A New Model of Retinal Pigment Epithelium Transplantation With Microspheres

Arutun Oganesian, MD; Karine Gabrielian, MD; J. Terry Ernest, MD, PhD; Samir C. Patel, MD

Objectives: To develop a 3-dimensional carrier system for subretinal transplantation of human fetal retinal pigment epithelial (HFRPE) cells and to assess their growth pattern in the rabbit subretinal space.

Methods: After a standard 3-port vitrectomy, HFRPE cells grown as microspheres on cross-linked fibrinogen were introduced into the subretinal space of rabbits. The eyes were studied at 7, 14, and 30 days after surgery by ophthalmoscopy and light microscopy.

Results: Ophthalmoscopically, at day 7, 11 (61%) of the 18 eyes showed radiating hyperpigmentation around the transplanted HFRPE microspheres. The results of a histological examination revealed a monolayer outgrowth of HFRPE cells, overlying host retinal pigment epithelium. The control eyes revealed a patch of chorioretinal atrophy with lymphocytic infiltration around the microspheres.

Conclusions: Human fetal retinal pigment epithelial cells grown as microspheres on cross-linked fibrinogen can be successfully transplanted into the subretinal space. Cells can survive for at least 1 month and form a monolayer over the host retinal pigment epithelium cells, with a mild local inflammatory response. The difference in inflammatory responses between the eyes that underwent transplantation and the control eyes may suggest a modulating effect of the HFRPE cells on inflammation, immunity, or both. This new xenogenic model may have importance in the study of subretinal transplant cell biology and the associated immune response.

Clinical Relevance: The results of this study may be important for better understanding of the mechanisms of retinal pigment epithelium cell behavior after transplantation. The proposed model may be applicable for future clinical and experimental investigations in the area of retinal pigment epithelium transplantation.

MATERIALS AND METHODS

PREPARATION OF THE MATRIX

Cross-linked fibrinogen films were prepared under sterile conditions by mixing fibrinogen, 90 mg, and flavin mononucleotide, 1.3 mg (Sigma-Aldrich Corp, St Louis, Mo), in 5 mL of deionized water. \(^3\) Four drops (≈80 µL) of the mixture were spread evenly on the bottom of a 30-cm Petri dish. The mixture was left under UV light for 12 hours. This allowed the formation of 20- to 30-g thick, yellowish, transparent, slightly sticky films that could easily be separated from the bottom of the dish with fine forceps. The film was cut into smaller 1 × 1-mm pieces that were used for HFRPE monolayer implantation.

SEPARATION AND CULTURE OF HFRPE CELLS AS MICROSPHERES

Human fetal eyes at 17 to 22 weeks of gestation were used in this study. The eyes were obtained from the Anatomic Gift Foundation, Laurel, Md, and from the University of Chicago Hospitals, Chicago, Ill, after therapeutic abortions. The eyes were enucleated and processed under aseptic conditions by mixing fibrinogen, 90 mg, and flavin mononucleotide, 1.3 mg (Sigma-Aldrich Corp, St Louis, Mo), in 5 mL of deionized water. \(^3\) Four drops (≈80 µL) of the mixture were spread evenly on the bottom of a 30-cm Petri dish. The mixture was left under UV light for 12 hours. This allowed the formation of 20- to 30-g thick, yellowish, transparent, slightly sticky films that could easily be separated from the bottom of the dish with fine forceps. The film was cut into smaller 1 × 1-mm pieces that were used for HFRPE monolayer implantation.

RESULTS

Three albino and 3 pigmented rabbits (6 eyes) were studied in each group that underwent transplantation and in each control group at each period. In total, 36 eyes were studied.

OPHTHALMOSCOPY

0 to 7 Days

The transplanted microsphere (Figure 1, A) appeared as a pigmented subretinal lesion (Figure 1, B and C). On day 7, subretinal hyperpigmentation was noted in 2 rabbit eyes in proximity to the transplanted HFRPE.
microspheres. None of the eyes showed intraocular inflammation.

14 Days

Four of 6 eyes showed hyperpigmentation around the transplanted microsphere (Figure 1, D). In those eyes in which hyperpigmentation was present from day 7, an increase in its size with the formation of pseudopodia was noted around the donor tissue source. No intraocular inflammation was seen.

30 Days

Five of 6 eyes showed hyperpigmentation around the microspheres. No ophthalmoscopic evidence of inflammation or infection was noted at the 30-day follow-up.

In summary, the extent of hyperpigmentation varied among the eyes, ranging from no hyperpigmentation (7 eyes: 3 albino and 4 pigmented eyes) to prominent hyperpigmentation, with some extending as far as 3 to 4 disc diameters away from the initial donor site (Figure 2, A-C). A total of 11 of 18 rabbits that underwent transplantation showed subretinal hyperpigmentation adjacent to the microspheres.

In the control eyes, no subretinal hyperpigmentation was noted. Starting from day 7, prominent whitening due to chorioretinal atrophy, with no change in size with time, was seen in all control pigmented rabbit eyes around the transplanted matrix (Figure 2, D). Similar chorioretinal atrophy was noted in the control albino eyes at the site of the transplanted matrix.

HISTOPATHOLOGIC CHARACTERISTICS

There were no notable differences noted in the inflammatory response at various times after transplantation. For better assessment of the sections, we defined 3 tissue regions: a region over the microsphere, which included only the microsphere with overlying retina and underlying choroid; a region close to the microsphere, which included the area where the microsphere was always seen with adjacent migrated cells; and a farther region, which included sections where only donor cell monolayer was seen.

Light microscopy showed that the areas corresponding to the transplanted microspheres were composed of a circumscribed region of highly pigmented HFRPE cells. The cells were residing in the subretinal space as thick multilayers (Figure 3, A). Loss of photoreceptors was typically noted immediately above and in close proxim-
ity to the transplanted tissue. Migration of transplanted HFRPE cells into the overlying neurosensory retina was noted at the site of microsphere implantation. Fragments of the matrix appeared as eosinophilic material between the HFRPE cells. Human fetal retinal pigment epithelial cells were seen in 2 eyes in close proximity to the initial donor tissue at 7 days after the transplantation. At 14 days after the transplantation, the HFRPE cells were identified at farther regions from the microsphere. In both albino and pigmented eyes, the donor cells formed a monolayer in the subretinal space. This corresponded to the hyperpigmentation site that was seen ophthalmoscopically around the microsphere (Figure 3, B-F, and Figure 4, A and C). The cells forming the transplanted microspheres as well as pigmented cells seen at the subretinal space showed positive immunostaining for HLA-ABC monoclonal antibodies (Figure 4, C, and Figure 5, A-D). Neurosensory retina was preserved above the pigmented cell monolayers located at the distant site from the microsphere (Figure 3, C).

The area with the transplanted microsphere was also studied by scanning electron microscopy at 30 days after the transplantation (Figure 6, A and B) in 2 albino eyes. The retina was locally “glued” to the underlying microsphere, and the microsphere appeared flattened. Two layers of RPE could be identified. The cells in the top layer appeared more rounded, with filamentous cell-cell junctions, possibly representing donor tissue, while the bottom layer showed flatter, more polygonal cells, probably the host RPE. The HFRPE cells that grew out from the microsphere appeared rounded and were of different sizes compared with the host RPE cells. They showed monolayer formation around the microsphere, and they formed long filamentous cell-cell junctions. In some areas, the monolayers were not continuous; and in some areas, only 2 or 3 donor cells were seen.

The cellular response to the transplanted tissue was strictly local and was present around the microsphere, mainly in the underlying choroid. Compared with the controls, there was minimal choroidal thickening with mononuclear cell infiltration beneath the microsphere itself. No inflammatory or lymphocytic responses were seen in the areas where the HFRPE cells were distributed as monolayers.

A striking difference was noted in the control eyes in which only bare matrix microspheres were trans-
planted (Figure 7, A and B). A markedly thickened, infiltrated choroid was evident under the transplanted matrix, with loss of photoreceptors in the overlying retina. Lymphocytes and other mononuclear cells invaded the entire area of the subretinal space and the retina around the matrix. This could represent a mixed nonspecific inflammatory and immune response. This cellular response was local and was confined to the area of the transplanted bare matrix. The reaction was more severe than in the eyes that underwent HFRPE transplantation. Multiple cells with engulfed eosinophilic material, possibly the matrix, were identified in the area. At 30 days after the surgery, some of the control eyes showed chorioretinal atrophy, with no extracellular matrix present. Immunostaining with CD5 monoclonal antibody, which recognizes rabbit panlymphocytes, showed more intense lymphocytic infiltration in control eyes than in the eyes that underwent HFRPE transplantation (Figure 8, A-C).

**COMMENT**

Subretinal RPE transplantation has shown promise in the rescue of overlying receptors in some experimental degenerative retinal diseases. This may be important in the management of various diseases affecting the RPE. Our 3-dimensional culture system offers a new approach for the provision of donor tissue into the subretinal space.

The transfer of the HFRPE cell-containing microspheres into the subretinal space is simple and reproducible. The adjustable size and spherical shape of the donor tissue makes it easy to insert into the subretinal space. Because the HFRPE cells in our model form compact tissue conglomerates, there is less chance for cell reflux. To the best of our knowledge, in previous studies of RPE transplantation, there was no evidence of donor cell proliferation or migration in the subretinal space. Some studies suggest that the subretinal space...
is an immune-privileged environment where cell proliferation is kept under strict control. Transplanted cells may need some kind of initial stimulation to migrate or proliferate actively in this environment. Fibrinogen and the 3-dimensional state of HFRPE tissue could contribute to the ability of the cells in our model to grow out from the initial source. Recent studies done in our laboratory provide indirect evidence of the importance of the modulatory effect of the matrix on cell behavior in the subretinal space. Human fetal retinal pigment epithelial cells grown as microspheres on a synthetic polymer matrix showed notably less potential for subretinal spread compared with cells grown on a fibrinogen matrix. Some cell types reexpress their original in vivo characteristics in a 3-dimensional culture and maintain cell-specific functions that are lost in monolayer cultures. The cells in 3-dimensional cultures express increased DNA synthesis and proliferation. Donor cells provided to the recipient as 3-dimensional culture systems show prolonged survival and the ability to migrate from the initial source and establish themselves among the host tissues. In addition, fibrinogen is a known potent stimulator for cell proliferation and migration. Related to it, fibronectin is an important constituent of the Bruch membrane and the surrounding RPE. Human fetal retinal pigment epithelial cells grown on cross-linked fibrinogen matrix in a 3-dimensional state with tight cell-cell contacts may become activated and possess the potential for migration and proliferation after being brought into the subretinal space. Although there was notable damage to the overlying retina, it appeared to be only local and restricted to the site of the microsphere. The damage can be comparable in size with a large laser burn. The retina appears preserved, however, above the HFRPE monolayers at more distant sites from the microsphere. The growth of HFRPE cells outside the maternal source of donor tissue, shown in our studies, may provide an opportunity to transplant microspheres in an extrafoveal region with secondary spreading to the subfoveal space.

The ophthalmoscopic observation of subretinal hyperpigmentation around the transplanted microspheres corresponded histologically to a monolayer of pigmented cells in albino rabbits. Immunohistochemically, the transplanted cells were identified by staining for HLA-ABC antibody, and similarly showed migrating HFRPE cells from the initial source with monolayer formation close to the transplanted microsphere.

All control eyes transplanted with bare matrix showed a notably higher inflammatory response and increased lymphocytic infiltration compared with the HFRPE transplanted eyes. Retinal pigment epithelium can modulate the functions and behavior of other cells, such as lymphocytes, vascular endothelial cells, and macrophages. Recent studies have shown that some tumor cells grown as spheroids show increased resistance to lymphocyte lysis and inhibition of lymphocyte penetration compared with the cells grown as monolayers. Human fetal retinal pigment epithelial cells transplanted as multicellular spheroids, ie, microspheres, may similarly possess lymphocyte inhibitory qualities. Retinal pigment epithelium cells have been shown to release transforming growth factor β family proteins that have immunosuppressive functions and that can inhibit neovascularization. In addition, cell types grown in a 3-dimensional culture system show increased levels of intracellular cytokines, including transforming growth factors. Human fetal retinal pigment epithelial cells cover the matrix, possibly preventing its direct contact with the
subretinal tissues, resulting in less intense inflammation compared with controls. Some studies explain the rescuing effect of RPE transplantation to cytokine release by healthy donor cells. Three-dimensional cultures of HFRPE cells may be a better source of the cumulative release of different trophic cytokines, compared with monolayers, due to the high accumulation of healthy cells.

In conclusion, the provision of donor cells into the subretinal space as microspheres is reproducible and tech-
Figure 6. Scanning electron micrographs of the area transplanted with a human fetal retinal pigment epithelial (HFRPE) microsphere in an albino rabbit at day 30. A, Short arrows indicate the top layer with rounded HFRPE cells; long arrows, the more polygonal bottom layer with host retinal pigment epithelium cells; R, turned over retina; and M, turned over transplanted microsphere (original magnification ×250). B, Higher magnification of the area with possible HFRPE outgrowth, top cell layer. The arrows point to the intercellular junctions (original magnification ×2200).

Figure 7. A, The subretinal human fetal retinal pigment epithelial microsphere (long arrows) in an albino rabbit eye. Short arrows point to the donor cells that migrated from the initial source. Choroidal infiltration appears less compared with the control (original magnification ×200). B, The control pigmented rabbit eye transplanted with bare microsphere matrix. The whole area is infiltrated with inflammatory cells. Arrows point to the matrix.

Figure 8. A, Fluorescence image from an eye that underwent transplantation shows only minimal staining against CD5 compared with the control eye (B) transplanted with matrix only (arrows) (original magnification ×200). The arrows point to positively stained inflammatory cells. Ch denotes choroid. C, Negative control stained with irrelevant antibody (original magnification ×400).
nically easy, and it may decrease the chances for iatro-
genic damage to the retina. The donor cells can spread
and survive in the subretinal space for at least 1 month.
Subretinal transplantation of HFRPE cells as a 3-dimen-
sional culture system may become a useful model for
studying donor and host cell interactions.

Accepted for publication June 8, 1999.

Supported in part by Research to Prevent Blindness Inc,

Presented in part at the Chicago Ophthalmological So-
ciety Meeting, Chicago, Ill, May 20, 1998; and was awarded
the Beem-Fisher prize.

Reprints: Arutun Oganesian, MD, Department of
Ophthalmology and Visual Sciences Center, University
of Chicago, 939 E 57th St, Chicago, IL 60637 (e-mail:
aoganesi@midway.uchicago.edu).

REFERENCES

1. Tezel TH, Del Priore LV. Reattachment to a substrate prevents apoptosis of hu-
41-47.


3. Li L, Turner JE. Functional and structural characteristics of pho-
toceptor cells rescued in RPE-cell grafted retinas of RCS dystrophic rats. Exp

4. Sheedlo HJ, Li LX, Turner JE. Functional and structural characteristics of pho-
toceptor cells rescued in RPE-cell grafted retinas of RCS dystrophic rats. Exp

5. Gabrielian K, Oganesian A, Patel SC, Verp MS, Ernest JT. Cellular response in
rabbit eyes after human fetal RPE transplantation. Graefes Arch Clin Exp Oph-


internal approaches for transplantation of autologous retinal pigment epide-

8. Gouas P, Huijung C, Yaohua S, Tenuyo T, Eremova Y, Kjeldbye H. Patch cultur-
ing and transfer of human fetal retinal epithelium. Graefes Arch Clin Exp Oph-

235:103-110.

10. Wintemantel E, Cima L, Schloo B, Langer R. Angiopolarity of cell carriers: di-
11. Hoffman RM. To do tissue culture in two or three dimensions? that is the ques-

12. Spier RE, Maroudas N. Microcarriers for animal cell biotechnology: an unful-

13. Peshwa MV, Wu FJ, Sharp HL, Derra FB, Hu WS. Mechanics of formation and
32:197-203.


15. Ruoslahti E, Hayman EG, Fierschbacher M. Extracellular matrices and cell ad-

16. Spector DH, Boss BD, Strecker RE. A model three-dimensional culture system
for mammalian dopaminergic precursor cells: application for functional intrace-

17. Hoffman RM. The three-dimensional question: can clinically relevant tumor drug

18. Kleinman HK, Klebe RJ, Martin GR. Role of collagenous matrices in the adhe-

19. Gray AJ, Bishop JE, Reeves JT, Laurent GJ. Alpha and Beta chains of fibrinogen

20. Hoffman RM. To do tissue culture in two or three dimensions? that is the ques-

13:405-410.

22. He S, Wang HM, Ogden TE, Ryan SJ. Transplantation of cultured human retinal
231:737-742.

23. el Dinini AA, Wang HM, Ogden TE, Ryan SJ. Retinal pigment epithelium implan-
tation in the rabbit: technique and morphology. Graefes Arch Clin Exp Ophthal-


25. Gouas P, Lopez R, Brittis M, Kjeldbye H. The ultrastructure of transplanted rab-


27. Jiang LG, Jiorgura M, Streilein JW. Subretinal space and vitreous cavity as im-
munologically privileged sites for retinal allografts. Invest Ophthalmol Vis Sci.
1993;34:3347-3354.


29. Williamson E, Rezai KA, Farrokhi-Siar L, et al. Biodegradable polymer film as a
source of adhesion and formation of human retinal pigment epithelium sphero-
ides. Paper presented at: Association of Research in Vision and Ophthalmo-
lology meeting; May 1998; Fort Lauderdale, Fla. 1998;39:100. Abstract 472.

30. Mere D, Albrecht P, Hopp HE. Cell growth optimization in microcarrier cul-


32. Sorn LA, Bunce LA, Francis OW. Cell proliferation on fibrin: modulation by fi-

33. Campochiaro PA, Jordon JA, Glaser BM. The extracellular matrix of human reti-

34. Koller MR, Papoutsakis ET. Cell adhesion in animal cell culture: physiological

35. Olive PL, Durand RE. Drug and radiation resistance in spheroids: cell contact

36. Liversidge J, Grabowski P, Ralston S, Benjamin N, Forrester JV. Retinal pig-
ment epithelial cells express an inducible form of nitric oxide synthase and pro-
duce nitric oxide in response to inflammatory cytokines and activated T cells.
Immunology. 1994;83:404-409.

37. Liversidge J, McKay D, Mullien G, Forrester JV. Retinal pigment epithelial cells
modulate lymphocyte function at the blood-retina barrier by autocrine PGE2 and

38. Sakamoto T, Sakamoto H, Murphy TL, et al. Vessel formation by choroidal en-
dotheelial cells in vitro is modulated by retinal pigment epithelial cells. Arch Oph-
thalmol. 1995;113:512-520.

39. Ochalek T, von Kleist S. Study of the resistance of tumor-cell spheroids to pen-

40. Anderson DH, Guerin CJ, Hageman GS, Pfeffer BA, Flanders KC. Distribution of
transforming growth factor-beta isoforms in the mammalian retina. J Neurosci

41. Yoshimura N, Matsumoto M, Shimizu H, Manda M, Hata Y, Ishibashi T. Photoso-
agulated human retinal pigment epithelial cells produce an inhibitor of vascular en-

42. Seaton AD, Sheedlo HJ, Turner JE. A primary role for RPE transplants in the in-
hibition and regression of neoangiogenesis in the RCS rat. Invest Ophthalmol

43. Ness GO, Pedersen PH, Bjerkvig R, Laerum OD, Lillehaug JR. Three-
dimensional growth of glial cell lines affects growth factor and growth factor re-