity, as a patch having a selected size;
   transferring at least one of (i) a retinal pigment epithelial cell, referred to as an RPE cell, (ii) an iris pigment epithelial cell, referred to as an IPE cell and (iii) a stem cell, to the support material, to provide a cell-covered support material; and
   attaching the cell-covered support material to a selected region in a sub-retinal space of an eye that is to be repaired.

2. The method of claim 1, wherein said step of providing said bucky paper comprises:
   using a high pressure carbon monoxide process to prepare a plurality of single-wall and multi-wall carbon nanotubes (referred to as “CNTs”);
   immersing the plurality of CNTs in a selected acid for a selected time interval, and allowing the CNTs to become purified;
   centrifuging the CNTs at least once to form at least one pellet containing primarily CNTs;
   suspending the CNT pellet at least once in a selected base for a selected time interval;
   immersing the CNT pellet in distilled water;
   removing substantially all liquid from the CNT pellet by:
   providing a solid support filter having an array of filter apertures therein in a selected pattern;
   providing a filter paper, having a selected porosity, contiguous to the filter apertures;
   positioning the CNT pellet adjacent to the filter paper so that the filter paper lies between the CNT pellet and the filter; and
   applying a selected vacuum to the filter and filter apertures so that liquid associated with the CNT pellet is removed from the CNT pellets through the filter paper and through at least one filter aperture;
   mechanically forming the CNT pellet into at least one scrap of said bucky paper; and
   allowing at least one dimple to form in a surface of said bucky paper adjacent to a location of one of said apertures in said solid support filter.

3. The method of claim 1, wherein said step of providing said cell-covered support material comprises:
   transferring a plurality of at least one of RPE cells, IPE cells and/or stem cells to the cell-covered support material;
   pressing said cell-covered support material against said sub-retinal space comprises:
   pressing said cell-covered support material against said selected region in said sub-retinal space so that said cell-covered support material becomes mechanically attached to said selected region.

4. The method of claim 3, wherein said step of attaching said cell-covered support material to said selected region in said sub-retinal space comprises:
   pressing said cell-covered support material against said selected region in said sub-retinal space so that said cell-covered support material becomes mechanically attached to said selected region.

5. The method of claim 3, wherein said step of attaching said cell-covered support material to said selected region in said sub-retinal space comprises:
   pressing said cell-covered support material against said selected region in said sub-retinal space so that at least one of said RPE cells, said IPE cells and said stem cells is attached to the at least two connected regions of said support material.

6. The method of claim 3, further comprising selecting said pattern from the group of patterns consisting of triangles, quadrilaterals, pentagons, hexagons, n-sided polygons, with n at least equal to 7, circles and ovals.

7. The method of claim 1, wherein said step of providing said cell-covered support material comprises:
   providing said bucky paper in a selected pattern on said support material, the selected pattern comprising at least two connected regions of bucky paper separated by at least one interstitial region that is substantially free of bucky paper, where said selected thickness of said bucky paper lies in a range 25-200 µm; and
   transferring a liquid solution containing at least one of said RPE cells, said IPE cells and said stem cells in a selected pattern to said support material.

whereby the at least one of said RPE cells, said IPE cells and said stem cells is attached to the at least two connected regions of bucky paper and is not attached to the at least one interstitial region.

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