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ScienceDirect
Experimental Eye Research 86 (2008) 661–668
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Maintenance of adult porcine retina and retinal pigment epithelium in perfusion culture: Characterisation of an organotypic in vitro model☆

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Received 26 April 2007; accepted in revised form 10 January 2008
Available online 18 January 2008

Abstract

The purpose of this study was to characterise an ex-vivo adult porcine retina–retinal pigment epithelium (RPE) perfusion organ culture model. Fresh porcine full-thickness retina–RPE–choroid tissue samples were clamped into tissue carriers and mounted in two-compartment containers. The retinal and choroidal sides were continuously perfused with culture medium. pO_2, $[Na^+]$, $[K^+]$, $[Cl^-]$, [glucose], [lactate], and pH were measured in the medium. Tissue samples were examined after 24 h, 4, 7, and 10 days in culture. The morphology of the retina and the RPE was examined by light and electron microscopy (LM, EM). The retinal cellular integrity was further examined by immunohistochemistry (Ki 67, GFAP, rhodopsin, synaptophysin, syntaxin, NF 200, TUNEL-test). Fresh porcine full-thickness retina–RPE–choroid tissue samples and tissue samples in static organ culture served as controls. LM, EM, and immunohistochemistry showed intact retinal and RPE cytoarchitecture kept in perfusion culture. Photoreceptor outer segments showed first signs of degeneration after 24 h, significant signs of apoptosis and necrosis appeared in the retina after 4 days in perfusion culture. Control tissue samples kept in static culture showed disintegration of the retinal cytoarchitecture after 4 days in culture. The data show that adult porcine retina–RPE tissue can be maintained morphologically intact in perfusion organ culture for at least 10 days. Although first signs of degeneration set in after 24 h the structural preservation of the tissue in perfusion organ culture is superior to that in static culture. The perfusion culture model of the retina refines organotypic in vitro test systems and may help to reduce the number of necessary animal experiments in retina and RPE research. It offers new perspectives for the safety testing of substances designed for intraocular application.

Keywords: retina; retinal pigment epithelium; perfusion organ culture; model; porcine

1. Introduction

There is a continuous demand in ophthalmic research for the investigation of new treatment modalities of retinal disease such as pharmaceutical agents or vitreous substitutes used in vitreo-retinal surgery. The mainstays of such investigations are cell culture studies and animal experiments.

Cell culture models are often used as the first step in the investigation of new treatment modalities but there are some principal limitations to this method. Most retinal cell or retinal pigment epithelium (RPE) cell culture models are based on proliferating cell types cultivated under conventional static culture conditions (Kim and Takahashi, 1988; Martini et al., 1992; Gaudin et al., 1996; Engelmann and Valtink, 2004; Jackson et al, 2004; Levin, 2005). The degree of cellular differentiation differs considerably from the in vivo situation because cells are removed from their natural organ-specific environment; hence the effect of intercellular interactions is reduced or lost. Furthermore, cell culture

☆ Presented in part at the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, Florida, USA, May 9–14, 1999 and at the annual meeting of the German Ophthalmologic Society (DOG), Berlin, Germany, September 23–26, 1999.

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doi:10.1016/j.exer.2008.01.011

models allow only the investigation of single cell types. Therefore, the functional unit of a retina-RPE model with intact cell-cell associations could be superior to cell culture models in the testing of the effect of a treatment which may affect the entire retina and the RPE. However, in contrast to cell culture it is difficult to maintain the highly differentiated, multilayered tissue of adult retina and RPE in conventional static organ culture because of insufficient nutrient and oxygen supply.

Animal models are often used as a second step in the investigation of new treatment modalities following cell culture studies. However, animal experiments have been widely criticised for ethical and economical reasons. For example, in order to compare the pharmacokinetic profiles of a drug in two different delivery systems using six animals for each delivery system and measuring 8 time points, either 48 or 96 animals have to be sacrificed, depending on whether one or both eyes are used for the study. In retina and RPE research, a reliable organotypic retina–RPE–choroid organ culture model could possibly close the gap between first investigations in cell culture and secondary tests in animal models, thereby helping to avoid animal experiments or to reduce the number of necessary animal experiments.

The aim of the present study was to characterise an ex-vivo adult porcine retina–RPE perfusion organ culture model. The perfusion organ culture system used has been originally developed and established by Minuth et al. for long-term culture of embryonic renal epithelia (Minuth et al., 1992). In our laboratory we adapted this system for perfusion organ culture of adult porcine retina–RPE–choroid tissue specimens. The purpose of the model is to preserve the unit of the retina and RPE cytoarchitecture for morphological studies and for safety testing of substances designed for intraocular application.

2. Materials and methods

2.1. Tissue preparation

Eyes of pigs aged 3–6 months were obtained from a local abattoir, transported on ice in 1% penicillin/streptomycin-supplemented balanced salt solution (NaCl 0.9%, Braun-Melsungen, Melsungen, Germany) in darkness to the laboratory within 1 h of death. Whole globes were dissected under an operating microscope (Zeiss Universal S3, Jena, Germany) under sterile conditions. The anterior portion of the eye was carefully removed by a circumferential incision at the pars plana and the cornea, together with the lens, iris, and vitreous, discarded.

The posterior segment was dissected into a temporal and a nasal half perpendicular through the optic disc. The sclera was fixed with pins to an underlying section support. Tissue preparation was performed in the same area of the retina for all experiments. The choroid together with the RPE and the retina were gently separated from the sclera with fine forceps and a microsurgical knife (Fig. 1A). Next, the retina–RPE–choroid tissue complex was clamped in a double ring tissue carrier with an outer diameter of 13 mm and inner diameter of 9 mm (Minucells and Minutissue, Bad Abbach, Germany) without additional support. First, the smaller white ring was placed between sclera and choroid. Then the bigger black ring was pressed onto the white ring from the inner side of the retina, so that the tissue was fixed between the carrier rings, the retina well attached to the RPE (Fig. 1B,C).

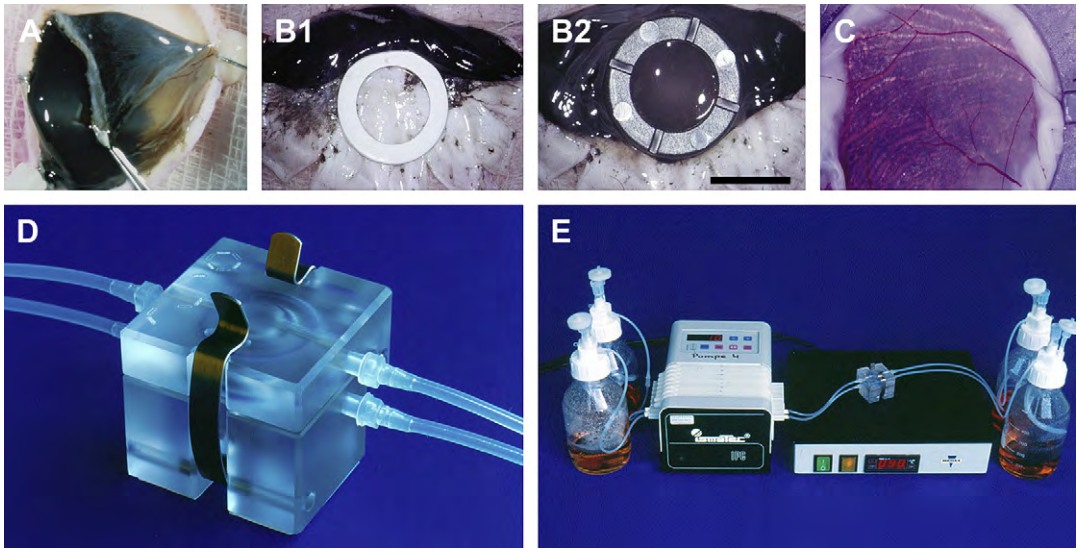

Fig. 1. (A) Microsurgical preparation of a retina–RPE–choroid sheet from a porcine eye. (B) Mounting the retina–RPE–choroid sheet into a double ring tissue carrier (outer diameter 13 mm, inner diameter 9 mm). B_1: The white ring of the tissue carrier is placed on the sclera, the retina is pulled over the white ring. B_2: The outer black ring is pressed gently over the retina and the white ring. The retina is fixed in the tissue carrier without additional support (scale bar: 8 mm). (C) The retina is well attached to the underlying RPE and choroid. (D) The tissue carrier is mounted in the two-compartment perfusion container. The tissue separates the upper and lower compartment of the perfusion container. (E) The perfusion line consists of the sterile medium-supply (bottles on the left), the peristaltic pump, the warming plate with the perfusion container covered by a Perspex lid for constant temperature. Medium drains on the opposite side of the chamber (bottles on the right).

2.2. Perfusion culture

Tissue carriers were transferred into a two-compartment perfusion container holding a single tissue carrier (Minucells and Minutissue, Bad Abbach, Germany) (Fig. 1D). The upper (retinal) and lower (choroidal) compartment of the container was thus separated by the tissue. Tissue culture was initiated within 2 h of death. Both compartments were separately and continuously superfused with medium from a sterile supply of Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich, Seelze, Germany) supplemented with 15% porcine serum, 2.5% HEPES-buffer solution, and 1% penicillin/streptomycin (Gibco Life Technologies, Eggenstein, Germany). The medium was pumped through 1 mm inner diameter thin, gas-permeable silicone tubes, which allowed the continuous exchange of atmospheric gases. The medium was transported at a rate of 1 ml/h driven by a peristaltic pump (IPC N8, Ismatec, Wertheim, Germany). The culture container was placed on a warming plate (Medax, Kiel, Germany) set to a constant temperature of 37 °C under atmospheric conditions (Fig. 1E). The sealed perfusion container was perfused for 24 h, 4, 7 and 10 days. The culture system was kept under room light with a physiological light/dark rhythm.

pO_2 was measured with a fibre-optic microsensor (Presens, Regensburg, Germany) at the inlet and at the outlet of the upper and the lower compartment of the perfusion container after 4 days in culture. [Na^+], [K^+], [glucose], [lactate], and pH were measured in medium samples obtained from the inlet, the outlet, and from the upper compartment of the perfusion container with a chemistry analyser (ADVIA 1650, Bayer, Leverkusen, Germany) after 4 days, 7 days and 10 days in culture. Representative data are reported after 4 days.

2.3. Static culture

For static culture, retina–RPE–choroid sheets were prepared as described above. Specimens were mounted in the same tissue carriers as for perfusion and maintained in Petri dishes (Greiner, Frickenhausen, Germany) in a CO_2-incubator (37 °C, 95% air, 5% CO_2, 100% humidity) (WTB Binder 3615, Binder, Tuttlingen, Germany). Medium (DMEM, Sigma–Aldrich) supplemented with 15% porcine serum and 1% penicillin/streptomycin (Gibco Life Technologies) was changed daily. Cultures were kept for 4, 7, and 10 days.

2.4. Histology

Upon completion of the perfusion period, tissue specimens were removed from the tissue carrier by opening the double ring. The tissue was fixed in paraformaldehyde 4% in phosphate buffered saline (PBS, Sigma–Aldrich) overnight, washed in PBS 0.1 M, dehydrated in ascending alcohol and embedded in paraffin. After deparaffinisation in xylol and rehydration, 2–5-μm tissue sections were cut and mounted on microscope slides (SuperFrost Plus, Menzel, Braunschweig, Germany). Samples of each specimen were stained with haematoxylin–eosin and viewed with a light microscope (Axiotech, Zeiss) (LM). The preservation of the tissue was evaluated using a grading score (Table 1).

Following perfusion culture for 10 days, three specimens were fixed in Karnovsky (glutaraldehyde 2,5%, paraformaldehyde 2% in cacodylate buffer) and embedded in Epon (EMBED 812 kit, Electron Microscopy Science, Science Services, Munich, Germany). Ultrathin sections were processed for electron microscopy.

2.5. Immunohistochemistry

In order to evaluate the preservation of different retinal cell types and neuronal structures, specimens were evaluated by immunohistochemistry after 24 h (TUNEL-test only), 4 and 10 days in perfusion culture and after 4 days in static culture.

After fixation, paraffin embedding and sectioning as described above, 2–5-μm sections were processed with the specific antibodies in a disposable immunostaining chamber-system (Shandon Coverplate®, Thermo Electron, Pittsburgh, PA, USA).

A horseradish peroxidase-based kit with AEC (3-amino-9-ethylcarbazol) as chromogen was used according to the manufacturer's protocol for qualitative identification of the antigens by LM, resulting in a red-coloured precipitate at the antigen site (LSAB2 System-HRP®, DakoCytomation, Glostrup, Denmark). The following antibodies were applied: Ki 67 (Clone MIB 1) (Code No. M 7240, DakoCytomation) for the identification of proliferating cells; glial fibrillary acidic protein (GFAP, clone GA-5; G 3893, Sigma–Aldrich) for the identification of Müller cells; rhodopsin (mouse monoclonal antibody Rho 4D2 against the N-terminal region of rhodopsin) for the identification of rod photoreceptor cells (kind

Table 1
Grading score for the evaluation of tissue preservation of the retina and the RPE by light microscopy

Score	Retinal pigment epithelium	Photoreceptor outer segments	Photoreceptor inner segments	Outer nuclear layer	Inner nuclear layer	Inner and outer limiting membrane
0	Polar pigment distribution, microvilli intact	Outer segments intact	Inner segments intact	<5% pycnotic cell nuclei	<5% pycnotic cell nuclei	Fully intact
1	Polar pigment distribution, microvilli reduced	Outer segments swollen	Inner segments swollen	<10% pycnotic cell nuclei	<10% pycnotic cell nuclei	Segmental defects <10%
2	Polarity of pigment distribution reduced, mildly vacuolised	Outer segments vacuolised	Inner segments vacuolised	<30% pycnotic cell nuclei	<30% pycnotic cell nuclei	Segmental defects >10%
3	Polarity of pigment distribution lost, vacuolised	Outer segments disintegrated	Inner segments disintegrated	> 30% pycnotic cell nuclei	> 30% pycnotic cell nuclei	Not detectable

donation by R.S. Molday, Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada); synaptophysin (Ab-1, Cat. # NB06; Oncogene Calbiochem, Merck Biosciences, Boston, MA, USA) for the identification of synaptic vesicle proteins in the process of neurotransmitter release in the inner and outer plexiform layer of the retina; syntaxin (clone HPC-1 S 0664, Sigma–Aldrich) for the identification of the membrane of amacrine cell bodies and the inner plexiform layer in the retina; NF 200 (clone NE14, N 5389, Sigma–Aldrich) for the identification of neurofilaments of 200 kDa molecular weight which are synthesised in the neuronal perikarya, assembled to filaments and then slowly transported within the axons towards the synaptic terminals. This antibody was used either on flat specimens of the retina to demonstrate the longitudinal axonal condition or on paraffin sections.

Detection of apoptotic cell death was performed using the TUNEL-test according to the manufacturer's protocol (*in situ* Cell Death Detection Kit, Roche, Penzberg, Germany). Sections were permeabilised with freshly prepared 0.1% Triton X-100 solution containing 0.1% sodium citrate and treated with 3% hydrogen peroxide in ethanol for 20 min to block endogenous peroxidase. Sections incubated with 3, 300, and 3000 units of DNAse served as positive controls. Sections were counterstained with haematoxylin–eosin. The presence of apoptosis was defined as shrunken, highly condensed and sometimes fragmented cell nuclei.

All sections were viewed using a light microscope (Axiotech, Zeiss). A total number of 176 retinae was used for the experiments. Twenty retinae were examined in each group (perfusion culture 24 h, 4 days, 7 days, 10 days; static culture 24 h, 4 days, 7 days; fresh tissue as control group). In addition, a number of 4 retinae for each perfusion interval was prepared for electron microscopy.

3. Results

A set of preliminary experiments showed that preservation results of quality could only be achieved with a post-mortem time of less than 2 h. Furthermore, a comparison of the preservation of three specimens connected in series in one perfusion container (Minucells and Minutissue, Bad Abbach, Germany) by LM showed that the preservation of the first specimen was usually superior to that of the following ones. In the present study we therefore used perfusion containers holding a single tissue carrier (Fig. 1D,E).

After 24 h in perfusion culture no light-microscopic signs or mild signs of cell necrosis and no labelling to mild degree labelling of nuclei by the TUNEL method were found (Fig. 3A). A significant number of apoptotic nuclei was first seen after 4 days in perfusion culture predominantly in the outer nuclear layer (Fig. 3B). After 10 days in perfusion organ culture a significant number of apoptotic nuclei was found in all nuclear retinal layers (Fig. 3C).

The tissue preservation as evaluated by LM showed that the retinal cytoarchitecture and the RPE remained well preserved for the entire culture period of 10 days (Fig. 2). After 10 days in perfusion culture, the RPE remained a fully differentiated monolayer adherent to Bruch's membrane with preserved polarity and apical position of the pigment granules. Electron microscopy showed RPE cells with intact intercellular junctions, apical microvilli and phagocytosis of photoreceptor outer segments (Fig. 4).

The photoreceptor outer and inner segments proved to be the most vulnerable retinal structure as demonstrated by first signs of degeneration such as swelling and vacuolisation after 24 h and more pronounced morphological alterations after 4 days with vacuolisation of inner segments and disintegration of most outer segments (Table 2). The retina remained attached to the RPE in most specimens which seemed to be a precondition for preservation of the photoreceptors for up to 4 days in perfusion culture. Despite careful handling of the tissue the retina detached in some specimens (compare Fig. 2A, column 3: photoreceptors in *attached* retina after 4 days in perfusion culture and Fig. 3A: photoreceptors in *attached* retina after 24 h in perfusion culture, with Fig. 3B: photoreceptors in *detached* retina after 4 days in perfusion culture).

After 7–10 days in perfusion culture photoreceptor inner and outer segments had fully disintegrated in most specimens (Table 2) although an excellent preservation of the outer segments and intact mitochondria could be found in some areas (Fig. 4B).

LM showed an increasing number of pycnotic cell nuclei in the inner and outer nuclear layer during the culture period with time (Table 2) whereas in the ganglion cell layer only single pycnotic cell nuclei were observed after 10 days in perfusion culture.

Tissue preservation as evaluated by LM and immunohistochemistry was clearly superior in perfusion culture compared to static culture (Tables 2 and 3, Fig. 2). Labelling with GFAP, rhodopsin, synaptophysin, syntaxin and NF 200 showed intact retinal cytoarchitecture (Fig. 2). Labelling with Ki 67 showed no signs of cell proliferation after 4 and 10 days in perfusion culture (not shown).

Following static culture, signs of pronounced cell necrosis and cytoarchitecture disintegration were observed after 4 days (Fig. 2). After 10 days the retinal cytoarchitecture had fully disintegrated. RPE cells were atypically rounded, had lost contact to Bruch's membrane and showed lack of polarity, microvilli and intercellular junctions. Photoreceptor cells were completely degenerated. Outer and inner limiting membranes could not be detected (Table 3).

In perfusion culture analysis of the medium showed that pO_2 equilibrated with the oxygen pressure in room air ranging from 157 mmHg to 165 mmHg (mean 161 ± 3.0 mmHg, $n = 10$) in the silicon tube leading to the perfusion container. In the perfusion container a decline of pO_2 as a sign of oxygen uptake by the cultured tissue was observed from 160 mmHg to 76.6 ± 40 mmHg in the upper compartment and to 61.4 ± 34 in the lower compartment. The electrolyte concentrations and pH remained stable during perfusion culture. As a sign of active cell metabolism the concentration of lactate increased following tissue perfusion (Table 4).

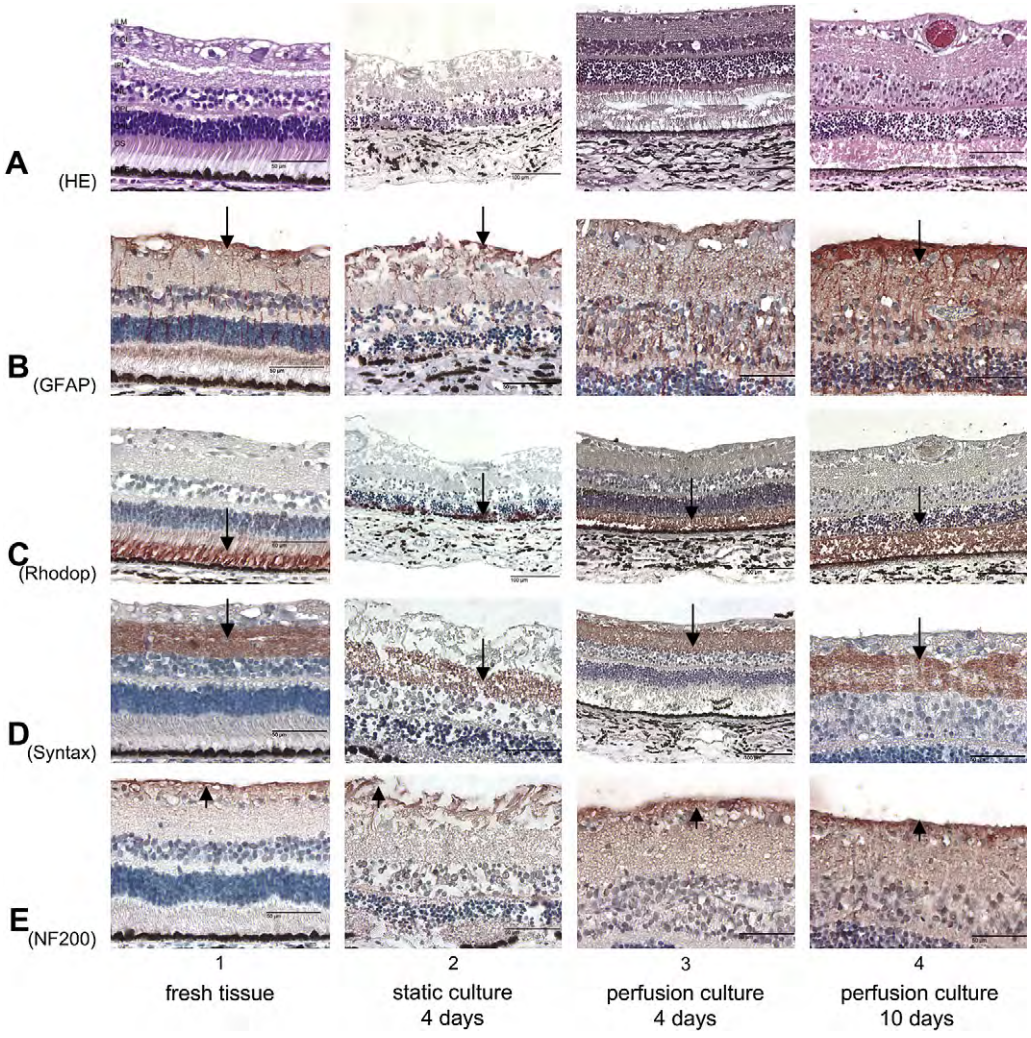

Fig. 2. Light photomicrographs of porcine retina stained with haematoxylin–eosin (HE) and processed with immunohistochemistry markers. Column 1: fresh porcine retina; column 2: porcine retina after 4 days in static culture; column 3: porcine retina after 4 days in perfusion culture; column 4: porcine retina after 10 days in perfusion culture. ILM, internal limiting membrane; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segments; RPE, retinal pigment epithelium.

4. Discussion

The findings of the present study demonstrate that adult porcine retina–RPE tissue can be kept morphologically intact in perfusion organ culture for at least 10 days. The structural preservation of retinal tissue in perfusion organ culture was clearly superior to that in stationary culture. However, a limitation of the present system is that a possible effect on photoreceptor inner and outer segments can only be tested short-term since this most vulnerable retinal layer cannot be preserved over a longer period of time. Since we observed significant signs of apoptosis and cell necrosis in the retina after 4 days in perfusion culture, the safety testing of substances which potentially damage the retina by induction of apoptosis and necrosis may be limited to a testing period of less than 4 days. We used an established test system (Minucells and Minutissue, Bad Abbach, Germany) (Minuth et al., 1992) which has among other appliances so far been applied in organ culture

Table 2
Grading score of tissue preservation in perfusion culture after 24 h and after 4, 7, and 10 days ($n = 7$ each)

Score	Retinal pigment epithelium	Photoreceptor outer segments	Photoreceptor inner segments	Outer nuclear layer	Inner nuclear layer	Inner and outer limiting membrane
24 h	0.14 ± 0.38	2.29 ± 0.49	1.57 ± 0.79	0.57 ± 0.79	0.29 ± 0.49	0 ± 0
4 days	0.57 ± 0.53	2.86 ± 0.38	2.14 ± 0.69	2.00 ± 0.82	1.57 ± 0.53	0 ± 0
7 days	0.71 ± 0.76	3.0 ± 0	2.43 ± 0.53	2.43 ± 0.79	1.86 ± 0.69	0.43 ± 0.79
10 days	0.86 ± 0.69	3.0 ± 0	2.43 ± 0.53	2.71 ± 0.76	1.86 ± 0.69	0.14 ± 0.38

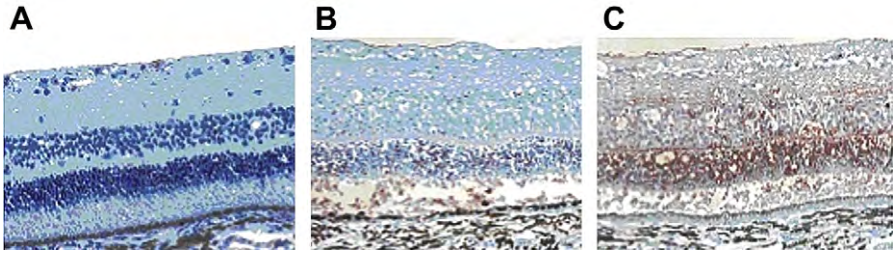

Fig. 3. Light photomicrographs of porcine retina processed with the TUNEL-technique and counterstained with haematoxylin–eosin show an increasing number of apoptotic cells predominantly in the outer nuclear layer after (A) 24 h, (B) 4 days and (C) 10 days in perfusion culture (magnification 20×, $n = 5$ each).

of embryonic renal epithelia (Minuth et al., 1992; Schumacher et al., 2002), the RPE (Framme et al., 2002), and in tissue engineering (Minuth et al., 2000). The perfusion culture system used in the present study proved easy to use. Contamination, a common problem in tissue culture, was not observed, perhaps because the perfusion container remains closed for the entire time of culture. The comparatively short post-mortem time of two hours, keeping the enucleated eyes on ice during transport to the laboratory and careful handling of the tissue were crucial to achieving reproducible tissue preservation results.

In this study, we used morphological parameters for the evaluation of the retinal tissue in the perfusion system. Light microscopy, immunohistochemistry and electron microscopy revealed a superior preservation of the complex structure of retina, RPE and choroid as compared to other retina explant models. Intact functional properties of the RPE in this culture system are demonstrated by the preserved polarity of the apical and basal side of the RPE, apical position of the pigment, presence of microvilli, basal infoldings, junctional complexes as well as intact mitochondria and rod outer segments (Fig. 4A,C). However, as electron microscopy and immunohistochemistry do not allow a judgement on the function of the RPE-tight junctions, further functional assessment of the outer blood–retinal barrier is limited in the present system for the following reasons. The two-compartment culture container which we used for our experiments is separated by the tissue and the carrier ring in an upper and lower compartment and perfused with medium separately from the upper and lower side. Minimal leakage between the two compartments cannot be excluded because of the design of the carrier ring and because the retinal tissue may be squeezed and damaged at the edges by the ring to an unpredictable degree. So further evaluation of the preservation of retinal barrier functions, diffusion processes or of electrophysiological parameters is limited under the present conditions. Watertight sealing of the upper and the lower compartment would require major alterations within the system and would require a new systemic evaluation.

In vivo the retina and the RPE are inherently dependent on a continuous dual supply of nutrients from the retinal and choroidal blood circulations. This dual supply system is not sufficiently mimicked by conventional static organ culture methods. An effective oxygen and nutrient supply together with an effective removal of carbon dioxide and other waste products is advantageous in the organ culture of a multilayered tissue such as the retina. In static organ culture unilateral medium supply may not provide sufficient nutritional and oxygen supply for deeper tissue layers and may lead to an accumulation of metabolites within the tissue. Therefore, the continuous supply of fresh medium to both the retinal and the choroidal sides of retina–RPE–choroid specimens together with constant removal of metabolites is a strength of the present perfusion organ culture system. Ahmed at al. have shown that 90% of the oxygen requirement of the photoreceptors in the macaque retina is supplied by the choroidal circulation and 10% by the retinal circulation. Moving from the photoreceptor layer towards the inner retina, oxygen tension decreased reaching a minimum value at about 70% retinal depth, and then gradually increased more proximally (Ahmed et al., 1993). The present model resembles the in vivo supply system; however, the path length of oxygen and nutrient supply from

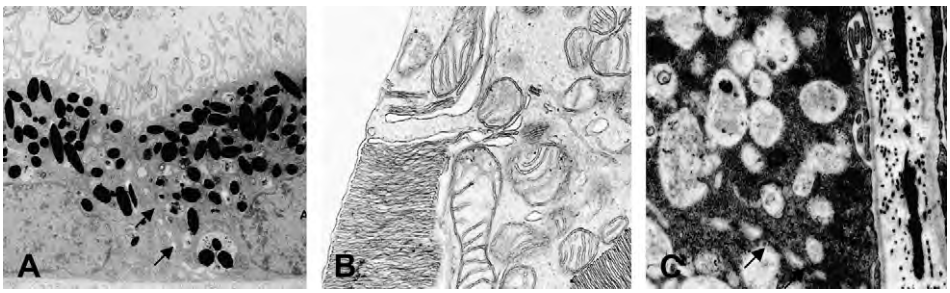

Fig. 4. Electronmicrographs of porcine retina after 10 days in perfusion culture show well preserved RPE with apical pigment and microvilli (A) and intact intercellular tight junctions (A, C). Phagocytotic activity of the RPE is evidenced by intracellular vacuoles and basal infoldings (A). In some areas, the photoreceptor outer segments and mitochondria are well preserved even after 10 days in culture (B) ($n = 3$).

Table 3
Grading score of tissue preservation in static culture after 4, 7, and 10 days ($n = 7$ each)

Score	Retinal pigment epithelium	Photoreceptor outer segments	Photoreceptor inner segments	Outer nuclear layer	Inner nuclear layer	Inner and outer limiting membrane
4 days	2.43 ± 0.79	3.00 ± 0.0	2.71 ± 0.49	3.00 ± 0.0	2.71 ± 0.49	2.14 ± 1.07
7 days	2.86 ± 0.38	3.00 ± 0.0	2.86 ± 0.38	3.00 ± 0.0	3.00 ± 0.0	2.71 ± 0.49
10 days	3.00 ± 0.0	3.00 ± 0.0	3.00 ± 0.0	3.00 ± 0.0	3.00 ± 0.0	3.00 ± 0.0

the choroidal side is increased by the bulk of choroidal connective tissue. The increased path length of oxygen and nutrient supply may partially explain why we observed first degenerative signs in the retina after 4 days in culture and why the photoreceptors proved to degenerate even earlier. It has been shown that a complete removal of the choroid without causing damage to Bruch's membrane and the overlying RPE is technically difficult if not impossible. Pilot studies undertaken to strip the choroidal vessels and fibres by microdissection invariably led to holes, tearing and permanent deformation of the specimens (Moore et al., 1995). Schütt et al. described the ablation of choroidal tissue from Bruch's membrane using an Excimer laser. Completely denuding Bruch's membrane without damaging its structure could not be achieved but it was possible to substantially debulk choroidal tissue (Schütt and Holz, 2002). Partially removing choroidal tissue from full-thickness retina–RPE–choroid specimens could shorten the oxygen and nutrient supply path length on the choroidal side of the present model and thereby possibly increase the longevity of the cultured retinal tissue.

Static organ culture of embryonic (Sparrow et al., 1990) and neonatal rat (Feigenspan et al., 1993; Johansson and Ehinger, 2005) or neonatal mouse (Mosinger Ogilvie et al., 1999) and rabbit retina (Rowe-Rendleman et al., 1996; Germer et al., 1997) has been described. Embryonic and neonatal retinal tissue instead of adult retinal tissue has been used, in part, due to the perceived difficulty in setting up and maintaining a retina preparation. The retinal explants with or without RPE were usually placed on filter paper or porous membranes immersed in medium in an incubator. While in culture, the developing retina undergoes a process of structural remodelling which is different in culture and in vivo (Johansson and Ehinger, 2005). Minuth et al. described that the differentiation of embryonic epithelia in perfusion culture principally depends on specific culture conditions (Minuth et al., 1999). Although cultured embryonic and neonatal tissue exhibited an excellent vitality, the developing embryonic or neonatal retina cannot be directly compared to the fully differentiated adult retina. Therefore, cultured embryonic or neonatal retinas are of limited relevance as models of the adult retina.

In the 1970s Remé and Niemeyer presented the isolated perfused mammalian eye model. The authors perfused the choroidal, retinal, and ciliary vascular network of enucleated mammalian eyes via the ophthalmociliary artery with culture medium. Light- and electronmicroscopy studies showed a well-preserved retinal ultrastructure after 2 h of perfusion (Remé and Niemeyer, 1975; Niemeyer, 1977,2001). We cannot conclude from the published data whether the preservation of the retinal structure could potentially last longer than 2 h, the model having been designed for short-term studies (Niemeyer, 2001).

Compared to blood where oxygen is transported in combination with haemoglobin, the oxygen transport capacity of medium is reduced by a factor of 30 to 100 and the transport capacity of CO_2 is reduced by a factor of 15 to 20 (Guyton and Hall, 1996). Therefore, the present model where the entire choroidal and retinal surfaces of the retina are continuously exposed to medium may be superior to the perfused eye model in long-term tissue preservation. Cabanas et al. presented a perfusion system for isolated bovine retina. NMR spectroscopy showed stable signals for 20 h (Cabanas et al., 1993) but the tissue was not examined by microscopy. Also, separation of the retina from the RPE may lead to a decreased longevity of the retina in culture.

In conclusion, the present system allows to maintain adult retina and the RPE morphologically intact for at least 10 days. The maintenance of intact adult retina as opposed to embryonic or neonatal retina is a strength of the present system. Physiological parameters of the cultured tissue may be monitored by continuous analysis of the medium. The culture system of a two-compartment container allows for polar application of test substances either to the retinal or to the choroidal side. Therefore this model is especially of interest for the evaluation of local effects of intraocular treatments (Framme et al., 2002) and intraocular substances (Saikia et al., 2006). Furthermore, the system has been used as an in vitro model for light damage in age related macular degeneration, toxic retinopathies or for retinal transplantation (Croxen et al., 2005; Hammer et al., 2005; Kobuch et al., 2005). Although the system is limited by the onset of necrosis and apoptosis in

Table 4
Analysis of the culture medium after 4 days of perfusion culture ($n = 10$)

	$[Na^+]$ (mmol/l)	$[K^+]$ (mmol/l)	$[Cl^-]$ (mmol/l)	[lactate] (mg/dl)	[glucose] mg/dl	pH
Inlet	157.7 ± 2.51	5.30 ± 0.34	124.0 ± 4	7.63 ± 0.71	424.7 ± 6.5	7.28 ± 0.04
Chamber	159.8 ± 5.31	5.28 ± 0.26	124.6 ± 351	15.9 ± 8.32	413.8 ± 13.75	7.6 ± 0.15
Outlet	160.3 ± 0.58	5.45 ± 0.32	125.0 ± 1.73	29.2 ± 2.95	399.7 ± 6.11	7.64 ± 0.08

the retina after 4 days in culture it may help to reduce the number of necessary animal experiments in retina and RPE research, and it offers new perspectives for the safety testing of substances designed for intraocular application.

Acknowledgements

The authors would like to thank Will Minuth, Department of Anatomy, University of Regensburg, Germany for his advice and Elfriede Eckert, Andrea Dannullis, Brigitte Wild and Josef Schröder for their expert technical assistance.

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