



HAL
open science

Sources of RPE for replacement therapy

Edward Lee, Robert E Maclaren

► **To cite this version:**

Edward Lee, Robert E Maclaren. Sources of RPE for replacement therapy. *British Journal of Ophthalmology*, 2010, 95 (4), pp.445. 10.1136/bjo.2009.171918 . hal-00577961

HAL Id: hal-00577961

<https://hal.science/hal-00577961>

Submitted on 18 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Sources of RPE for replacement therapy

Key words: iPS, stem cell, AMD, cloning, Yamanaka, transplantation

Edward Lee MA FRCOphth,^{1,2} Robert E MacLaren DPhil FRCS
FRCOphth^{1,2,3,4}

¹UCL Institute of Ophthalmology, ²Moorfields Eye Hospital Foundation Trust
NIHR Biomedical Research Centre, ³Nuffield Laboratory of Ophthalmology
University of Oxford, ⁴Oxford Eye Hospital

Correspondence to Prof Robert MacLaren at:
Nuffield Laboratory of Ophthalmology University of Oxford
Level 6 John Radcliffe Hospital, Headley Way, Oxford OX3 9DU

Tel: +447702727853

email: robert.maclaren@merton.ox.ac.uk (not for publication)

Commissioned review

Summary: 194 words

Main text: 2958 words

References: 59

Figure legend: 1

Figure: 1 x 600 dpi, 8 bit RGB colour, 18.50 cm x 9.41 cm JPEG

Summary

Stem cells with their capacity to regenerate and replace diseased tissues have recently been proposed as having great potential in the treatment of age-related macular degeneration (AMD). A stem cell therapeutic approach could operate either to replace the retinal pigment epithelium (RPE), or the neurosensory retina, or a combination of both. From the scientific perspective, RPE replacement alone is likely to be far more straightforward than rebuilding the complex circuitry of the neurosensory retina. Furthermore, recent advances with induced pluripotent stem (iPS) cells have raised the real possibility of transplanting healthy "young" autologous RPE into patients with early signs of AMD. At this stage, however, it is useful to reconsider some of the earlier clinical studies that used suspensions of autologous RPE cells harvested from the peripheral retina. These showed that isolated RPE cell suspensions had little capacity to recreate a monolayer on the diseased Bruch's membrane of AMD. To counter this problem, researchers from Southampton in the UK report use of a synthetic polymer alternative to Bruch's membrane,¹ which could provide a scaffold for future RPE derived from stem cells, or possibly reopen opportunities for autologous RPE cells harvested from the peripheral retina.

Introduction

Current surgical treatments for AMD act on the principle of intervention when foveal photoreceptors are acutely compromised by diseased RPE, with a view to restoring contact with healthy RPE before significant degeneration has occurred. This has traditionally involved macular translocation,²⁻⁴ RPE suspension,⁵⁻⁶ or patch graft techniques,⁷⁻¹¹ developed by vitreoretinal surgeons over the last two decades. Surgery was typically most effective following the acute development of choroidal new vessels (CNV) and haemorrhage, because this provided a window of opportunity for RPE replacement before secondary degeneration of the overlying neurosensory retina. The advent of effective anti-vascular endothelial growth factor (VEGF) therapies, however, has made it difficult to justify surgical interventions which have potentially severe complications, except for specific mechanical conditions such as RPE tears.¹² This applies also for non-exudative AMD, because some patients will retain good vision for many years, which could otherwise be compromised acutely by a surgical complication. For

surgical treatments to be re-established as a potential treatment for AMD it will be necessary to find some way of simplifying the process to reduce the risks of severe sight threatening complications, such as proliferative vitreoretinopathy (PVR), sub macular haemorrhage and retinal detachment. Stem cells have been proposed as providing a simpler surgical alternative, but this concept is also likely to be complex, because some means of restoring Bruch's membrane would almost certainly still be necessary and this would require further surgical manipulations beyond a simple subretinal injection of a cell suspension.

Here we discuss the essential physiological properties of Bruch's membrane that would need to be replaced by a polymer-based scaffold and the potential sources of RPE cells that could be combined with this new technology as a future treatment for AMD.

Morphology and function of the RPE-Bruch's interface

In the healthy retina, RPE cells form a continuous layer of polarised cells, adherent through intervening tight junctions to create a physiological barrier between the choriocapillaris and outer retina. By controlling the passage of water, ions and selected molecules, the RPE cell layer actively maintains the chemical environment and homeostasis of the outer retina. Integral to this barrier function is the polarised arrangement of the cells, with different proteins expressed on the apical and basal surfaces and adjoining tight junctions.¹³ The apical microvilli interdigitate between photoreceptors within the extracellular matrix and phagocytose outer segments. RPE replacement should aim as closely as possible to restore this polarised arrangement, which is crucial for normal RPE function and maintenance of the outer blood-retina barrier. This can be assumed following full-thickness RPE-choroid grafts, because scanning electron microscopy has demonstrated the maintenance of RPE polarity on graft tissue removed from the eye during surgery.¹¹ In contrast, RPE cells differentiated from human embryonic stem (hES) cells need to be differentiated on a basal substrate, such as matrigel,¹⁴ or synthetic transwell membranes in order to re-establish polarity.¹⁵ Although a soluble substrate such as Matrigel might be more biocompatible, in the subretinal space any trophic properties of an RPE substrate may induce CNV formation,¹⁶ which would rapidly compromise any transplanted RPE cell sheet even if optimally polarised.

The search for a suitable RPE substrate is further complicated by the multiple roles that the normal Bruch's membrane has on RPE cell function beyond inducing polarity, which include powerful influences on cell survival and differentiation. In the absence of protein binding interactions with Bruch's membrane, transplanted RPE cells may de-differentiate to become fibroblast-like and undergo increased rates of apoptosis.¹⁷ It is doubtful whether the host Bruch's membrane could act as a substrate for transplanted cells, because in AMD it is likely that compromise of this layer is at least in part the reason for RPE cell death in the first place. Evidence for this comes from two key studies of human aged Bruch's membrane. First, when single dissociated RPE cells were harvested from the nasal retina in patients with macular degeneration and transplanted into the damaged foveal region, the cells were unable to attach to the damaged Bruch's membrane under the fovea.⁵ Significantly however the donor site in the nasal retina was able to restore RPE continuity, presumably because these RPE cells were able to divide and adhere to the undamaged Bruch's membrane.⁵ This clinical observation implies that it is not the age of the RPE donor cells but the damaged host Bruch's membrane which is the limiting factor for adherence of isolated cells. In support of this clinical observation, an in vitro study also showed that embryonic RPE cells could adhere onto normal but not aged Bruch's membrane obtained from post-mortem specimens.¹⁸ Similarly the concept of repairing a pigment epithelial detachment (PED) with RPE derived from stem cells is not a logical approach if the pathogenesis of the PED is due to changes in conductance of the underlying Bruch's membrane.¹⁹ Hence we might conclude from these longstanding observations that any successful RPE replacement therapy for AMD must also take into consideration the need to replace the underlying Bruch's membrane.

The pentilaminar structure of Bruch's membrane, with the layers being formed from distinct origins and at different stages of development,²⁰ is a relatively complex structure to repair or replace. Its composition varies considerably with geographic location in the eye, as the sub-macular membrane is significantly thinner than that found more peripherally.²¹ With age additional proteins and lipids are seen to be deposited within the membrane leading to increased thickness, and there are degenerative changes including fragmentation of the elastins and the development of abnormal cross-links within the

proteins.²²⁻²³ These aging changes are most pronounced in the macular region and have an adverse effect on RPE cell binding, function and survival and conductivity across the membrane.²⁴ Gullapalli and colleagues studied cultured foetal human RPE cells in combination with segments of Bruch's membrane extracted from 27 donor eyes (55 to 75 years of age), and found the RPE cell coverage and morphology to be poor on aged submacular Bruch's membrane, and to deteriorate further with time; the binding was worse still in the membrane of an eye with large soft drusen.¹⁸ The finding that aged Bruch's membrane does not support transplanted RPE survival and differentiation is of fundamental importance for RPE transplantation strategies as ultimately the fate of donor cells will depend on whether they can maintain or develop attachments to suitable substrates. These age-related changes in Bruch's membrane also represent one of the critical differences between potential patients and the laboratory animals in which many of the successes of RPE transplantation experiments have been reported, because the latter are generally young and have healthy Bruch's membrane.^{15, 25-26}

An advantage of the autologous RPE grafting technique in use clinically, is that the RPE is harvested from the equatorial region en bloc with attached Bruch's membrane and superficial choroid.^{7-11, 27} Age-related changes are less pronounced in peripheral Bruch's membrane which although different in structure,²³ can support continued RPE function when transplanted into the macular region as evidenced by pigmentation and continued autofluorescence.^{11, 27} A limitation of the procedure however is the surgical morbidity, with a high proportion of patients undergoing repeat surgery because of PVR, macular pucker, retinal detachment or vitreous haemorrhage.¹⁰⁻¹¹ In one series there was also evidence of further loss of function, possibly related to the initial trauma of surgery.²⁸ Unless modifications can be made to reduce the surgical complexity and risks involved of full thickness RPE-choroid grafts, the applicability of this procedure is likely to remain limited when compared to the new medical therapies currently being developed.

Other approaches have been to manipulate molecules such as the integrins to facilitate RPE cell integration on damaged Bruch's membrane,²⁹⁻³¹ or to develop biological or synthetic membranes that can be used in combination with replacement cells from a variety of sources.³²⁻³³ There are considerable

challenges for any artificial substrate on which RPE cells are transplanted; it must be well tolerated in the subretinal space, and either biodegrade with time, or integrate. There will also be discerning membrane transport and permeability requirements, and any artificial substrate must be both robust enough to withstand surgical manipulation and remain in place under the fovea, whilst also being flexible enough to allow delivery through a small retinotomy. These requirements may still present a limiting factor in the clinical application of stem cell derived or other sources of cultured RPE cell suspensions.

Immunological considerations for subretinal RPE transplantation

Although immune privilege have been demonstrated in the sub-retinal space of animal transplantation models, the features of local tolerance are best described as relative rather than absolute.³⁴ The RPE cells are central to maintaining the features of immune privilege seen in healthy eyes, by making up the outer blood-retinal barrier and expressing and secreting a variety of immuno-modulatory molecules; these include surface bound molecules such as FasL (which induces apoptosis of CD94+ inflammatory cells) and immuno-modulatory factors such as transforming growth factor- β , thrombospondin-1, somatostatin and pigment epithelial derived factor.³⁵⁻³⁷ Loss of RPE cells, aging, and disease within the retina have all been demonstrated in animal models to increase the risk of immunogenetically mismatched grafts in the subretinal space undergoing rejection.³⁸⁻³⁹ The resultant inflammation may lead to further damage to the host tissues.⁴⁰ Studies in animals have demonstrated that aggressive immunosuppressant dosing may be required. In a xenotransplantation study (donor and recipient of different species) Del Priore and colleagues transplanted porcine foetal RPE cells into the subretinal space of albino rabbits and found that even with the combination of prednisolone, cyclosporine and azathioprine from the day of surgery, the transplanted cell survival remained poor.⁴¹ Similarly Crafoord and co-workers studied RPE allografts (donor and recipient of same species) in rabbits, and found that immunosuppression with cyclosporin was inadequate to prevent rejection.⁴² It therefore seems highly likely that immune tolerance cannot be assumed for embryonic stem cell treatments that use cells

from an allograft donor, particularly when the subretinal environment is already in a heightened inflammatory state due to the active AMD process.

In keeping with these animal studies a trial some years ago of non-HLA matched fetal RPE cells in humans without immunosuppression, demonstrated post-operative macula oedema (likely to be a manifestation of host-graft rejection) in those who had undergone prior removal of fibrovascular membranes.⁴³ However, the corresponding clinical changes of leak were not seen in patients with dry AMD undergoing the same procedure, suggesting the threshold for rejection may be higher in non-exudative AMD because there was less pathogenic disruption to the RPE layer and Bruch's membrane prior to transplantation.⁴³ Similarly transplantation of intact sheets of foetal retina appear to survive in the subretinal space of patients with retinitis pigmentosa, possibly because the RPE-Bruch interface is relatively intact in these patients.⁴⁴ Conversely a recent study suggested that non-HLA matched foetal grafts did not undergo immunological rejection when placed in the subretinal space of four patients with AMD,⁴⁵ however, it was also suggested that rejection might still occur, but more slowly, as evidenced by progressive RPE atrophy rather than vasculitis in these patients.⁴⁶

The question of rejection is inherently difficult to determine in patients where no histology is available and against the background of a disease such as AMD, which is itself inflammatory. Nevertheless, there is convincing histological evidence from animal studies that non-matched cells are rejected from the sub-retinal space in time when not accompanied by systemic immune suppression,^{38, 40, 42, 47} albeit less floridly than may be expected in other parts of the body. The use of immune suppressants in clinical practice is possible, but brings risks of malignancy, susceptibility to infection and hepatic or renal toxicity, rendering a non-immunogenic source of replacement RPE cells far preferable. In this regard, using RPE obtained by therapeutic cloning or induction of pluripotent stem cells would be ideal, because donor cells could ultimately be derived from the patient and therefore provide a precise HLA match.

Sources of autologous RPE from stem cells

The cloning of Dolly the sheep by somatic cell nuclear transfer (SCNT) in 1997 was a landmark scientific discovery, because it showed for the first time that a

single cell taken from an adult mammal could be reprogrammed into an embryonic stem cell that could subsequently give rise to every cell type in the mammalian body.⁴⁸ In somatic cell nuclear transfer, DNA is taken from a donor cell (in the case of Dolly the sheep - a mammary gland cell from her genetic mother) and transferred into an oocyte which has had its original nucleus removed. This oocyte is then able to reprogram the DNA via undefined cytoplasmic factors and differentiate into an embryonic stem cell following the normal pattern of embryonic development. Although the mitochondrial DNA will be different as it is derived from the oocyte, all chromosomal DNA in the fully cloned animal should be identical to the DNA of the donor cell and could thus provide ideally matched tissue for transplantation. Dolly the sheep could see and had a normal functional retina which meant that the DNA from the mammary gland donor cell had been reprogrammed to express the correct genes required for normal RPE cell differentiation and function.⁴⁸ A decade later the first cloning of primate DNA was achieved using a similar method,⁴⁹ raising the possibility of cloning human cells.

One major drawback however with cloning through somatic cell nuclear transfer, is that the reprogramming goes back to the embryonic state and must begin with a donor oocyte.⁵⁰ This raises additional ethical questions because another life-form is involved during the cloning process. In 2006 however a major breakthrough occurred in deriving stem cells directly from adult differentiated cells without the need for an oocyte, by transducing cells directly with a series of four transcription factors (Oct4, Sox2, Klf4 and c-Myc) capable of reprogramming DNA without need for an oocyte.⁵¹ Essentially this process involves manipulation of the DNA binding proteins known as histones to inactivate the genes active in an adult skin cell and reactivating developmentally regulated genes – reprogramming the cell directly to become an embryonic ‘induced’ pluripotent stem (iPS) cell (see Figure 1).

As more is understood about the molecular mechanisms of differentiation, recent progress has shown how embryonic and pluripotent stem cells can be differentiated into adult cell types with a particular morphology and phenotype without needing to recreate the whole organism. Fortunately human embryonic stem cells can differentiate relatively easily into RPE cells, possibly because they arise early in during embryogenesis,⁵² and similar techniques can be applied to

iPS cells to regenerate RPE cells in vitro,⁵³ which show similar properties to ES-cell derived RPE in vivo.¹⁵ A drawback of using stem cells, however, is that these cells may de-differentiate within the subretinal space, potentially giving rise to teratoma formation. In one study of embryonic stem cell derived neural precursor cells injected into the mouse subretinal space, over 50% of eyes had developed teratomas within 8 weeks following injection.⁵⁴ Conversely, another study using 18 different human embryonic stem cell lines showed no evidence of tumour formation,⁵⁵ perhaps due to more stringent selection of appropriate cell lines. With induced pluripotent stem cells there are additional concerns about using the oncogenic c-Myc transcription factor, but recent developments have shown that iPS cells can be derived without c-Myc⁵⁶⁻⁵⁸, albeit with a reduced yield of cells. Furthermore, there is now evidence that human iPS cells may be derived using non-integrating episomal vectors,⁵⁹ which theoretically reduces the risk of tumour formation and host gene mutagenesis. Hence earlier safety concerns with iPS cells are now being addressed.

Conclusions

Stem cell technology is moving rapidly and it is highly likely that within the next decade we may have the ability to take a skin sample from an AMD patient and return it to them as a fully differentiated and healthy RPE monolayer suitable for autologous transplantation. The unknown question relates again to the scaffold on which these cells might be placed to restore the function of Bruch's membrane and the technical challenges of transplanting RPE cells into the subretinal space without causing damage to surrounding tissues. These particular problems and solutions are best addressed by ophthalmologists and it is therefore vital that we take time to follow developments in the stem cell field carefully and use our clinical expertise to guide progress towards future treatments. It is also possible that if we can solve the scaffold problem, we may be able to go back and reassess the option of using not stem cells, but suspensions of autologous RPE cells harvested from the retinal equator in patients with AMD. Hence we may be led into solving an old problem by developing a new surgical technique that may not actually require stem cells at all.

Acknowledgements

EL is a Wellcome Trust Clinical Training Fellow; REM is a Health Foundation Clinician Scientist Fellow. This research is also supported by the Medical Research Council, the Royal College of Surgeons of Edinburgh, Fight for Sight, the John Fell Fund of Oxford University Press, the Special Trustees of Moorfields Eye Hospital and the NIHR Ophthalmology Biomedical Research Centre. The authors declare no conflicts of interest with anything discussed in this article.

Licence for Publication

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in BJO editions and any other BMJPGJL products to exploit all subsidiary rights, as set out in the licence (<http://group.bmj.com/products/journals/instructions-for-authors/licence-forms/>).

References

1. Thomson H, Treherne A, Walker P, et al. Optimisation of polymer scaffolds for RPE cell transplantation. *Br J Ophthalmol* 2010 [in press]
2. Machemer R, Steinhorst UH. Retinal separation, retinotomy, and macular relocation: II. A surgical approach for age-related macular degeneration? *Graefes Arch Clin Exp Ophthalmol* 1993;231:635-41.
3. Wolf S, Lappas A, Weinberger AW et al. Macular translocation for surgical management of subfoveal choroidal neovascularizations in patients with AMD: first results. *Graefes Arch.Clin.Exp.Ophthalmol* 1999;237:51-7.
4. Eckardt C, Eckardt U Conrad HG. Macular rotation with and without counter-rotation of the globe in patients with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol.* 237, 313-325. 1999.
5. Binder S, Stolba U, Krebs I et al. Transplantation of autologous retinal pigment epithelium in eyes with foveal neovascularization resulting from age-related macular degeneration: a pilot study. *Am.J.Ophthalmol.* 2002;133:215-25.
6. van Meurs JC, ter AE, Hofland LJ et al. Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes. *Br.J Ophthalmol* 2004;88:110-3.

7. Peyman GA, Blinder KJ, Paris CL et al. A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. *Ophthalmic Surg.* 1991;22:102-8.
8. Stanga PE, Kychenthal A, Fitzke FW et al. Retinal pigment epithelium translocation after choroidal neovascular membrane removal in age-related macular degeneration. *Ophthalmology* 2002;109:1492-8.
9. van Meurs JC, Van Den Biesen PR. Autologous retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: short-term follow-up. *Am J Ophthalmol* 2003;136:688-95.
10. Jousseaume AM, Heussen FM, Joeres S et al. Autologous translocation of the choroid and retinal pigment epithelium in age-related macular degeneration. *Am J Ophthalmol* 2006;142:17-30.
11. MacLaren RE, Uppal GS, Balaggan KS, Tufail A, Munro PM, Milliken AB, Ali RR, Rubin GS, Aylward GW, da Cruz L. Autologous transplantation of the retinal pigment epithelium and choroid in the treatment of neovascular age-related macular degeneration. *Ophthalmology* 14(3), 561-570. 2007.
12. Maaijwee K, Jousseaume AM, Kirchhof B et al. Retinal pigment epithelium (RPE)-choroid graft translocation in the treatment of an RPE tear: preliminary results. *Br.J Ophthalmol* 2008;92:526-9.
13. Economopoulou M, Hammer J, Wang F et al. Expression, localization, and function of junctional adhesion molecule-C (JAM-C) in human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 2009;50:1454-63.

14. Vugler A, Carr AJ, Lawrence J et al. Elucidating the phenomenon of HESC-derived RPE: anatomy of cell genesis, expansion and retinal transplantation. *Exp.Neurol.* 2008;214:347-61.
15. Carr AJ, Vugler AA, Hikita ST et al. Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. *PLoS.ONE.* 2009;4:e8152.
16. Qiu G, Stewart JM, Sadda S et al. A new model of experimental subretinal neovascularization in the rabbit. *Exp.Eye Res.* 2006;83:141-52.
17. Tezel TH, Del Priore LV. Reattachment to a substrate prevents apoptosis of human retinal pigment epithelium. *Graefes Arch.Clin.Exp.Ophthalmol.* 1997;235:41-7.
18. Gullapalli VK, Sugino IK, Van PY et al. Impaired RPE survival on aged submacular human Bruch's membrane. *Exp.Eye Res.* 2005;80:235-48.
19. Bird AC, Marshall J. Retinal pigment epithelial detachments in the elderly. *Trans.Ophthalmol Soc.U.K.* 1986;105 (Pt 6):674-82.
20. Takei Y, Ozanics V. Origin and development of Bruch's membrane in monkey fetuses: an electron microscopic study. *Invest Ophthalmol.* 1975;14:903-16.
21. Nakaizumi Y. The ultrastructure of Bruch's Membrane. I. Human, Monkey, Guinea pig and rat eyes. *Arch.Ophthalmol.* 1964;72:380-7.
22. Spraul CW, Lang GE, Grossniklaus HE et al. Histologic and morphometric analysis of the choroid, Bruch's membrane, and retinal pigment epithelium

in postmortem eyes with age-related macular degeneration and histologic examination of surgically excised choroidal neovascular membranes.

Surv.Ophthalmol. 1999;44 Suppl 1:S10-S32.

23. Guymer R, Luthert P, Bird A. Changes in Bruch's membrane and related structures with age. Prog.Retin.Eye Res. 1999;18:59-90.
24. Zarbin MA. Current concepts in the pathogenesis of age-related macular degeneration. Arch.Ophthalmol. 2004;122:598-614.
25. Li LX, Turner JE. Inherited retinal dystrophy in the RCS rat: prevention of photoreceptor degeneration by pigment epithelial cell transplantation. Exp.Eye Res. 1988;47:911-7.
26. Girman SV, Wang S, Lund RD. Cortical visual functions can be preserved by subretinal RPE cell grafting in RCS rats. Vision Res. 2003;43:1817-27.
27. Jousseaume AM, Joeres S, Fawzy N et al. Autologous translocation of the choroid and retinal pigment epithelium in patients with geographic atrophy. Ophthalmology 2007;114:551-60.
28. Chen FK, Uppal GS, MacLaren RE et al. Long-term visual and microperimetry outcomes following autologous retinal pigment epithelium choroid graft for neovascular age-related macular degeneration. Clin.Experiment.Ophthalmol 2009;37:275-85.
29. Afshari FT, Fawcett JW. Improving RPE adhesion to Bruch's membrane. Eye (Lond) 2009;23:1890-3.

30. Fang IM, Yang CH, Yang CM et al. Overexpression of integrin alpha6 and beta4 enhances adhesion and proliferation of human retinal pigment epithelial cells on layers of porcine Bruch's membrane. *Exp.Eye Res.* 2009;88:12-21.
31. Gullapalli VK, Sugino IK, Zarbin MA. Culture-induced increase in alpha integrin subunit expression in retinal pigment epithelium is important for improved resurfacing of aged human Bruch's membrane. *Exp.Eye Res.* 2008;86:189-200.
32. Lu L, Garcia CA, Mikos AG. Retinal pigment epithelium cell culture on thin biodegradable poly(DL-lactic-co-glycolic acid) films. *J Biomater.Sci Polym.Ed* 1998;9:1187-205.
33. Tomita M, Lavik E, Klassen H et al. Biodegradable polymer composite grafts promote the survival and differentiation of retinal progenitor cells. *Stem Cells* 2005;23:1579-88.
34. Zhang X, Bok D. Transplantation of retinal pigment epithelial cells and immune response in the subretinal space. *Invest Ophthalmol.Vis.Sci.* 1998;39:1021-7.
35. Zamiri P, Sugita S, Streilein JW. Immunosuppressive properties of the pigmented epithelial cells and the subretinal space. *Chem.Immunol.Allergy* 2007;92:86-93.
36. Zamiri P, Masli S, Streilein JW et al. Pigment epithelial growth factor suppresses inflammation by modulating macrophage activation. *Invest Ophthalmol Vis Sci* 2006;47:3912-8.

37. Jorgensen A, Wiencke AK, la CM et al. Human retinal pigment epithelial cell-induced apoptosis in activated T cells. *Invest Ophthalmol.Vis.Sci.* 1998;39:1590-9.
38. Enzmann V, Faude F, Wiedemann P et al. Immunological problems of transplantation into the subretinal space. *Acta Anat.(Basel)* 1998;162:178-83.
39. Lund RD, Rao K, Kunz HW et al. Immunological considerations in neural transplantation. *Transplant.Proc.* 1989;21:3159-62.
40. Jiang LQ, Jorquera M, Streilein JW. Immunologic consequences of intraocular implantation of retinal pigment epithelial allografts. *Exp Eye Res.* 1994;58:719-28.
41. Del Priore LV, Ishida O, Johnson EW et al. Triple immune suppression increases short-term survival of porcine fetal retinal pigment epithelium xenografts. *Invest Ophthalmol.Vis.Sci.* 2003;44:4044-53.
42. Crafoord S, Algvere PV, Seregard S et al. Long-term outcome of RPE allografts to the subretinal space of rabbits. *Acta Ophthalmol.Scand.* 1999;77:247-54.
43. Algvere PV, Berglin L, Gouras P et al. Transplantation of RPE in age-related macular degeneration: observations in disciform lesions and dry RPE atrophy. *Graefes Arch.Clin.Exp.Ophthalmol.* 1997;235:149-58.

44. Radtke ND, Seiler MJ, Aramant RB et al. Transplantation of intact sheets of fetal neural retina with its retinal pigment epithelium in retinitis pigmentosa patients. *Am J Ophthalmol* 2002;133:544-50.
45. Radtke ND, Aramant RB, Petry HM et al. Vision improvement in retinal degeneration patients by implantation of retina together with retinal pigment epithelium. *Am J Ophthalmol* 2008;146:172-82.
46. Zarbin MA. Retinal pigment epithelium-retina transplantation for retinal degenerative disease. *Am J Ophthalmol* 2008;146:151-3.
47. Grisanti S, Szurman P, Jordan J et al. Xenotransplantation of retinal pigment epithelial cells into RCS rats. *Jpn.J.Ophthalmol.* 2002;46:36-44.
48. Wilmut I, Schnieke AE, McWhir J et al. Viable offspring derived from fetal and adult mammalian cells. *Nature* 1997;385:810-3.
49. Byrne JA, Pedersen DA, Clepper LL et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature* 2007;450:497-502.
50. MacLaren RE, Pearson RA. Stem cell therapy and the retina. *Eye* 2007;21:1352-9.
51. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-76.
52. Haruta M, Sasai Y, Kawasaki H et al. In vitro and in vivo characterization of pigment epithelial cells differentiated from primate embryonic stem cells. *Invest Ophthalmol Vis Sci* 2004;45:1020-5.

53. Buchholz DE, Hikita ST, Rowland TJ et al. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. *Stem Cells* 2009;27:2427-34.
54. Arnhold S, Klein H, Semkova I et al. Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. *Invest Ophthalmol Vis Sci* 2004;45:4251-5.
55. Lund RD, Wang S, Klimanskaya I et al. Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats. *Cloning Stem Cells* 2006;8:189-99.
56. Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;318:1917-20.
57. Nakagawa M, Koyanagi M, Tanabe K et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat.Biotechnol.* 2008;26:101-6.
58. Ebert AD, Yu J, Rose FF, Jr. et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 2009;457:277-80.
59. Yu J, Hu K, Smuga-Otto K et al. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 2009;324:797-801.

Figure 1 – DNA reprogramming

Diagrammatic representation of DNA reprogramming by four transcription factors able to de-differentiate fibroblast cells into induced pluripotent stem (iPS)

cells by reactivating embryonic genes: Key: Klf4, Kruppel-like factor 4; Oct 4, Octamer 4; RA, SOX2, sex determining region Y-box 2; c-Myc, chromosomal-Myelocytomatosis gene. These transcription factors are believed to act through methylation mechanisms on the histone proteins that bind DNA into secondary coils. Collectively they provide a molecular switch to reactivate embryonic genes that are otherwise dormant in adult differentiated cells. In somatic cell nuclear transfer (the method used to clone Dolly the sheep) undefined factors in oocyte cytoplasm reprogram DNA extracted from the adult cell and injected into the oocyte through a similar process. The advantage of applying these transcription factors directly to the adult cell is that no oocyte is required to make iPS cells. These iPS cells could subsequently be differentiated into retinal pigment epithelial cells for autologous transplantation back into a patient with age-related macular degeneration.

