Autologous Translocation of the Retinal Pigment Epithelium and Choroid in the Treatment of Exudative Age-related Macular Degeneration

Kristel Johanna Maria Maaijwee

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Autologe translocatie van het retina pigment epitheel en choroidea in de behandeling van exsudatieve leeftijdsgebonden macula degeneratie

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Aan mijn ouders
Voor Wouter en Lore

PUBLICATIONS AND MANUSCRIPTS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

Chapter 2.1

Retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: long-term results.

Maaijwee K, Heimann H, Missotten T, Mulder P, Joussen A, van Meurs JC. *Graefes Arch Clin Exp Ophthalmol* 2007;245:1681-9.

Chapter 2.2

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Chapter 2.3

Influence of intraoperative course on visual outcome after an RPE-choroid translocation.

Maaijwee K, Missotten T, Mulder P, van Meurs JC. *Invest Ophthalmol Vis Sci* 2008;49:758-61.

Chapter 2.4

Retinal pigment epithelium (RPE)–choroid graft translocation in the treatment of an RPE tear: preliminary results.

Maaijwee K, Joussen AM, Kirchhof B, van Meurs JC. *Br J Ophthalmol* 2008; in press.

Chapter 2.5

Angiographic evidence for revascularization of an RPE-choroid graft in patients with age-related macular degeneration.

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Chapter 2.6

Hyperfluorescence of the optic disc with indocyanine green angiography.

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Chapter 2.7

Submacular surgery.

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Chapter 3.1

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Chapter 4.1

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Chapter 5.1

Threshold amplitude and frequency for ocular tissue release from a vibrating instrument: an experimental study.

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Chapter 6.1

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Chapter 6.2

Reliability testing of the Dutch version of the Radner Reading Charts.

Maaijwee K, Mulder P, Radner W, van Meurs JC. *Optom Vis Sci* 2008; in press.

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1. Introduction

1.1 The macula and visual function

In the human eye, light enters the eyeball through the cornea and passes through an opening known as the pupil. Subsequently, the light passes the crystalline lens, the corpus vitreum (a clear gel, which occupies the posterior compartment of the eye) and finally arrives at the retina (Fig. 1).

The retina is a multi-layered sensory tissue at the inner surface of the eye and contains the photoreceptor cells, which convert light into nerve signals.[1] These nerve impulses travel along the optic nerve to the brain where they are turned into images.

The macula (diameter of about 1.5 mm) is located in the center of the retina, temporal to the optic nerve (Fig. 1). The macula contains a higher concentration of photoreceptor cells compared to the surrounding retina.[1] Therefore, this tiny macula is a highly sensitive and vital part of the retina responsible for detailed central vision. The macula allows us to appreciate detail and perform tasks such as reading. At the very center of the macula is a depression, called the fovea, that contains the largest concentration of cone cells (Fig. 1).[1]

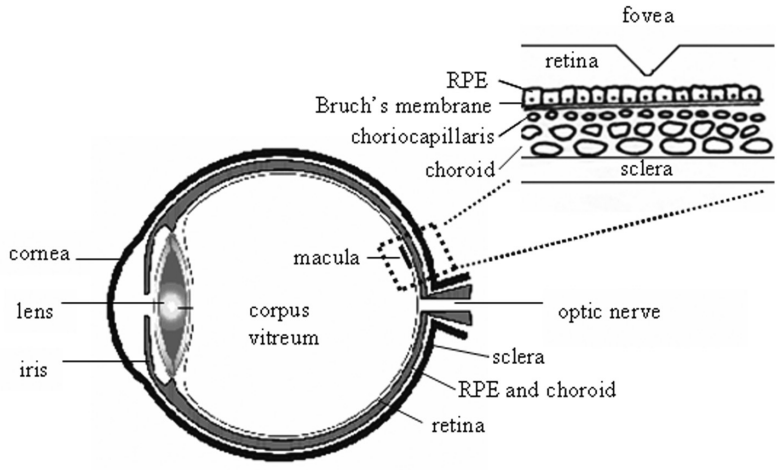

Figure 1. Anatomy of the eye with a detailed figure of the macula.

Underneath the photoreceptors is a dark monolayer of hexagonal cells called the retinal pigment epithelium (RPE)(Fig. 1). RPE cells absorb excess light and transport oxygen, nutrients and cellular waste between the photoreceptors and the choroid.

The Bruch's membrane separates the RPE layer from the choroid. The choroid is composed of

layers of blood vessels that provides oxygen and nourishment to the outer layers of the retina. The innermost layer of the choroid consists of capillaries, called the choriocapillaris, and the outermost layer consists of larger diameter blood vessels adjacent to the sclera (Fig. 1).[2]
The sclera is the tough, opaque tissue that may serve as the eye's protective outer coat.
This highly compact introduction of macular function serves to outline the clinical importance of the most common disease affecting the macula, i.e. age-related macular degeneration (AMD).

1.2 Epidemiology of age-related macular degeneration

Age-related macular degeneration (AMD) is the final stage of age related maculopathy (ARM). Advanced AMD, which is the most important cause of irreversible legal blindness (defined as visual acuity of ≤ 0.05, or a visual field of ≤ 10 degrees in the better eye with best possible correction) in elderly persons in industrialized countries, has two forms: atrophic (dry) and exudative (wet).[3-5] The overall prevalence of AMD is 1.7% and increases strongly from 55 years of age onwards.[6] This is reflected by the 15-year cumulative AMD incidence of 3.1% in a group of subjects aged 43-86 years at baseline up to 8% in people ≥ 75 years of age at baseline.[7] The exudative form leads faster to a deeper and larger scotoma than the atrophic form.[8,9] For example, if untreated, the exudative form is responsible for severe vision loss (> 6 ETDRS lines) in 21% of the patients after six months and 42% after three years from baseline.[10] Moreover, after one to four years, exudative AMD develops in 12%, respectively, 27 to 37% of the fellow eyes that were initially free of neovascular maculopathy.[10-12] As a 2% yearly increase is expected up to 2025 due to population aging and the 1950s baby boom, these data emphasize the impact of AMD on the society and health care.[12]
This thesis focuses on a new surgical treatment for exudative AMD.

1.3 Pathophysiology of exudative AMD

Exudative AMD involves central vision loss due to abnormal blood vessel growth (neovascularization) in the choriocapillaris underneath the macula. The vessels grow through ruptures in the Bruch's membrane, into the sub-retinal pigment epithelium (RPE) space or into the sub-retinal space, or a combination of both (Fig. 2). Ultimately the choroidal neovascularization (CNV) leads to blood and serum leakage below the RPE (causing a pigment epithelium detachment (PED) or an RPE tear) and/or below and within the retina.[13-18] The retina becomes disorganized and the patient notices this as metamorphopsia. The bleeding, leaking, and scarring from the CNV eventually cause irreversible damage to the photoreceptors of the overlying macula which results in a central scotoma.

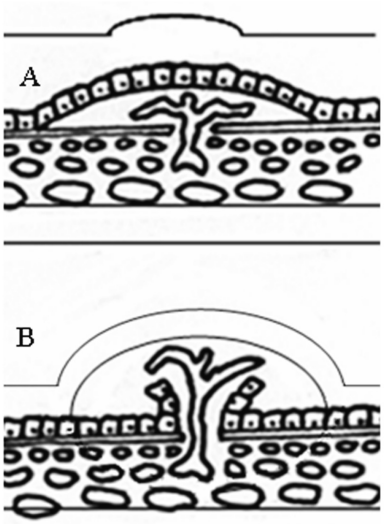

Figure 2. Schematic representation of choroidal neovascular growth in age-related macular degeneration. Vessels grow A, into the sub-retinal pigment epithelium space or B, into the sub-retinal space.

1.4 Classification of exudative AMD

Choroidal neovascularizations (CNVs) are classified according to its leakage properties as seen on fluorescein angiography (FA). The main features of a subfoveal CNV studied by FA are size and pattern (classic or occult). A classic pattern is defined as a well-demarcated focal area of hyperfluorescence emerging in the early phase of the angiogram progressing to a more intense and more extensive late stainig.[19,20] The occult pattern is defined as a poorly demarcated late-phase leakage of an undetermined source.[19,20]

The contribution to the total lesion of the classic and/or occult pattern (including possible presence of blood, serous PED, fibrous and/or scar tissue) determines the lesion composition.

The lesion composition was the major pre-treatment variable and guideline in most treatments studied for AMD, e.g. laser photocoagulation (the Macular Photocoagulation Study), photodynamic therapy (TAP and VIP studies), and more recently inhibitors of vascular endothelial growth factor (VEGF)(VISION, ANCHOR and MARINA studies).[19, 21-29]

1.5 Current therapies for patients with subfoveal exudative AMD

The pathophysiology of choroidal neovascularization is multifactorial, involving a complex interaction of anatomic, metabolic, genetic and environmental factors. This interferes with the development of one single therapy. Therefore, the current treatments of subfoveal CNV

secondary to AMD only aim to delay or temporarily reverse vision loss.

Since October 2006, the most effective proven therapy for subfoveal exudative AMD is intra-ocular injection of an inhibitor of VEGF.[27-30] A second choice for predominantly classic lesions is photodynamic therapy (PDT) with verteporfine.[21-25] However, there are patients not responding to or not eligible for these therapies. The latter group consists of patients with a visual acuity (VA) of < 20/320, lesion size of ≥ 9 macular photocoagulation study (MPS) disc areas, presence of an RPE tear, and a subretinal hemorrhage, PED or fibrosis that comprises ≥ 50% of the lesion (MARINA and ANCOR study), as such patients were excluded from these treatment series.[27,28] For these patients, a surgical approach may be used as a last resort option.

1.6 Surgery for AMD

Because up till 2006 non-surgical treatments (such as thermal laser and PDT) only decreased or retarded visual loss, numerous surgical techniques have been developed for AMD. Two surgical techniques have been extensively studied: submacular surgery and macular translocation.

The purpose of submacular surgery was to remove the subfoveal CNV and associated hemorrhage and was introduced in 1988.[31] The first randomized and controlled study was the multi-center submacular surgery trial (SST), that was initiated in 1998 to compare submacular surgery with observation in patients with exudative AMD over two years and more. The visual outcome in the surgery and observation arm were similarly poor for AMD patients in which more than 50% of the lesion consisted of CNV.[32] In patients with a predominantly hemorrhagic lesion submacular surgery reduced the risk of severe vision loss (> 6 ETDRS lines loss) (21%) compared to the observation arm (36%).[33] Significant complications in the non-hemorrhagic and hemorrhagic surgery group included retinal detachment (5 and 16 %, respectively) and almost half of the patients had cataract surgery by two years. In the observation arm, the retinal detachment occurred in 0.5 to 3% and cataract surgery was needed in 5% of the patients.[32,33]

The application of macular translocation surgery (MTS) in patients was first reported in 1993 by Machemer.[34] The concept of MTS is to move the fovea from the submacular diseased RPE-Bruch's membrane-choroid to a healthy extramacular RPE layer. This can be accomplished by vitrectomy, the creation of a total retinal detachment combined with 360 degree retinotomy (MTS 360) or by vitrectomy, the creation of a temporal retinal detachment combined with external sclerochoroidal foreshortening (the limited MTS).[35-37] Subsequent muscle surgery for counter-rotation of the eye is needed to prevent diplopia and a tilted image in MTS

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360. Only non-randomized studies have been reported. One and two-year outcomes revealed improvement or stabilization (< 3 ETDRS-lines loss) in half of the patients.[37-41] However, both techniques (MTS 360 and limited MTS) are surgically challenging and time-consuming and have many intraoperative and postoperative complications:

- intraoperative complications: failure to achieve complete hydraulic retinal detachment (38%), inadequate rotating of the retina with the fovea remaining above or adjacent to the RPE defect (13%) and macular hole formation (2%);
- postoperative complications: persistent diplopia (20-46%), retinal detachment (17-26 %), recurrent CNV (3-56%), proliferative retinopathy (PVR)(16-26%), macular pucker (5-7%), cystoid macular edema (CME) (8-29%), hypotony (3-28%), and secondary RPE and choroid atrophy underneath the new location of the fovea (61%).[36,38-41]

1.7 The development of RPE-choroid translocation

The poor visual outcomes of the SST trials emphasized that CNV removal, which is accompanied with subfoveal RPE loss in at least 90% of the patients, leads to atrophy and subsequent dysfunction of the overlying photoreceptors in the macula.[42]

The functional restoration achieved in some AMD patients after macular translocation proved the potential of creating a fresh surface of functioning RPE cells.[36-41] The drawbacks of this technique, however, remained the complex surgery and numerous complications. Therefore, several strategies were continued to reconstitute the submacular RPE after CNV extraction, such as transplantation of suspensions or sheets of allogenic fetal and adult RPE, autologous iris pigment epithelium or RPE cells (Table 1). However, results were equivocal: a lack of demonstrable presence or function of these transplants in most patients, to preserving vision and achieving modest visual improvement in a few studies. Of interest, the use of allogenic tissue, either fetal or cadaver, resulted in a immunogenic reaction or a fibrotic respons.[43-52]

Peyman et al. first reported in 1991 the translocation of a free full-thickness autologous pedicle graft after removal of the CNV. The graft consisted of RPE, Bruch's membrane, choriocapillaris and choroid and was taken from the paramacular region in one patient.[53] This approach was later refined in Moorfield's Eye Hospital by Aylward.[54-55] To minimize trauma to the macular area, van Meurs, in the Rotterdam Eye Hospital, pioneered a technique to cut out the graft from the midperiphery.[56-57]

This thesis studies the latter autologous RPE-choroid translocation.

Table 1. Studies reporting on techniques replacing the retinal pigment epithelium cells lost after CNV extraction in patients with exudative age-related macular degeneration.

Type of transplant	Publication	No. of eyes
suspension of autologous IPE	Thumann et al.[43], Lappas et al.[44], Aisenbrey et al.[45]	20, 56, 20
suspension of allogenic adult RPE	Valtink et al.[46]	7
suspension of autologous RPE	Binder et al.[47,48], van Meurs et al.[49]	14, 53, 8
sheet of allogenic fetal RPE	Algvere et al.[50]	5
sheet of allogenic adult RPE	Del Priore et al.[51], Tezel et al.[52]	1, 12
allogenic RPE-choroid graft	Peyman et al.[53]	1
autologous RPE-choroid graft	Peyman et al.[53], Stanga et al.[54,55], van Meurs et al.[56,57], MacLaren et al.[58,59], Joussen et al.[60], Maaijwee et al.[61], Heussen et al.[62]	1, 6, 9, 6, 18 4, 12, 45, 84, 30

1.8 The outline of this thesis

The main objective of this thesis was to evaluate whether the autologous RPE-choroid graft translocation would be a feasible technique in the treatment of patients with exudative AMD.

C**hapter 2** focuses on the clinical results. The intraoperative and postoperative complications and long-term functional outcome were studied (**chapter 2.1 and 2.2**). In order to be more selective in patients to treat, the preoperative conditions were correlated to visual outcome at one year after surgery (**chapter 2.1 and 2.2**). However, as the intraoperative course might have acted as confounder, another study was conducted to investigate whether this, in fact, was an independent variable (**chapter 2.3**).

We reported on a separate group of patients with an RPE tear secondary to AMD treated with an RPE-choroid translocation and who did better than expected: a population for which no other treatment was available till present (**chapter 2.4**).

The survival and function of the RPE-choroid graft is dependent on its revascularization. The graft perfusion was studied using preoperative and postoperative fluorescein angiography (FA) and indocyanine green angiography (ICGA) in patients treated with an RPE-choroid graft. Recommendations for assessment of revascularization of the graft were made (**chapter 2.5**). While examining these ICGAs, hyperfluorescent optic discs were observed in one fourth of the patients after macular surgery. This phenomenon was related to the intravitreal use of ICG during surgery (**chapter 2.6**). Although this finding had been previously described, a report was written as a hyperfluorescent optic disc with ICGA appeared unknown even under experienced medical retinal specialists.[63-65] The chapter ends with a letter to the editor hypothesising that improvement in surgical technique might improve long-term visual outcome (**chapter 2.7**).

Chapter 3 reports on the in vivo experiments performed in pigs to obtain histological evidence for revascularization of the RPE-choroid graft.

As the in vivo experiments in pigs were complicated, time-consuming and expensive, attempts to induce revascularization of an RPE-choroid graft in a perfusion tissue culture were made (**chapter 4**). Varied culture conditions were used to create a stimulus for angiogenesis.

During RPE-choroid translocation surgery, the adhesion of the graft to the translocation instrument complicated its submacular release. Vibration of the instrument appeared to improve the release of the graft. In **chapter 5**, the effectiveness of the principle of vibration was validated, and the threshold amplitude and frequency required for development of an optimized instrument were determined.

Reading performance tests can provide more detailed information about visual impairment and function than the routine single optotype distance visual acuity (VA) tests. For an international controlled study for RPE-choroid graft surgery in patients with AMD (in preparation), we needed a reading chart that was reliable, but would also have its validated counterparts in German and English. **Chapter 6** concerns the development (**chapter 6.1**) and reliability testing (**chapter 6.2**) of the Dutch version of the originally German language Radner Reading Charts.

Finally, in the general discussion (**chapter 7**), the findings in this thesis are placed in a broader context. The position of the RPE-choroid translocation among the other current treatments for AMD is discussed and views are given of the new developments and future research of the RPE-choroid translocation technique itself, but also of the perfusion tissue culture and Radner Reading charts.

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Chapter 2.1

Retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration: long-term results

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Abstract

Background

To study the results of the translocation of a free autologous retinal pigment epithelium (RPE)-choroid graft after removal of a subfoveal choroidal neovascular membrane in patients with exudative age-related macular degeneration (AMD), and to determine whether preoperative variables may predict visual outcome at 1 year after surgery.

Methods

Prospective interventional case series of 84 eyes of 83 consecutive eligible patients with exudative AMD with a minimal follow-up of 1 year after surgery. Of this group, 45, 24 and 11 patients reached a follow-up of respectively 2, 3 and 4 years. Pre- and postoperative evaluation included ETDRS visual acuity (VA), fixation testing and color fundus photography. Preoperative fluorescein angiograms were assessed by masked readers for lesion size, size of hemorrhage and lesion composition according to the MPS criteria. The relationship between lesion composition adjusted for preoperative delay and VA, lesion size, percentage of blood, and visual outcome at 1 year after surgery was analyzed.

Results

The mean VA (logMAR) improved slightly at 1 and 2 years (0.89, Δ = - 0.06), 3 years (0.79, Δ = - 0.16) and 4 years (0.74, Δ = - 0.21) after surgery. Five patients had a preoperative VA better than 20/80, compared to 19 out of 84, six out of 45, four out of 24 and two out of 11 after 1, 2, 3 and 4 years respectively. Fixation was located on the graft in 62 patients (74 %) up to the last examination. Predominantly classic and occult lesions had a significant better prognosis than minimally classic or hemorrhagic (≥50% blood) lesions. Retinal detachment occurred in seven patients; two caused by rhegmatogenous detachment and five caused by proliferative vitreoretinopathy. In 11 eyes, a recurrent or persisting neovascular membrane was observed.

Conclusion

An autologous free RPE-choroid graft may stabilize or improve vision in patients with exudative AMD up to 4 years after surgery.

Introduction

In industrialized countries, age-related macular degeneration (AMD) is the principal cause of irreversible legal blindness in elderly persons.[1-3] In the exudative form, the most promising treatment modality are pharmacological agents. Recently, anti-VEGF treatment has for the first time showed visual improvement.[4-6] Surgical approaches have also been reported, generally as pilot studies.

Submacular CNV removal was found ineffective in preserving or regaining useful vision.[7-10] The functional restoration achieved in some AMD patients after macular translocation with a 360^0 retinotomy proved the potential of creating a fresh surface of functioning RPE cells.[11-13] A tilted image in successfully operated patients, complex surgery and numerous complications, however, remained drawbacks of this technique.

Several strategies have been reported to reconstitute the submacular RPE after CNV extraction, with transplantation of sheets of homologous fetal RPE and suspensions of autologous iris pigment epithelium or RPE cells. However, there is a lack of demonstrable presence or function of these transplants in patients.[14-16] Peyman et al. first reported the translocation of a free full-thickness autologous graft consisting of RPE, Bruch's membrane, choriocapillaris and choroid taken from the paramacular region in one patient.[17] This approach was refined in Moorfield's Eye Hospital.[18,19] To minimize trauma to the macular area, we pioneered a technique to cut out the graft from the midperiphery.[20,21] The feasibility of this technique has recently been confirmed in other pilot studies.[22,23]

The present report concerns the results of the transplantation of a free RPE-choroid graft harvested from the midperiphery in a larger group of patients up to 4 years after surgery. We also wished to study whether we could identify preoperative conditions predictive for visual outcome.

Patients and Methods

Patients

We report the data of 84 eyes of 83 consecutive eligible patients with exudative AMD (one patient had both eyes treated). Patients were eligible if they had a subfoveal CNV membrane, with or without submacular blood, not treatable by other modalities available at that time in our hospital (laser, photodynamic therapy from May 2003 onwards) and before the introduction of Avastin (bevacizumab). Exclusion criteria for surgery included a CNV with

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an etiology other than AMD (e.g. angioid streaks), a visual acuity (VA) of >20/80 and a history of symptomatic visual loss for more than 6 months.

The Institutional Review Board of the Rotterdam Eye Hospital approved the study; written informed consent was obtained from all patients in accordance with the ethical standards laid down in the Declaration of Helsinki. The first patient was included in October 2001, and the censoring date was June 2006.

Preoperative examination included best-corrected ETDRS VA (Snellen and logMAR equivalents), dilated funduscopy, fluorescein angiography (FA) or indocyanine green (ICG) angiography. Postoperative visits were scheduled at 1, 3 and 6 weeks, at 3, 6, 9, 12, 18 months, as well as 2, 3 and 4 years. During each visit, best corrected ETDRS VA testing was performed.

Foveal fixation and retinal sensitivity over the graft were determined in all patients with biomicroscopy. In addition, depending on the temporary availability of this device in our hospital, fundus micro perimetry MP-1 (Nidek, Padova, Italy) was performed in selected patients to determine retinal sensitivity and fixation stability over the graft. Fixation was classified as stable, relatively unstable or unstable.[24]

Every year after surgery, color fundus pictures were taken. The graft was monitored with optical coherence tomography (OCT) by 6 mm radial scans (Zeiss stratum OCT, model 3000, Carl Zeiss, Jena, Germany).

Grading of preoperative images

Masked readers (HH and TM) independently assessed the preoperative stereo color fundus photographs and FAs. Pictures were imported into image analysis software (ImageJ for Mac OC X, National Institute of Health, Bethesda, MD, U.S.A.). The fundus pictures and FA were used for identification of disc area (DA), lesion size (in DA; all lesion components taken together) and composition, and size of hemorrhage (in DA and % of lesion). Lesion composition (predominantly classic, minimally classic or occult) was classified with FA according to the Macular Photocoagulation Study (MPS).[25] If a lesion was covered with an extensive hemorrhage (≥50% of the lesion), the lesion was labeled a "hemorrhagic lesion".

Surgery

After the induction of a posterior vitreous detachment, a complete vitrectomy was performed.

The choroidal membrane was removed from the subretinal space with a Thomas subretinal forceps through a paramacular retinotomy in the temporal raphe (Fig. 1a). After circular

heavy diathermia in the midperiphery at the 12 o'clock position and removal of the retina within the diathermia marks, vitreous scissors were used to cut a full-thickness graft of RPE-choroid of approximately 1.5-2.5 x 2-3 mm (Fig. 1b). The graft was loaded onto an aspiration-reflux spatula (Fig. 1c) (Dutch Ophthalmic Research Center (DORC), Zuidland, The Netherlands) and repositioned under the macula through the existing paramacular retinotomy (Fig. 1d). Perfluorocarbon liquid (PFCL) was injected to keep the graft in place and facilitate the release of the graft when retracting the instrument. The midperipheral donor site was surrounded with laser coagulation followed by a silicone oil tamponade. In a second procedure, approximately 3 months later, the silicone oil was removed. Lensectomy and insertion of an intraocular lens (IOL) were performed during the first or second surgery in phakic patients. One vitreoretinal surgeon (JvM) performed all surgeries.

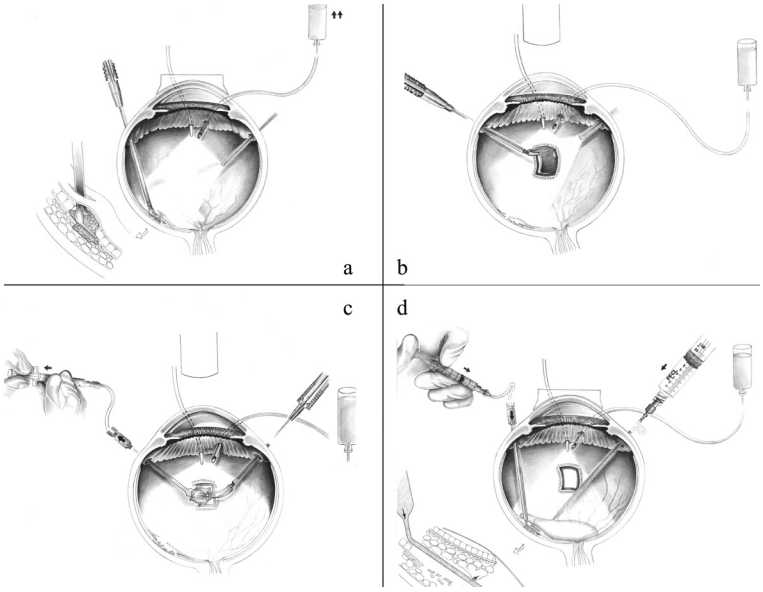

Figure 1. Schematic overview of the translocation of a free autologous full-thickness RPE-choroid graft in patients with exudative AMD. (A) Extraction of the subfoveal neovascular membrane. (B) The graft is cut out and prepared from the sclera in an area demarcated with coagulations in the midperiphery. (C) Loading of the graft on a spatula or grasped with forceps (not shown in figure). (D) Insertion of the graft in the submacular space while injecting perfluorocarbon liquid (PFCL). Medical art work by Dr. Jessica Leenen.

Statistical analysis

Analysis of covariance (ANCOVA) was used to analyze the relation between the four lesion composition groups (minimally classic, predominantly classic, occult or hemorrhagic), adjusted for the following variables: 1) preoperative delay (i.e. time between onset of symptoms and surgery), 2) preoperative VA, 3) lesion size, and 4) percentage of blood, and

the outcome variable: postoperative VA at 1 year after surgery. The postoperative VA was reported as logMAR and Snellen equivalents, or less than two or three ETDRS lines loss.
All analyses were performed using SPSS (Windows version 12.0; SPSS Inc., Chicago, USA).

Results

Completion of scheduled examinations

Of 92 consecutive AMD patients treated with an RPE-choroid graft translocation for ≥ 1 year after initiation of this study, 83 patients (84 eyes) (91%) completed the 1-year follow-up. Fifty-seven of these 83 patients had reached the 2-year follow-up. Complete data were available in 45 of these 57 (79%) patients. The 3-year follow-up was completed in 24 out of 27 (89%) patients, and all 11 patients completed the 4-year follow-up.
Reasons for loss of follow-up were: deceased of unrelated causes (n = 7), physically unable to return for the follow-up (n = 8) and loss of contact (n = 8).
The visual outcomes of the patients lost during follow-up are incorporated in the Kaplan-Meier plot (Fig. 3).

Baseline characteristics

The age of the 83 patients (49 females and 34 males) was 79 ± 8 (mean ± SD) years (range 57-95 years). The preoperative delay in the operated eye (n = 84) ranged from 1 week to 6 months (mean of 10 weeks). Patients with a hemorrhagic lesion (≥50% blood) had a mean delay of 5 weeks after onset of symptoms, compared to 10 to 14 weeks in the other groups.
Forty patients used anticoagulants. They were asked to discontinue these medications 2 weeks prior to surgery. Twenty-two patients were initially pseudophakic. None of the patients remained phakic.

Grading of preoperative images

The preoperative FAs together with a fundus image were available in 68 (out of 84) eyes. FA lacked in 16 patients: erroneously, in seven patients only an ICG was obtained because of macular hemorrhages. In eight patients, FAs were made and evaluated before surgery, but were lost before evaluation by our masked readers; and in one patient the FA was made 6 months before surgery and therefore excluded. The lesions of these 16 eyes were not analyzed.
The two independent masked readers classified the FA differently in 7 eyes, but consensus was

reached after consultation. According to the MPS criteria, nine patients had a predominantly classic, 14 a minimally classic, 18 an occult subfoveal neovascularization and 27 were classified as hemorrhagic lesion (≥50% blood). The size of the total lesion varied from 1.5 to 72 MPS disk areas (DA)(mean 10 DA). Subretinal blood was present in all but 6 (out of 68 available) angiograms.

Visual outcome

Mean preoperative VA was 0.95 logMAR (range 1/300 to 20/63), and 0.89 logMAR (range 1/300 to 20/32) after 1 and 2 years, 0.79 logMAR (range 1/60 to 20/25) after 3 years, and 0.74 logMAR (range 20/800 to 20/32) after 4 years. This represents no average change in ETDRS lines. Five patients had a preoperative vision of 20/80, whereas 19 out of 84 patients had a VA of 20/80 or better after 1 year, and six out of 45, four out of 24 and two out of 11 after 2, 3 and 4 years respectively (Fig. 2).

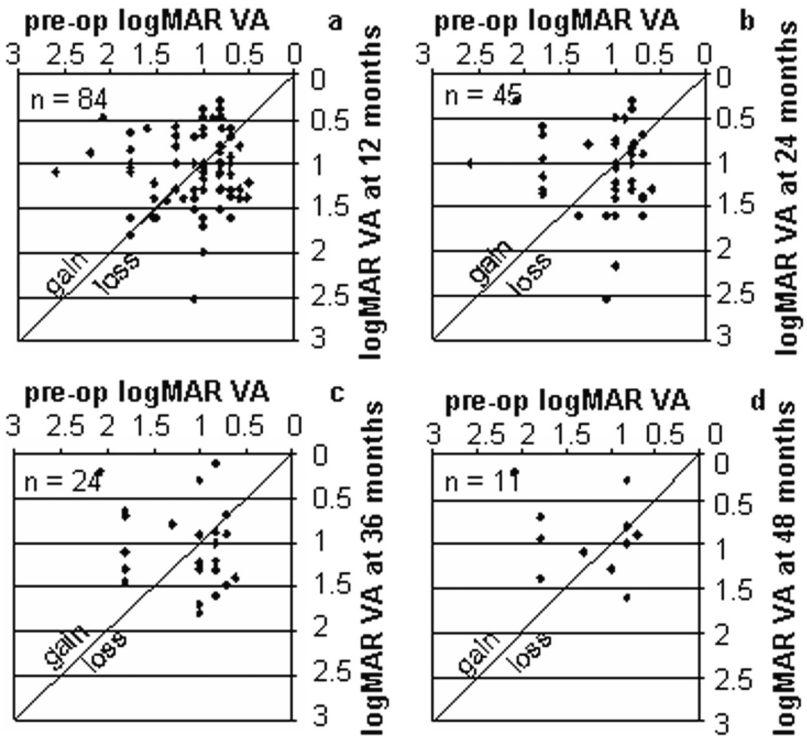

Figure 2. Preoperative versus postoperative visual acuity (logMAR). **(A-D)** At 1, 2, 3 and 4 years after surgery (n = 84, 45, 24 and 11 respectively). Data points above the diagonal line indicate patients with visual improvement.

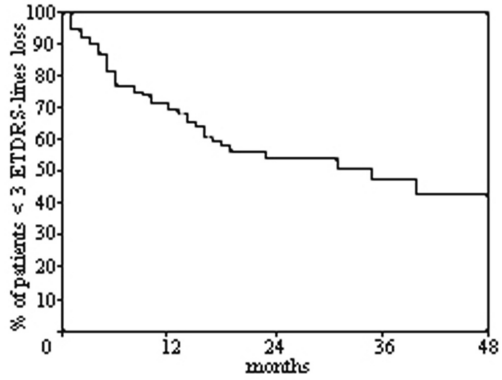

Figure 3. Kaplan-Meier plot of percentage of patients (n = 92) who had <3 (≤2) ETDRS-lines loss. The patients lost within the 4-year follow-up are included.

A loss of ≤ 2 (< 3) ETDRS lines was observed in 76% (64 out of 84) to 82% (9 out of 11) from 1 to 4 years after surgery respectively (Fig. 3).

The mean VA at 1 year after surgery in the four lesion composition groups was 1.09 logMAR in the minimally classic, 0.96 logMAR in the hemorrhagic, 0.82 logMAR in the predominantly classic and 0.77 logMAR in the occult lesion group.

If a last observation carried forward method was applied to these data (n = 92) (to correct for patients lost to follow-up), mean VA was 0.91, 0.93, 0.92 and 0.93 logMAR at 1, 2, 3 and 4 years after surgery respectively.

Foveal fixation on the graft viewed by biomicroscopy was present in 74% (62 out of 84) of the eyes up to the last examination. Twelve patients lost their fixation over the graft during follow-up, associated with a decrease in VA (mean 1.48 logMAR).

Retinal sensitivity could be demonstrated over the graft in 16 of 19 patients examined with the NIDEK MP-1 (Fig. 4)**.** Nine of these patients had fixation over the graft; three of them were graded as stable and six as relatively unstable. Of the ten eyes without fixation over the graft at the time of examination with the MP-1 microperimeter, six eyes gained fixation during follow-up.

Imaging

Postoperative angiograms were performed in 27 patients; in 14 both FA and ICG, in eight FA only and in five ICG only. Indications for a postoperative angiography were suspicion of a central retinal vein occlusion (n = 1) or recurrent/persistent CNV (n = 17), an unexplained loss of VA (n = 2), research purposes (n = 4) and suspicion of pathology in the fellow eye (n = 3).

Early fluorescence of the graft was seen in 21 of the 22 FAs obtained from 1 month to 4

years after surgery (Fig. 4). In 10 of 19 ICGs, obtained from 1 to 27 months after surgery, perfusion of the parallel oriented graft vasculature could be identified in the early phases of the ICG (Fig. 4). In seven patients without visualization of the graft vasculature with ICG, FA showed early fluorescence (in one patient the FA was not performed). The graft that lacked fluorescence with early FA also had no signs of choroidal graft perfusion with ICG.

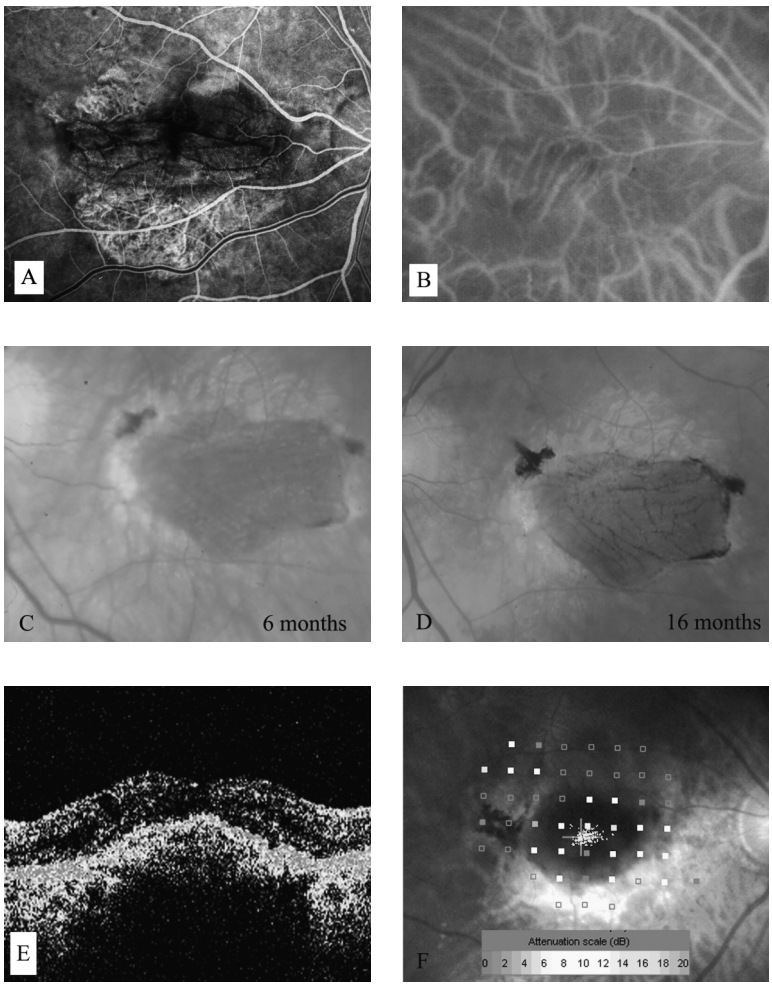

Figure 4. Imaging of a free RPE-choroid graft. (A) Fluorescein angiography. The graft fluorescence emerges simultaneously with the surrounding fundus in the early phase. (B) Indocyanine green angiography reveals perfusion of the parallel oriented vessels of the graft. (C-D) Fundus images with typical accumulations of pigmentation alongside and on the graft increasing over time. (E) OCT image. The retina overlying the graft has a layered structure, foveal depression, present but suboptimal photoreceptor layer. (F) Microperimetry shows function on the graft. Color figure can be found in the appendix. See page 196.

The graft had a brown, velvety appearance in 60 eyes. In three patients the graft had a convex shape, in two eyes one side was folded, in two eyes the graft was small (< 1 DA), and in two patients the graft had become fibrotic. In these nine patients, VA was ≤ 20/250 (mean 20/640) after 1 year. Irregular accumulation of hyperpigmentation at the margins or near the graft, and sometimes on the graft, could be seen in 59 patients, starting from 3 months after surgery (mean 7 months) and increasing in time (Fig. 4). Except for this hyperpigmentation, the position, size and shape of the graft did not change during follow-up.

OCT of the graft was performed in 55 patients. The graft RPE layer was observed in all 55 patients up to 4 years after surgery (Fig. 4). The photoreceptor layer was absent in 14 (25%) patients with a mean VA of 1.05 logMAR, and present but suboptimal in 41 (75%) with a mean VA of 0.77 logMAR (P = 0,012). In 16 (29%) patients, cystic spaces were present at time of OCT-imaging.

Complications during surgery

A complete posterior vitreous detachment (PVD) was present in only ten patients at the start of surgery; in all others a PVD had to be induced, with remaining hyaloid (as a result of hyaloidal schizis) covering the retina in 42 patients.

Complications during surgery occurred in 17 % (14 out of 84) of the patients and consisted of a CNV-membrane attached to the retina (n = 1), a large choroidal defect after removing the CNV-membrane (n = 1), damage to the papillomacular bundle (n = 1), trauma of the subfoveal choroid with bleeding (n = 6, two of which were caused by manipulation of the graft during repositioning), tearing of the retinotomy with bleeding (n =2), a peripheral retinal tear (n = 1), a retinal detachment (n = 1) and loss of the graft through the sclerotomies with the need to prepare a second graft (n = 3).

The main problems encountered were failure of the subfoveal release of the graft (n = 18), an inadequate positioning or incomplete flattening of the graft (n = 56), which was partly folded or wrinkled underneath the fovea. In 12 of these cases the graft had to be removed and was re-inserted. To optimize the position or to unfold the graft, reposition by manipulating the subfoveal graft was performed in these 74 patients (88%).

Postoperative complications

After surgery, a recurrent or persistent CNV membrane was observed in 11 patients (13%) (range 2 to 27 months after surgery). Ten CNV membranes were closed by thermal laser treatment and recently (June 2006) in one patient, Avastin (bevacizumab) was injected. In these 11 recurrences, vision dropped to counting fingers in seven patients, three remained

stable after treatment (VA of 20/100) and one patient even improved to a VA of 20/80, with 20/125 at the last examination 2 years after surgery.

Retinal detachment (RD) occurred in seven patients (8 %); five due to proliferative vitreoretinopathy (PVR) and two with a rhegmatogenous cause. One rhegmatogenous-RD developed before silicone oil removal, one rhegmatogenous-RD and three PVR-RDs occurred after, and in two patients PVR-RD occurred both before and after silicone oil removal. Two of these seven patients had ≥ 3 lines increase in vision, one had two lines increase, three remained stable and one had lost two lines in vision before the development of RD. Membrane peeling and repeat silicone oil tamponade were performed. Compared to preoperative VA, at the last examination after RD-repair, six of these seven patients lost more than three lines and only one had three lines visual improvement up to the last examination.

In one patient, a local macular RD developed secondary to a macular pucker before silicone oil removal. After reattachment, VA improved from 20/800 to 20/125 at the last examination at 17 months after surgery.

In nine patients, postoperative hemorrhages occurred: one suprachoroidal bleeding, seven subretinal and one patient both a subretinal and vitreous hemorrhage. These hemorrhages were self-limiting, except for two that needed surgical intervention; a suprachoroidal bleeding and one caused by a large subfoveal CNV (see above). These hemorrhages occurred between 1 week and 6 months after surgery both in patients with (n = 6) or without (n = 3) anticoagulant use.

Optic disc atrophy was observed in one patient, and acute glaucoma in five patients. Two of the latter needed peripheral iridectomy. A non-ischemic central retinal vein occlusion occurred in one patient after 8 months. One patient suffered from a retinal artery occlusion after surgery.

Silicone oil had been removed in all patients, typically 3 to 4 months after the first procedure. When removing the silicone oil, some degree of retinal puckering/cellophane maculopathy was present in 90% (76 out of 84) of the patients. The ILM was removed in these patients using ICG staining.

Predictors for visual outcome

ANCOVA analysis revealed a significant correlation between the preoperative CNV classification (n = 68), adjusted for preoperative delay, baseline VA, lesion size, and percentage of blood, and the outcome variable: postoperative VA 1 year after surgery defined as logMAR (P = 0.026) or Snellen equivalents (P = 0.014); predominantly classic and occult lesions had a significant better prognosis than minimally classic or hemorrhagic (≥50% blood) lesions.

There was no significant difference between the four lesion composition groups if the outcome was defined as less than two (P = 0.129) or three (P = 0.333) ETDRS lines loss 1 year after surgery.

By analyzing the correlation between all preoperative variables (except lesion composition) and visual outcome with ANCOVA, the preoperative VA turned out to be the strongest (but not statistically significant) prognostic factor.

Also, no significant correlation was found when preoperative delay was assessed as intervals of more or less than 4 and 6 (the latter is the median delay) weeks and postoperative visual outcome (defined as ETDRS-VA or less than two or three ETDRS lines loss) at 1 year after surgery.

Discussion

A free autologous RPE-choroid graft stabilized the mean VA up to 4 years after surgery (0.74 logMAR, Δ = - 0.21) after CNV removal in patients with exudative AMD. Despite the occurrence of serious intra- and postoperative complications, such as 8 % retinal detachments (RD), a VA of 20/80 or better was observed in some patients up to 4 years after surgery. Fixation over the graft was achieved in 74% of the eyes up to the last examination.

This study did not reveal a significant correlation between preoperative delay, even when analyzed in time-intervals (delay more or less than 4 or 6 weeks). This is surprisingly, as one would assume that the longer the CNV-membrane would be present beneath the fovea prior to surgical intervention, the more extensive the photoreceptor loss and the less beneficial that an RPE-choroid graft would be to visual acuity. We assume that either the number of patients was too small to detect a correlation, or that other variables not included (such as intraoperative variables) acted as confounding factors.

As a predictor for better visual outcome in order to be more selective in patients to treat, predominantly classical membranes were identified in this study as the best candidate, as with photodynamic therapy.[26-28] In the SST-trial, and in studies on full macular translocation, no obvious best candidates were found.[7,9,29]

Our functional results compare favorably with two other groups recently reporting on graft translocation in AMD patients, possibly because our RD rate of 8% is low compared to theirs of 31% and 42%.[22,23] This difference may be related to a difference in operating technique, as Joussen et al. harvested the graft from the inferior midperiphery. Another possible surgical cause may be our painstaking removal of all remaining hyaloid (which requires visualization

by triamcinolon acetonide crystals when working with a chandelier illumination) to prevent remaining hyaloid forming a scaffold for epiretinal membranes. Remarkably, a complete posterior vitreous detachment was rare in these patients.

Our visual results, as well as our rate of RD, also compare favorably with those obtained by simple membrane extraction alone (RD rate of 5-16%), as demonstrated by the submacular surgery trial (SST).[7,9] In full macular translocation, better functional improvements have been described in some reports, but a considerably higher rate of RD may occur (8-26%).[11,13,29-31] This technique is also more complex and time-consuming compared to the translocation of a free RPE-choroid graft.

This study also had a better outcome in terms of loss of ≥ 2 lines and average vision compared with the Photodynamic Therapy (TAP) Investigation and the Verteporfin in Photodynamic Therapy (VIP) Trial, even despite the large size (median 10 DA) of the lesions we treated with an RPE-choroid graft.[26,28,32]

However, one has to be careful to compare the results of this extended pilot study to the other treatment modalities that were studied in prospective controlled trials, whereas all surgical approaches (except the SST studies) have been uncontrolled single-center pilot studies.

The free RPE-choroid graft was revascularized in our patients as revealed by the early fluorescence of the graft by FA and the perfused parallel oriented vessels of the graft by ICG (Fig. 4) (Maaijwee et al., unpublished data). This was histologically confirmed in a pig model by demonstration of bridging vessels arising from the underlying recipient layer into the graft and not into the edge of the graft.[33] However, Maclaren et al. reported on one patient with a graft perfused from its periphery by blood entering horizontally through a contact point with the surrounding macular choroid as estimated by high speed angiography.[23] This is plausible, as in skin grafts the major contribution to revascularization of the graft originates from the underlying bed, and also, but less often, from the graft margins.[34,35]

Irregular accumulation of hyperpigmentation developed typically from 3 months after surgery onwards, at the margins or beside the graft (in the atrophic area), and increased with time (Fig. 4). RPE wound repair in the elderly probably occurs by cell migration and elongation.[36] We suggest that this hyperpigmentation at the margins of the graft may be explained by a crowding phenomenon of the RPE-cells, because the relatively normal Bruch's membrane ends abruptly at the graft's margin. The accumulation of dark pigment in the atrophic area adjacent to the graft might be a reaction to the membrane extraction, and may consist of choroidal melanin as well as remnants of RPE-cells.

MacLaren et al. reported on the long-term (≥5 years) function of a free RPE-choroid graft taken from the paramacular area in four patients, with decline in VA in three patients and

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loss of fixation in all.[18] As OCT suggested loss of photoreceptor outer segments, as was also observed in this study, they hypothesized that this may be explained by chronic photoreceptor apoptosis, initiated by either surgery or the disease process itself.[18] They also warned for unduly optimistic expectations from studies with a shorter follow-up. Nevertheless, several of their findings may be interpreted positively. Firstly, fibrosis and recurrent choroidal membranes were not a major problem, whereas this occurs regularly after laser coagulation, membrane extraction and macular translocation surgery. The recurrence rate in our study was 13 %, but is probably underestimated, as angiography was only performed if pathology was clinically suspected. Secondly, if surgical trauma indeed causes photoreceptor damage, less surgical damage might improve the long-term results.[37] In our study, better photoreceptor survival might be expected, as the free graft was taken from the midperiphery, which reduces submacular manipulation, compared to the use of a juxta-foveal site as described by Stanga and MacLaren et al.[18,19]

In fact, at this moment the main challenge is to find a reproducible and safe way to deliver the free graft under the macula in one go without further manipulation. We hypothesize that, with improved instrumentation and subsequent less trauma, the functional results may further improve and enable us to start a controlled trial. With promising pharmaceutical agents like anti-VEGF being developed and available at present, RPE-choroid graft surgery should be perfected and be available as a last resort option to patients not treatable or no longer responding to approaches with less potential complications.

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Chapter 2.2

Retinal pigment epithelium and choroid translocation in patients with exudative age-related macular degeneration

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Introduction

In industrialized countries, age-related macular degeneration (AMD), the end-stage of age-related maculopathy, is the principal cause of irreversible legal blindness in elderly persons.[1] This end-stage disease occurs in two forms: the exudative and the atrophic form.

Pathology

Although the etiology and pathogenesis of AMD are not yet fully understood, the resulting pathology is well defined. In the exudative form, choroidal neovascular ingrowth occurs under the retinal pigment epithelium (RPE) and through the RPE under the retina causing a hemorrhagic RPE and retinal detachment, and eventually a fibrovascular scar with subsequent dysfunction of the overlying neurosensory retina (fovea, macula).[2] In the atrophic form, a gradual loss of submacular RPE cells finally leads to macular dysfunction.

Surgical treatment approaches of exudative age-related macular degeneration

Neovascular membrane removal

In some young patients with a submacular choroidal neovascular (CNV) membrane secondary to e.g. the presumed histoplasmosis syndrome, subfoveal CNV grows through a focal extrafoveal break in Bruch's membrane. In such patients, surgical removal of the neovascular membrane may spare the subfoveal RPE and may result in a preserved foveal function. This concept, however, did not hold true in a recent controlled randomized trial.[3] The situation is even less favorable in AMD patients, where the neovascular tissue grows under the RPE, as well as under the retina. Therefore, simple surgical removal of CNV membranes in patients with AMD almost invariably leads to damage of the subfoveal RPE, as well as the Bruch's membrane/choriocapillaris complex, and could not restore visual function. Spontaneous RPE cell repopulation of the damaged area was ineffective or too late, if at all present.[4] Moreover, in 40 % of the patients recurrent membranes were detected within two years after CNV membrane removal. Despite these undesired effects, the resulting scotoma may be less disturbing than an untreated progressive exudative CNV membrane. Therefore, simple membrane removal has been studied in a controlled randomized manner in the U.S.A., in the submacular surgery trials (SST). Surgery was shown to be of some measurable benefit with reducing severe visual loss in predominantly hemorrhagic lesions and to have marginally higher visual quality-of-life scores.[5,6]

Membrane removal with reconstitution of the RPE layer

The spectacular functional restoration achieved in some patients with exudative age-related macular detachment after macular rotation with a 360 degree retinotomy proved the potential of creating a fresh undersurface of functioning RPE cells. A tilted image in successful cases, complex and time-consuming surgery and a high percentage of vision threatening complications because of proliferative vitreoretinopathy, however, remained drawbacks of this technique. A controlled trial in France of minimal rotation, a more modest variation of macular rotation, also confirmed the surgery's potential for an improvement of vision.[7]

Other arguments

Other cornerstones in the concept of restoring the RPE layer of the macula:

- Functioning RPE cells were shown to be essential for the preservation of Bruch's membrane and the survival of the choriocapillaris in rabbits;[8]
- Schlingemann showed that human RPE cells secreted VEGF on their basal side and that the facing choriocapillaris had VEGF-receptors;[9]
- Subretinal RPE injection was able to postpone photoreceptor death in RCS rats.[10]

Consequently, several different surgical approaches to recreate a functioning RPE layer of the macula have been tried. We could subdivide these approaches: autografts versus allografts, loose cells in suspension versus cell sheets or grafts, and RPE versus iris pigment epithelium cells.

Pigment Epithelium transplantation techniques

Homologous versus *autologous pigment epithelium transplantation*

Fibrosis with edema and persistent dye leakage with fluorescein angiography (FA) was observed in patients with a fetal RPE graft, HLA-typed RPE cell suspension or cadaver graft, which was thought to result from an immune rejection.[11,12] Therefore, autologous tissue would be preferable. Immune involvement and inflammation may nevertheless occur, because of the surgical trauma, as not only self and non-self, but also damaged tissue may trigger an immune response (the danger model).[13] However, it makes sense to reduce both factors by using autologous tissue and trying to minimize surgical manipulation.

Iris pigment epithelium **versus** *RPE:*

Using iris pigment epithelium would have the advantage of an relatively easy way of harvesting by performing a surgical peripheral iridectomy. Iris pigment epithelium, however, may not have all the functions required of RPE.[14,15]

Cell suspension **versus** *a cell sheet*

A formidable metamorphosis is required of injected RPE cells in suspension to reconstitute a RPE layer after CNV membrane extraction. After being scraped off their native Bruch's membrane or culture substratum, the cells are expected to adhere to a damaged Bruch's membrane, to survive and redifferentiate into a functional monolayer. In vitro studies show that RPE cells adhere poorly to damaged Bruch's membrane.[16-18] RPE cells from patients with exudative AMD, moreover, may even have less ability to proliferate than RPE cells from patients without AMD.[19] The results from autologous RPE cell suspension transplantation have never been convincing.[20-22]

RPE cells on some substratum, on the contrary, are already adhered and differentiated. Though, the delivery of a sheet is more problematic than a cell suspension through a small-bore canula.

Translocation of a full-thickness graft from the midperiphery

With the current lack of a demonstrable presence or function of autologous RPE suspension transplants in patients, we decided to pursue the use of a sheet of autologous RPE on its own substratum. Peyman reported on a full-thickness pedicle flap in a patient.[23] The follow-up was six months and stabilization of vision of 20/400 was reported. Aylward, in eight patients, used a full-thickness graft cut out from a location adjacent to the removed subfoveal CNV membrane. In four patients, some function was observed over the graft with microperimetry.[24,25] Fibrosis of the graft, however, developed in the second year of follow-up in most patients. In Aylward's patients, the grafted paramacular choriocapillary appeared sclerotic and damaged by the surgery and we speculated that it was therefore less likely to be successfully revascularized. We thought to improve on Aylward's technique by harvesting a relatively healthy midperipheral full-thickness RPE and choroid graft with the advantage of an easy accessibility to cut out the graft, and a direct control of bleeding from the donor site.[26,27]

Patients and Methods

Inclusion

Patients eligible for RPE translocation had a classic or occult subfoveal choroidal neovascular membrane on fluorescein angiography (FA), with or without submacular blood, and were excluded from other treatments like laser or photodynamic therapy (anti-VEGF was not available at that time). This study had been approved by the Institutional Review Board of the Rotterdam Eye Hospital and written informed consent was obtained from all patients, in accordance with the ethical standards laid down in the Declaration of Helsinki. The first patient was included in October 2001, and the censoring date was June 2006. The present report concerns 83 patients (84 eyes) who had a follow-up of 12 months or longer.

Preoperative examination included general and ophthalmologic history taking, and a ophthalmologic examination, including best corrected ETDRS vision and dilated funduscopy.

Postoperative visits were scheduled at one, three and six weeks, and 3, 6, 9 , 12, 18, and 24 months. During each visit best corrected ETDRS vision testing and a comprehensive examination were performed. At six and twelve months, the graft was monitored with optical coherence tomography (OCT). In selected patients, we recorded fundus autofluorescence (HRA, Heidelberg Retina Angiograph, Engineering GmbH, Dossenhein, Germany) and fundus perimetry (MP-1, Nidek, Padova, Italy).

FA or indocyanine green angiography (ICGA) were only performed after surgery to exclude regrowth of a CNV membrane. To compare preoperative and postoperative vision, and to be able to express visual outcome in terms of change in ETDRS lines, we transformed ETDRS visual acuity into logMAR.

Surgery

After the induction of a posterior vitreous detachment, a complete vitrectomy was performed.

Through a paramacular retinotomy, the CNV membrane was removed from the subretinal space with a Thomas subretinal forceps (Fig. 1). After circular heavy diathermia in the midperiphery at the 12 o'clock position and removal of the retina within the diathermia marks, we used vitreous scissors to cut a full-thickness RPE-choroid graft of approximately 2 x 3 mm (Fig. 2). We then loaded the RPE-choroid graft on an aspirating spatula (DORC, Zuidland, the Netherlands) (Fig. 3) and repositioned the graft under the macula through the existing paramacular retinotomy (Fig. 4).

We surrounded the midperipheral retinotomy site with laser coagulation, and left a silicone oil tamponade. In a second procedure, approximately three months later, we would remove the silicone oil, perform lensectomy and insert an intraocular lens.

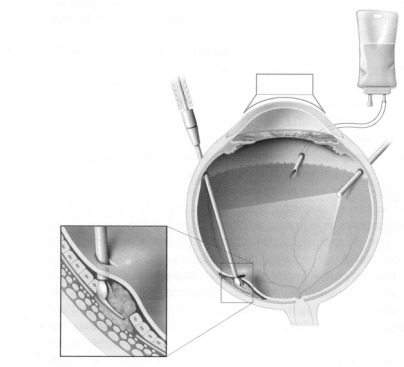

Figure 1. Drawing of extraction of the subfoveal neovascular membrane. Color figure can be found in the appendix. See page 197.

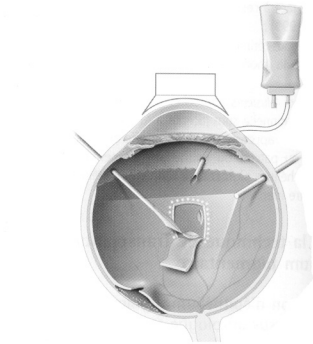

Figure 2. Drawing of preparation of the RPE-choroid graft. Color figure can be found in the appendix. See page 197.

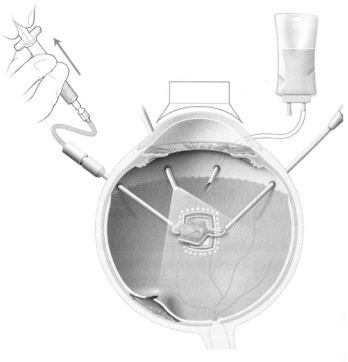

Figure 3. Loading of the graft on a spatula. Color figure can be found in the appendix. See page 197.

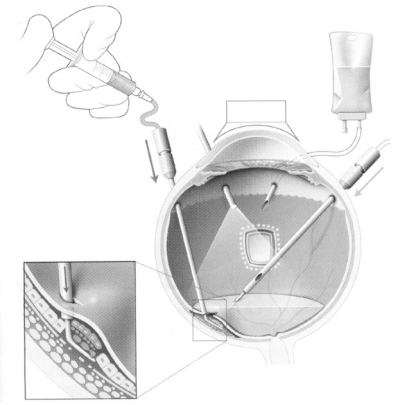

Figure 4. Submacular insertion of the graft. Color figure can be found in the appendix. See page 198.

Results

Baseline characteristics

The present report concerns the results of 83 patients (84 eyes) with a minimal follow-up of one year. The preoperative duration of visual loss in the operated eye ranged from one week to six months (mean of ten weeks). The mean preoperative visual acuity (VA) was 0.95 logMAR (range 1/300 to 20/80).

The preoperative FAs together with a fundus image were available in 68 (of 84) eyes. According to the Macular Photocoagulation Study Group criteria, nine eyes had a predominantly classic lesion, 14 a minimally classic lesion, 18 an occult lesion, and 27 were classified as hemorrhagic lesion (≥50% blood). The size of the neovascular membranes varied from 1.5 to 12 disk diameters. Forty patients used anticoagulants. They were asked to discontinue these medications two weeks before surgery.

Intraoperative course

The surgical techniques have evolved during the entire study period and notes on surgical technique will include surgical experience up till the present time.

Despite their advanced age, in only two eyes we did not have to actively induce a posterior hyaloid detachment. We raised the intraocular pressure to 120-140 mm Hg to prevent bleeding when removing the CNV membrane and slowly decreased the pressure afterwards. On the first sign of bleeding we would raise the bottle again (advise by Matthew Thomas, MD). Subretinal blood was best flushed away with a subretinal canula.

Removal of neovascular membrane

The area of damaged RPE resulting from CNV membrane and hemorrhage removal was approximately three to twelve disk diameters and included the area under the fovea in each patient. The RPE-choroid graft was smaller than the damaged RPE-Bruch's membrane-choriocapillary area in most patients.

Finding a cleavage plane between sclera and choroid

When we try to release the graft, remnants of connecting tissue between choroid and sclera may jeopardize a clean release. Before cutting out the graft, we now separate the graft from the sclera by introducing and sweeping a long spatula underneath the graft.

Preparation of the graft

Once all four sides of the rectangular graft and the collagenous connection of the choroid to the sclera have been cut with scissors, the graft has the tendency to curl up in a half cylinder with the RPE on the convex side, usually with the half cylinder limbus parallel.

This occurs in physiologic salt solution and the free floating graft may be subsequently difficult to position on the spatula. Moreover, the infusion bottle should be really low to minimize turbulence, and to prevent the graft to disappear through a sclerotomy when changing instruments. A great help is to use an extra ceiling illumination to be able to work with two hands, for example to hold the graft when changing instruments. We also used an additional infusion entrance for perfluorocarbon liquid (PFCL).

Positioning of the graft under the fovea

It helps to lift the foveal edge of the retinotomy with the PFCL aspirating canula to allow an easy insertion. Positioning of the graft under the fovea with a common spatula was difficult. Horizontal forceps, even when designed not to close entirely, proved to be unsuitable because the graft could not be released because it remained adhered to the forceps. The best instrument to hold and release the graft turned out to be a canulated spatula with one opening, with an assistant applying aspiration to hold the graft or reflux to release the graft. A single opening appeared better that more openings, since once occlusion is lost over one opening, release can no longer be effected by refluxing.

When releasing the graft by refluxing the spatula (at present manually by the assisting person, but foot control would more ideal), we simultaneously cover the macula with PFCL, to decrease the chance that the graft will move out again on withdrawal of the spatula.

Tamponade

Silicone oil was used to be able to better examine the patients in the early postoperative period. A gas tamponade would be possible too.

Postoperative course

One year vision results

Mean visual acuity (VA) at one year after surgery was 0.89 logMAR (range1/300 to 20/32), which is a gain of some ETDRS letters. Sixty-eight % of the patients had a stable or improved VA (loss of 2 ETDRS-lines or less). Five patients had a preoperative vision of 20/80, whereas 19 patients had a vision of 20/80 or better after one year (Fig. 5). Retinal sensitivity could be demonstrated over the graft in 16 of 19 patients examined with the NIDEK MP-1 (Fig. 6)

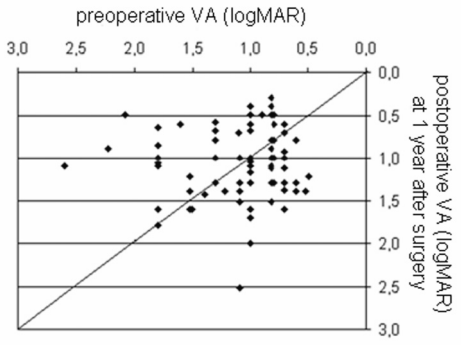

Figure 5. Preoperative versus postoperative visual acuity (logMAR) at one year after surgery.

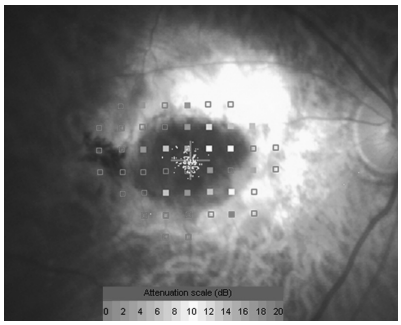

Figure 6. Microperimetry at eight months after surgery (VA 20/80). Color figure can be found in the appendix. See page 198.

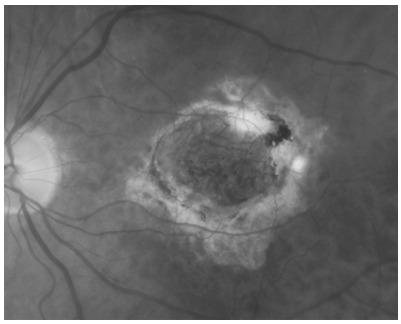

Figure 7. Fundus image shows an RPE-choroid graft at 26 months after surgery (VA 20/100). Color figure can be found in the appendix. See page 199.

Anatomical results

The graft was biomicroscopically flat in 75 patients, and had a brown, velvety appearance in the majority of eyes (Fig. 7). In 9 patients, a part of the graft appeared to be folded.

Postoperative angiograms were performed in 27 patients; in 14 both FA and ICGA, in eight FA only and in five ICGA only. Fluorescence of the graft in the early phase of FA was present in 21 of the 22 FAs, and a parallel orientation of the vascular pattern in the macular area was observed in 10 of the 19 ICGAs (Figs. 8 and 9). Both signs were considered to represent perfusion of the graft.

OCT images were not easy to read; the retina could be better evaluated than the RPE and choroid. There appeared to be a correlation between a thinner retina (less edema) and a better function (Fig. 10).

Confocal SLO showed almost normal autofluorescence over the graft in 6 out of 7 tested patients up to two years after surgery.

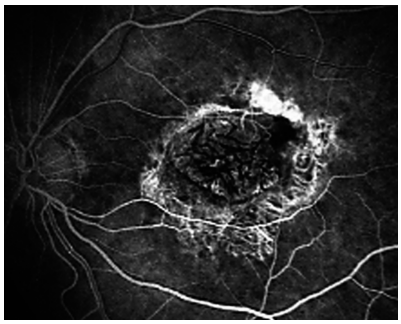

Figure 8. Fluorescein angiography at 13 months after surgery (VA 20/200).

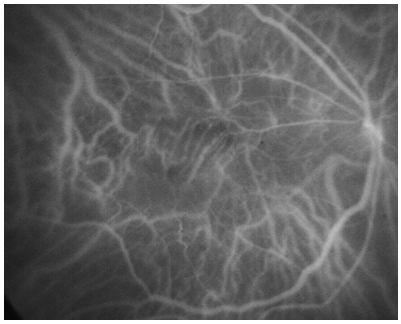

Figure 9. Indocyanine green angiography at three months after surgery (VA 20/40). This is the same eye as shown in figure 6.

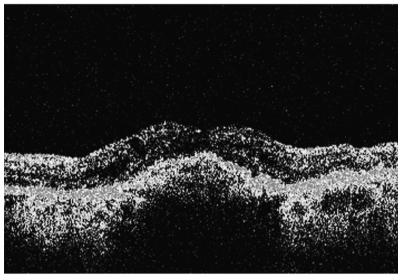

Figure 10. OCT image of RPE-choroid graft at 3 years after surgery (VA 20/25). The retina has its foveal depression. Color figure can be found in the appendix. See page 199.

Complications

In six patients recurrent or persistent CNV membranes were detected; despite laser treatment and closure of the membranes vision dropped to counting fingers in all six patients.

Retinal detachment occurred in 7 patients (8%); five due to proliferative retinopathy (PVR) and two with a rhegmatogenous cause. Revitrectomy, membrane peeling and silicone oil tamponade was performed. Six of these seven patients lost more than three ETDRS lines and only one had three ETDRS lines visual improvement up to the last examination.

Silicone oil has been removed in all eyes, typically 3 to 4 months after the first procedure. When removing silicone oil, some degree of retinal puckering/cellophane maculopathy was present in 76 eyes (90%). We removed the inner limiting membrane in these patients, with the help of ICG staining.

Comment

In our study, one fifth of patients reached a visual acuity of 20/80 or better after a follow-up of one year or longer, a level of visual acuity not likely to be expected in such patients. We were unable to identify patient characteristics that would predict a better outcome, because our

series was a pilot study with evolving surgical technique and experience, inducing numerous confounding factors besides patient selection.

The RPE-choroid graft appeared to be revascularized and viable, with fixation on and function of the graft in the majority of the patients. As there was a sustained two-line improvement in several patients with a follow-up of almost up to four years, our approach may be good way to go.[28]

The revascularization of the free graft we assumed to occur in our patients was, indeed, histologically confirmed in a pig model.[29]

Whereas laser and pharmacological treatments have been studied or are being studied in prospective controlled trials, all surgical approaches (except the SST studies) discussed in the preceding chapter (certainly including the discussed graft technique) have been uncontrolled single center pilot studies, without robust outcome measurements and varying follow-up. Therefore, data on visual results are not easily comparable to the data from the controlled studies.

Nevertheless, the above described surgical method combines several desirable objectives. Functioning, differentiated RPE cells on their native substrate were transplanted with relatively simple technology in a one step one hour surgical procedure, which was applicable to patients with a wide range of membranes (occult, very large), with or without subretinal blood and widespread RPE disease.

Although this surgery may only be an intermediate stage before more sophisticated upgraded cultivated RPE cells on a suitable artificial substratum are available, its concept and the surgical technique required may be useful for the future. If surgery will hold any place at all beside newer pharmacological biologicals, the graft technique may remain of interest.

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Chapter 2.3

Influence of intraoperative course on visual outcome after an RPE-choroid translocation

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Invest Ophthalmol Vis Sci 2008;49:758-61

Abstract

Purpose

In a previous study, preoperative variables were correlated with postoperative visual outcome after the translocation of a free RPE-choroid graft. The present study was conducted to investigate whether the intraoperative course was an independent factor influencing visual outcome in these patients.

Methods

This was a prospective interventional case series of 48 patients with exudative AMD treated with an RPE-choroid translocation. Preoperative and postoperative evaluation included ETDRS visual acuity (VA) and fixation testing by a masked examiner. Four critical surgical steps were evaluated, and the intraoperative course was graded from 0 (uncomplicated surgery) to 5 (most complicated surgery). The relationship between intraoperative course adjusted for preoperative delay/lesion composition and visual outcome at 3 months and 1 year after surgery was analyzed with multivariate analysis.

Results

The mean VA (logMAR) improved slightly from 0.99 before surgery to 1.00, 0.94, 0.89 and 0.91 after 3, 6, 9 and 12 months, respectively. Foveal fixation on the graft was present in 34 (71%) of the eyes at 1 year after surgery. The intraoperative course was statistically significantly associated with the ΔVA (logMAR) at 3 months (P = 0.037) and at 1 year after surgery (P = 0.020) and if measured as gain or loss of ≥ 2 ETDRS-lines (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.7 to 2.8, P = 0.027), and ≥ 3 ETDRS-lines (OR, 2.2, 95% CI 1.9-3.5, P = 0.003); better surgery was associated with visual gain whereas eventful surgery was associated with visual loss.

Conclusions

The intraoperative course adjusted for preoperative variables had a statistically significant influence on postoperative visual outcomes in patients treated with a free RPE-choroid translocation**.** Refining the surgery could improve results.

Introduction

Despite the availability of anti-VEGF drugs in the treatment of exudative age-related macular degeneration (AMD), there may still be a place for surgery through a retinal pigment epithelium (RPE)-choroid translocation (Figs. 1, 2).[1-3] This surgery should be available to patients whose conditions are not treatable or who are no longer responding to treatment, such as anti-VEGF or photodynamic therapy (PDT).

A study in 84 patients with a follow-up of more than 1 year revealed that RPE-choroid translocation might stabilize or even slightly improve mean visual acuity (VA) up to 4 years after surgery.[4] Of the preoperative variables (VA, duration of visual loss, lesion composition and size, percentage of blood), only predominantly classic and occult lesions were related to statistically significant better visual outcome 1 year after surgery.[4] We hypothesized, however, that other factors might influence visual outcome after surgery.

Therefore, the aim of this study was to investigate whether the intraoperative course might be an independent factor correlating with visual outcome after RPE-choroid translocation for exudative AMD.

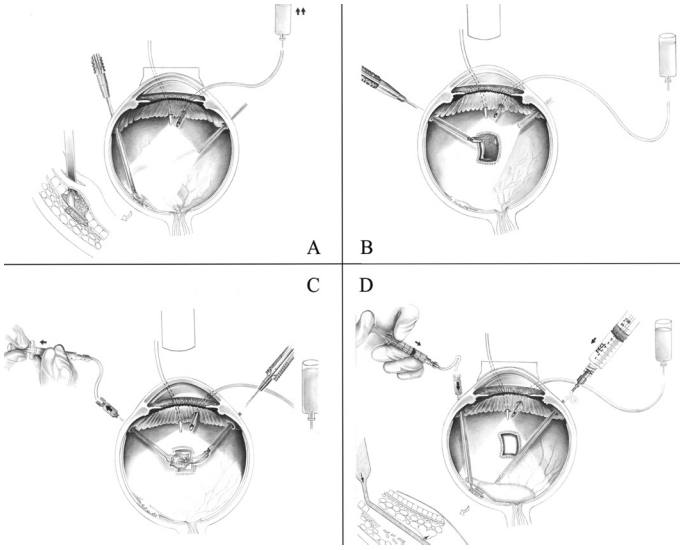

Figure 1. A drawing of the translocation of a free autologous full-thickness RPE-choroid graft in patients with exudative AMD. (A) Extraction of the subfoveal neovascular membrane. (B) The graft is cut out and prepared from the sclera in an area demarcated with coagulations in the midperiphery. (C) Loading of the graft on a spatula or grasped with forceps (not shown). (D) Insertion of the graft in the submacular space with simultaneous injection of perfluorocarbon liquid. Medical art work by Drs J. Leenen.

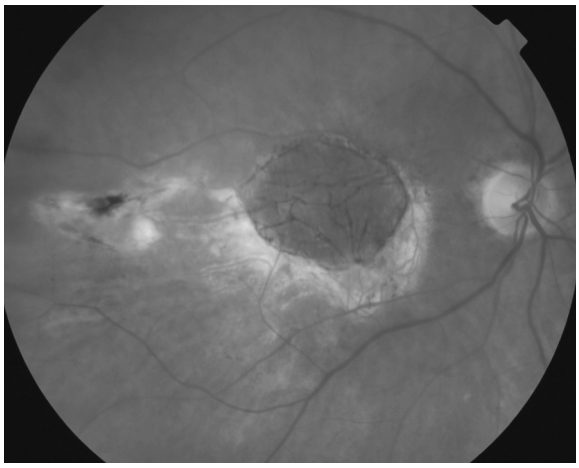

Figure 2. Color fundus photograph of the right eye showing an RPE-choroid graft at 12 months after surgery (VA 20/125). Color figure can be found in the appendix. See page 200.

Patients and Methods

Patients

An RPE-choroid translocation was performed in 48 eyes of 48 consecutive patients with exudative AMD (30 woman, 18 men) aged 79 ± 7 (mean ± SD) years (range, 63-96 years) between October 2005 and April 2006 by the same surgeon (JvM). At the time of analysis, all patients had been followed up for at least 1 year after surgery.

Patients were eligible if they had subfoveal choroidal neovascularization (CNV) membrane, with or without submacular blood, not treatable by or not responding to other modalities available at that time in our hospital: laser, PDT (in the Netherlands, this was only available for patients with predominantly classic lesions), or intravitreal bevacizumab (Avastin [Genentech, South San Francisco, CA], available March 2006). Exclusion criteria for surgery included choroidal neovascularization (CNV) with an etiology other than AMD, visual acuity (VA) >20/63, and history of symptomatic visual loss for more than 6 months. The Institutional Review Board of the Rotterdam Eye Hospital approved the study; informed consent was obtained from all patients in accordance with the ethical standards laid down in the Declaration of Helsinki.

Preoperative examination included best-corrected ETDRS VA (Snellen and logMAR equivalents), dilated funduscopy, and fluorescein angiography (FA). Postoperative visits were scheduled at 1, 3 and 6 weeks and at 3, 6, 9, and 12 months. During each visit, an

examiner masked to the intraoperative course performed best-corrected ETDRS VA testing and determined foveal fixation on the graft with biomicroscopy.

Grading of preoperative images

A reader (TM) masked for all other preoperative, intraoperative, and postoperative variables assessed the preoperative color fundus pictures and FAs. Lesion composition (predominantly/minimally classic or occult) was classified with FA according to the Macular Photocoagulation Study (MPS).[5] If a lesion was covered with an extensive hemorrhage (≥50% of the lesion), it was labeled a hemorrhagic lesion.

Surgery

After the induction of a posterior vitreous detachment, complete vitrectomy was performed.

The CNV was removed from the subretinal space with a Thomas subretinal forceps through a paramacular retinotomy in the temporal raphe (Fig. 1A). After circular heavy diathermia in the midperiphery at the 12 o'clock position and removal of the retina within the diathermia marks, vitreous scissors were used to cut a full-thickness graft of RPE-choroid of approximately 2 x 2 mm (Fig. 1B). The graft was grasped from the choroidal site with forceps (Fig. 1C) and repositioned under the macula through the existing paramacular retinotomy (Fig. 1D). Perfluorocarbon liquid was injected to keep the graft in place and to facilitate the release of the graft when retracting the instrument. The midperipheral donor site was surrounded with laser coagulation, followed by a silicone oil tamponade. In a second procedure, approximately 3 months later, the silicone oil was removed. In patients with phacic lenses, lensectomy was performed during the first surgery, and the intraocular lens (IOL) was inserted during the second surgery.

Grading of intraoperative course

Immediately after surgery, the surgeon graded the intraoperative course. Variables included in the grading process were removal of the entire CNV membrane in one extraction, without the need for further manipulation to extract the remaining fibrovascular tissue (yes, 0; no, 1); subretinal insertion of the RPE-choroid graft in one attempt (yes, 0; no, 1); submacular manipulation with a cannula to reposition or flatten the RPE-choroid graft (no, 0; one focused manipulation, 1; more than one manipulation needed, 2); and intraoperative submacular choroidal bleeding (no, 0; yes, 1).

These four variables were scored and added, resulting in an intraoperative course grade from 0 (ideal procedure) to 5 (complicated surgery).

Statistical analysis

Ordinary multiple linear regression analysis and multiple ordinal logistic regression analysis were used to analyze the relation between intraoperative course (grades 0-5, nominal values) adjusted for preoperative delay (i.e., time between onset of symptoms and surgery in weeks) and lesion composition (minimally classic, predominantly classic, occult, or hemorrhagic). Outcome variables were Δ VA (logMAR) at 3 months after surgery; Δ VA (logMAR) at 1 year after surgery; and ordinal categorical outcome variables "change of ≥ 2 or 3 ETDRS lines" defined as –1 (≥2 or ≥3 lines loss), 0 (< 2 or < 3 lines loss or gain [i.e., no change]), and 1 (≥2 or ≥3 lines gain) at 1 year after surgery.

To evaluate whether submacular manipulation during surgery could have influenced macular function while it excluded other postoperative complications related to the surgery that influenced the macula, patients with postoperative retinal detachment (RD) over the macula were excluded in a second analysis. All analyses were performed using SPSS (Windows version 12.0; SPSS Inc., Chicago, IL).

Results

Patients

Mean preoperative VA was 0.99 logMAR (range, 1/60-20/63). Mean postoperative VA was 1.00, 0.94, 0.89, and 0.91 logMAR after 3, 6, 9, and 12 months, respectively (Fig. 3). Four of the 48 patients had a preoperative VA ≥20/80, and eight had a VA ≥20/80 at 1 year after surgery. Foveal fixation on the graft was present in 71% (34 of 48) of the eyes up to the last examination.

According to the MPS criteria, two patients had a predominantly classic CNV, seven had a minimally classic CNV, 10 had occult CNV, and 29 had hemorrhagic lesion (≥50% blood).

Intraoperative course

Surgery was uneventful (score 0) in only six patients. No patient had a maximal possible score of 5 (Table 1).

Complications encountered during surgery (but not used for grading of the intraoperative course) consisted of a CNV membrane attached to the retina (n = 1), RPE damage on the graft (n = 4), entry site retinal tear (n = 1), macula schisis caused by fluid injection (n = 1), choroidal detachment (n = 1), local RD at the entry site tear (n = 1),and inadequate position or flattening of the graft (n = 3).

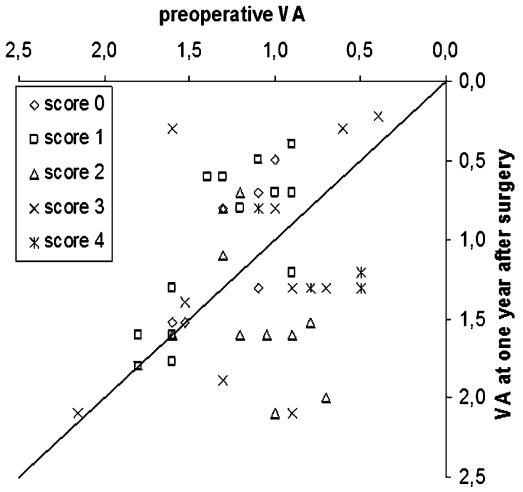

Figure 3. Preoperative VA versus VA at 1 year after surgery (logMAR). Patient group (n = 48) divided according to grading of intraoperative course (score 0 - 4). Data points above the diagonal line indicate patients with visual improvement.

Table 1. Grading of intraoperative course during an RPE-choroid translocation in patients with exudative AMD.

CNV removal in one piece	insertion of graft in one move	submacular manipulation of graft	submacular choroidal bleeding	total score
Yes (0) n = 42	Yes (0) n = 33	No (0) n = 11	No (0) n = 30	0 n = 6
				1 n = 13
No (1) n = 6	No (1) n = 15	One attempt (1) n = 20	Yes (1) n = 18	2 n = 11
				3 n = 14
		> one attempt (2) n = 17		4 n = 4
				5 n = 0

Complications after surgery

In 48 eyes, silicone oil was removed 4.6 ± 2.4 months after surgery. At the time of silicone oil removal in all but three patients, the inner limiting membrane was removed over the macula; four of these patients had a biomicroscopically manifest macular pucker.

RD resulting from proliferative vitreoretinopathy (PVR) and involving the macula developed in four patients before and in another six patients after silicone oil removal. Vision dropped to counting fingers in all these patients. Membrane peeling and a silicone oil (seven patients) or gas tamponade (three patients) were performed. In five patients, PVR-RD recurred after

revitrectomy. Revitrectomy was repeated and completed with a silicone oil (four patients) or gas tamponade (one patient). The silicone oil was still in five of the eyes at time of analysis. Rhegmatogenous RD originating from the retinotomy site developed in one patient after silicone oil removal. The vision remained stable after revitrectomy with a gas tamponade.

Four subretinal hemorrhages over or beside the graft and one vitreous hemorrhage occurred within 1 day after surgery, 2 after the RPE-choroid translocation, and 3 after silicone oil removal; all were considered related to the surgery.

CNV recurrence or persistence was detected angiographically in 8% (4 of 48) of the eyes from 3 to 12 months after surgery. Two patients were treated with bevacizumab, but in two patients no treatment had been initiated at the time of analysis.

Statistical analysis

Ordinary multiple linear regression and multiple ordinal logistic regression analysis revealed a statistically significant effect of intraoperative course on all the outcome variables adjusted for preoperative delay and lesion composition: Δ VA (logMAR) at 3 months after surgery (r^2 = 0.201; *P* = 0.037) and at 1 year after surgery (r^2 = 0.239; *P* = 0.020), change of ≥2 ETDRS lines (OR, 1.8; 95% CI, 1.7-2.8; *P* = 0.027), and change of ≥3 ETDRS lines (OR, 2.2; 95% CI, 1.9-3.5; *P* = 0.003). The effect showed that an increased number of intraoperative complications resulted in a lower postoperative visual outcome.

Some of the four lesion composition groups had low frequencies. Therefore, all analyses were repeated with the lesion groups *minimally classic, predominantly classic* and *occult* combined (n = 19), and results were compared with those of the hemorrhagic lesion group (n = 29). However, the results were the same as in the four separate lesion composition groups.

In the second analysis, the 10 patients with RD caused by PVR and involving the macula were excluded. The intraoperative course remained statistically significantly related to ΔVA at 3 months after surgery (r^2 = 0.317; *P* = 0.014) and to a change of ≥3 ETDRS lines (OR, 1.9; 95% CI, 1.8-3.1; *P* = 0.016) 1 year after surgery. De relation was not (though it was almost) significant with the outcome variables ΔVA (r^2 = 0.252; *P* = 0.052) and a change of ≥2 ETDRS lines (OR, 1.6; 95% CI, 1.5-2.6; *P* = 0.083) 1 year after surgery.

There was no relation between between lesion composition (minimally or predominantly classic, occult, or hemorrhagic) and intraoperative course (*P* = 0.98; Kruskal-Wallis test).

Discussion

This study showed a statistically significant relation between the intraoperative course of and the postoperative visual outcome after RPE-choroid translocation in patients with exudative AMD, suggesting that an improved surgical technique may help to improve the visual outcome.[6] This assumption is further supported by the poor long-term visual results in the first series of patients with a free RPE-choroid graft, in whom the graft was harvested from the juxtafoveal site.[2,7] This technique involved repeated manipulation to the macular area. Moreover, the adjacent RPE and choroid might have been affected by the often longstanding macular degeneration. Taking the graft from the midperiphery, however, decreased submacular surgical manipulations and their associated trauma, which may explain the sustained visual gain in several patients with the latter technique.[3,4,6]

In addition to direct tissue damage, manipulation of the graft or subfoveal site causes dispersion and proliferation of RPE cells, which may increase the risk for PVR formation.[8,9]

Intraoperative variable submacular choroidal bleeding was taken into account because such bleeding may increase intraoperative manipulations and shearing forces when clot removal is attempted, whereas the remaining blood may damage the retina by iron toxicity or by creating a diffusion barrier and may have a possible negative effect on graft revascularization.[10]

Given the high rate of PVR, it was unclear whether the relation between intraoperative course and postoperative visual outcome resulted from better macular function in patients with better intraoperative courses or by the greater number of postoperative complications in patients with worse intraoperative courses. Therefore, all analysis were repeated, excluding data on patients with RD involving the macula caused by PVR. However, there was still a statistically significant relation between intraoperative course and postoperative visual outcome. These data support the hypothesis of MacLaren et al. that late apoptosis of the photoreceptors and subsequent RPE graft failure may be a result not only of the disease process itself but of the trauma initiated by the surgery.[7]

The relationship between the intraoperative course and visual outcome was adjusted for preoperative variables as duration of visual loss and type of lesion composition. A previous study showed that lesion composition (predominantly classic and occult lesions did better than hemorrhagic and minimally classic ones) but not duration had a statistically significant influence on visual outcome after 1 year.[4] The present study confirmed that the intraoperative course probably acted as a confounding factor.

The CNV recurrence rate of 8% reported in this study might have been underestimated because fluorescein angiography was performed only if recurrent neovascularization was

clinically suspected.

This study showed that the intraoperative course statistically significantly influenced postoperative visual outcome after RPE-choroid translocation. This prompts us to optimize the surgical technique and instrumentation delivered by our multidisciplinary team. Surgery should be a last resort for patients whose conditions are no longer treatable or who are not responding to approaches that entail fewer potential complications, such as anti-VEGF or PDT.

With an optimized technique, RPE-choroid translocation may in the future be combined with local pharmaceutical therapeutics or gene or precursor cell transfer techniques.[11,12]

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Chapter 2.4

Retinal pigment epithelium (RPE)–choroid graft translocation in the treatment of an RPE tear: preliminary results

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Abstract

Purpose

To investigate whether retinal pigment epithelium (RPE)-choroid translocation would be a suitable treatment for RPE tears, which have a poor prognosis and are encountered more often since the introduction of anti-vascular endothelial growth factor (VEGF) therapy for exudative age-related macular degeneration (AMD).

Methods

Prospective interventional case series of six eyes of six AMD patients with an RPE tear treated with an RPE-choroid translocation. The RPE tear occurred in a vascularised pigment epithelium detachment in four patients and after treatment in the other two. Preoperative and postoperative evaluation included ETDRS visual acuity (VA) and fixation testing. The follow-up period ranged from 6 months to 2 years.

Results

The mean preoperative VA was 20/160 (range 20/400-20/80). The mean VA at the last examination after surgery was 20/80 (range 1/60-20/50). One of the six patients had a preoperative VA of ≥20/80, and four had a VA of 20/80 or better at their last examination. Foveal fixation on the graft was present in five out of the six eyes up to the last examination.

Conclusion

These preliminary data show that an RPE-choroid translocation may be a treatment option for patients with an RPE tear.

Introduction

A tear of the retinal pigment epithelium (RPE) may occur spontaneously (most commonly in the setting of a vascularised RPE detachment) or after treatments such as laser, photodynamic therapy (PDT) or, more recently, anti-vascular endothelial growth factor (VEGF) treatment.[1, 2]

There is a sudden onset of distortion and a decrease in central vision in most, but not all, of the patients in whom the RPE tear involves the fovea.[3,4] If the RPE tear is not subfoveal, visual acuity (VA) remains generally quite good shortly after onset.[4] However, after more than 2 years, both patients groups often suffer vision loss with a VA of 20/200 or less.[4-6] This poor prognosis is due to progressive scarring of the fibrovascular tissue.[1]

Photoreceptor viability depends on contact with the RPE. The mechanical disruption of this interface cannot be reversed with anti-VEGF treatment or PDT. However, creating a fresh layer of intact Bruch's membrane and RPE underneath the fovea might prevent or delay this long-term visual loss. Therefore, the surgical technique of RPE-choroid translocation, as used in patients with exudative age-related macular degeneration (AMD) to restore the subfoveal layers after removal of choroidal neovascularisation (CNV), may be a suitable approach.[7-9]

This study presents the data of the first six patients with an RPE tear treated with an RPE-choroid translocation.

Patients and Methods

Patients

An RPE-choroid translocation was performed in six eyes of six patients with an RPE tear (four female, two male; mean (SD) age 79 (4) years (range 71-82)) between June 2004 and October 2006 by the same surgeon (JvM). All had a history of exudative AMD. Four patients had spontaneous RPE tears associated with CNV, and in the other two a tear had developed after treatment (6 weeks after the second bevacizumab (Avastin) injection in one patient and 5 weeks after PDT in the other) (Table 1).

Preoperative examination included best-corrected ETDRS VA, dilated funduscopy and fluorescein angiography. Postoperative visits were scheduled at 1, 3 and 6 weeks, and at 3, 6, 9,12, 18 and 24 months. During each visit, best-corrected ETDRS VA and foveal fixation on the graft with biomicroscopy were examined. Postoperative fluorescein angiography was only performed if a recurrent CNV was suspected. At the time of analysis, the patients had a mean

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(SD) follow-up of 14 (6) months (range 6-24) (Table 1).

The removed CNV membrane had been histopathologically analysed in three patients (patient 1, 3 and 4; Table 1).

The institutional review board of the Rotterdam Eye Hospital approved the study. Informed consent was obtained from all patients in accordance with the ethics standards laid down in the Declaration of Helsinki.

Table 1. Clinical characteristics of the six patients with a retinal pigment epithelium (RPE) tear treated with an RPE-choroid translocation. CNV, choroidal neovascularisation; Delay, time between onset of RPE tear and surgery; PDT, photodynamic therapy; VA, visual acuity.

	1	2	3	4	5	6
M/F	M	F	M	F	F	F
Age (years)	82	71	82	81	80	79
Before surgery						
Treatment	No	No	2xAvastin	1xPDT	No	No
VA	20/100	20/400	20/250	20/400	20/80	20/160
Foveal fixation	Unknown	Yes	No	No	Yes	Yes
Size of RPE tear (disc areas)	1.7	7.3	1.5	3.2	3.0	3.2
Delay	9 days	3 months	5 weeks	3 weeks	5 months	6 months
Histology of removed CNV membrane	Yes	No	Yes	Yes	No	No
After surgery						
Follow-up	2 years	18 months	1 year	1 year	1 year	6 months
Best VA	20/63	20/50	20/63	20/200	20/63	20/50
Last VA	1/60	20/50	20/63	20/250	20/63	20/80
Foveal fixation	Lost	Yes	Yes	Yes	Yes	Yes

Surgery

After induction of a posterior vitreous detachment, a complete vitrectomy was performed.

The CNV membrane was removed from the subretinal space with Thomas subretinal forceps through a paramacular retinotomy in the temporal raphe. In selected patients, the removed tissue was fixed in formaldehyde, and the tissue sections were stained with H&E and periodic acid/Schiff.

After circular heavy diathermia in the midperiphery at the 12 o'clock position, vitreous scissors were used to cut a full-thickness graft of retina, RPE and choroid of approximately 2 x 2 mm. The graft was grasped from the choroidal side with modified subretinal forceps (Dutch Ophthalmic Research Center (DORC), Zuidland, The Netherlands), and the retina

was removed just before the graft was repositioned under the macula through the existing paramacular retinotomy. Perfluorocarbon liquid was injected, covering the macula, to keep the graft in place and facilitate release of the graft when the instrument was retracted. The mid-peripheral donor site was surrounded with laser coagulation followed by a silicone oil tamponade. In a second procedure, ~ 3 months later, the silicone oil was removed. Lensectomy and insertion of an intraocular lens were performed during the first or second surgery in phakic patients.

Results

Baseline characteristics

All patients had the pathognomic features of an RPE tear on biomicroscopy and fluorescein angiography (Fig. 1). All tears were centered on the fovea and covered 3.3 (2.1) (mean (SD)) disc areas (Table 1). In patients 1 and 3, the fovea was just above the border of folded RPE over bare choroid, and in the other four patients it was located above the folded RPE.

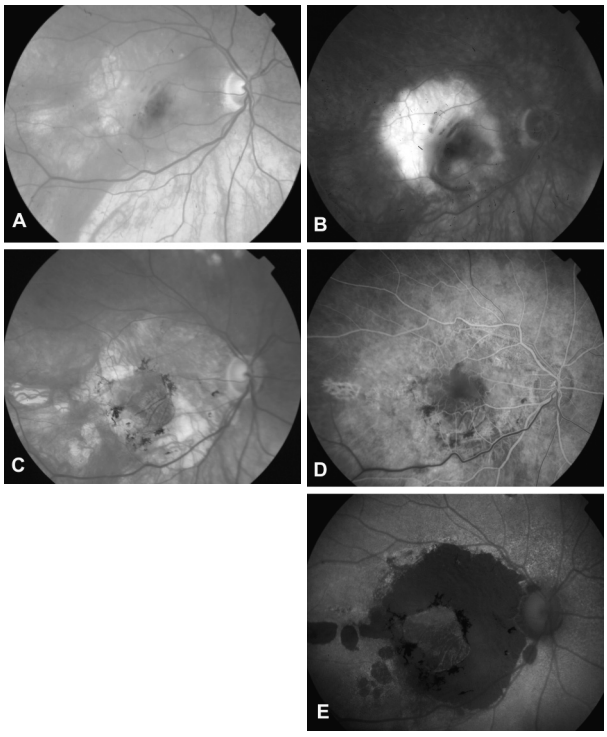

Figure 1. Preoperative fundus image (A) and fluorescein angiogram (B) of an RPE tear in a patient with exudative age-related macular degeneration (patient 2; Table 1). The dark and light bands represent the folded RPE, with temporal from it the hyperfluorescent window defect corresponding to the bare choriocapillaris. Hypofluorecence was observed nasal to the RPE folds due to the retracted RPE. (C) Postoperative fundus image, (D) fluorescein angiogram and (E) and autofluorescence of the RPE-choroid graft at 18 months after surgery of the same patient, with a visual acuity of 20/50. (C) RPE-choroid graft adjacent to areas of atrophic RPE and pigment accumulations. (D) Early fluorescence of the graft, which indicates its perfusion. (E) Black (non-autofluorescent) and hypo-autofluorescent areas surrounding the graft correspond to, respectively, pigment accumulations and atrophic RPE. Patient consent was obtained for publication of this figure. Color figure can be found in the appendix. See page 200.

In the four patients with a spontaneous RPE tear, the tearing had occurred along the margin of a pigment epithelium detachment.

None of the patients had significant cataract or submacular haemorrhage at presentation.

The histopathology of the removed CNV membranes in three patients revealed alternating atrophic and hyperplastic RPE cells, extensive basal laminar deposits and fibrovascular membranes in al three (Fig. 2). The fibrotic membrane of patient 3 (the patient previously treated with Avastin) also contained blood vessels and signs of inflammation.

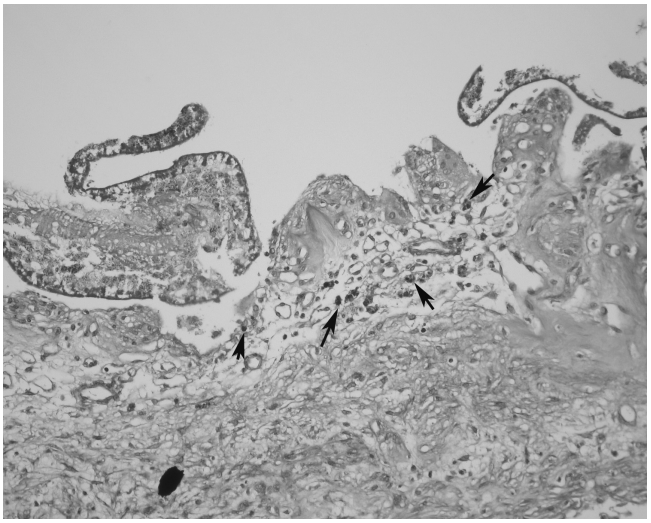

Figure 2. Histology of a retinal pigment epithelium (RPE) tear stained with periodic acid/ Schiff. Retracted and folded RPE borders bare choroid (the choriocapillaris has disappeared). The basal laminar deposit stains positive (arrows). There is a fibrovascular membrane present underneath the RPE. Patient consent was obtained for publication of this figure. Color figure can be found in the appendix. See page 200.

Visual outcome

Mean preoperative VA was 20/160 (range 20/400-20/80). The mean VA at the last examination was 20/80 (range 1/60-20/50)(Table 1). One of the six patients had a preoperative VA of ≥20/80, and four had a VA of 20/80 or better at their last examination. Foveal fixation on the graft was present in five out of the six eyes up to the last examination (Table 1).

Imaging

Preoperative imaging with colour fundus photography and fluorescein angiography showed typical RPE tear characteristics in all patients (Fig. 1). After surgery, the RPE grafts had the same appearance as previously seen in patients with AMD without an RPE tear treated

with an RPE-choroid translocation (Fig. 1).[9] Pigment accumulations alongside the grafts increased with time, and the RPE on the grafts revealed autofluorescence (Fig. 1). Early-phase fluorescein angiograms, obtained in the two patients with suspicion of a recurrent CNV, showed early fluorescence of the graft, a sign of perfusion (Fig. 1).[10]

Complications after surgery

In the six eyes, silicone oil was removed 3.3 (1.4) (mean (SD)) months after surgery. At this time, the inner limiting membrane was removed over the macula in all patients, and in two patients there was a biomicroscopically manifest macular pucker present.

A CNV recurrence or persistence was clinically suspected in two patients because of the presence of subretinal fluid. Subsequent fluorescein angiography did not show any sign of CNV in patient 2 (Fig. 1), but confirmed the diagnosis in the first patient at 3 months after surgery. This patient had foveal fixation with a VA of 20/63 before the CNV recurrence. During follow-up, a fibrotic membrane developed over the RPE-choroid graft and vision decreased to counting fingers with loss of foveal fixation.

Discussion

This study investigated RPE-choroid translocation as a treatment option for patients with an RPE tear. The visual outcome was favourable (mean VA of 20/80 at 13 (7) months after surgery) compared to the natural course as reported on other series (mean VA of 20/200 or worse).[4-6] We assume that the visual improvement was attributable to the translocation procedure, as no other variables, eg, significant cataract or submacular haemorrhage, influenced VA before surgery.

The visual outcome also appeared favourable compared with a study of patients with exudative AMD treated with an RPE-choroid translocation (mean VA of 20/160 after 1 and 2 years).[9] This is probably explained by the sudden onset of vision loss in patients with an RPE tear, with a consequently shorter preoperative delay and history of visual loss and retinal damage. Gibran et al. recently described a patient with exudative AMD treated with 360^0 macular translocation surgery. The histopathological evaluation of the excised CNV membrane revealed an RPE tear.[11] At 4 months after surgery, the patient had a VA of 20/80. However, we prefer to use the RPE-choroid translocation, as this surgery is less complex and time-consuming, with potentially fewer postoperative complications and without the need for counter-rotation of the globe.[9,12-15]

Although the number of patients with an RPE tear treated so far by these two surgical approaches is limited, these preliminary data show the potential to improve VA by creating a fresh layer of choroid, choriocapillaris, Bruch's membrane and RPE underneath the fovea in patients with an RPE tear.

Several mechanisms are involved in the pathogenesis of an RPE tear in patients with exudative AMD. An important role is played by the development of basal laminar deposits between Bruch's membrane and RPE layer, and drusen between Bruch's membrane and the inner collagenous layer of Bruch's membrane.[16] These deposits form a potential plane of cleavage.[17,18] Moreover, these deposits are hydrophobic and impede the passage of fluid at Bruch's membrane.[1] This may cause a serous pigment epithelium detachment even in the absence of CNV. CNV may also directly cause separation of the RPE with its basement membrane from the outer layers of Bruch's membrane by invasion of new vessels. Subsequent fibrotic contractile forces on the detached RPE may cause a tear. In our three excised CNV membranes, the extensively present basal laminar deposits as well as the CNVs could have contributed to the RPE tear.

In the six patients presented in this study, the lesion area of the RPE tear was located underneath the fovea. To replace this lesion area with an intact RPE-choroid layer by graft translocation is of particular value in this patient group. However, an RPE-choroid graft translocation may also be of use in patients with RPE tears that do not yet involve the fovea, as the folded RPE and fibrovascular membrane progressively affect foveal function by contraction and scarring.[4]

RPE tears are observed after intravitreal anti-VEGF injections in the treatment of exudative AMD.[12] This may be explained by tractional forces caused by a contracting CNV membrane or interruption of tight junction maintenance by VEGF.[19] It has recently been shown that it is not only VEGF, but also its relation to transforming growth factor (TGF-β1) and connective tissue growth factor (CTGF), that determines fibrovascular growth-that is, a decrease in VEGF resulted in upregulation of mRNA concentrations and subsequent protein expression of TGF-β1 and CTGF.[20] We hypothesise that an injection of anti-VEGF may disturb the VEGF/TGF-β1 and CTGF balance and contribute to a fibrovascular membrane contraction.

In conclusion, this study shows that an RPE-choroid graft translocation may be a treatment option for RPE tears.

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Chapter 2.5

Angiographic evidence for revascularization of an RPE-choroid graft in patients with age-related macular degeneration

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Abstract

Purpose

To study graft perfusion using fluorescein angiography (FA) and indocyanine green angiography (ICG) after the translocation of an autologous retinal pigment epithelium (RPE)-choroid graft in patients with exudative age-related macular degeneration (AMD).

Methods

Retrospective observational case series of 31 patients with AMD who had FA and/or ICG performed after an RPE-choroid graft translocation. The FAs (n = 25) and ICGs (n = 23) were assessed by an independent masked reader for the presence of early fluorescence of the graft in FA, and for perfusion of the choroidal vessels of the graft and recipient bed in ICG.

Results

Early fluorescence of the graft was present in 23 of the 25 FAs. Perfusion of the graft vasculature was observed in 12 of the 23 ICGs. The two grafts that lacked early fluorescence in FA also had no signs of choroidal perfusion of the graft and the recipient bed with ICG.

Conclusion

Revascularization of the RPE-choroid graft was observed in all but 2 of the 31 patients either by early fluorescence of the graft by FA or by identification of perfused choroidal graft vessels with ICG from 1 week up to 3 years after surgery. For assessment of revascularization of the graft evaluation of the early phase of the FA is recommended.

Introduction

In industrialized countries, age-related macular degeneration (AMD) is the principal cause of irreversible legal blindness in elderly persons.[1-3] In the exudative form, the most promising treatment modality is pharmacological agents. Recently, anti-VEGF treatment has for the first time showed visual improvement.[4-6] At present, surgery is used in our hospital as a last resort option for patients with AMD not treatable or no longer responding to these pharmaceutical agents or other approaches with less potential complications like photodynamic therapy (PDT). During this surgery the choroidal neovascular (CNV) membrane is removed and a free autologous graft consisting of a sheet of retinal pigment epithelium (RPE) on its own substratum (Bruch membrane, choriocapillaris, and choroid) is translocated to the subfoveal site. This technique, first reported by Peyman et al and taken up by Stanga et al, was modified by harvesting the graft from the midperiphery instead of the paramacular region.[7-8] RPE-choroid graft translocation is used in an increasing number of ophthalmologic centers (currently 12) and its feasibility has been further documented.[9-12] In this study, the postoperative angiographic data of patients with exudative AMD treated with an RPE-choroid graft translocation were evaluated for evidence of revascularization of the graft.

Methods

Subjects

Thirty-one patients with exudative AMD treated with an RPE-choroid graft translocation with a follow-up period of 1 year or more, and who had postoperative fluorescein angiography (FA) and/or indocyanine green angiography (ICG), were included in this study. In this surgery, initial surgery with removal of the neovascular membrane with subsequent insertion of the RPE-choroid graft (as previously described by van Meurs et al) is followed by silicone oil removal after 3-4 months, at which time ICG-assisted ILM removal is routinely performed.[9,10]

The eligibility criterion for surgery was a subfoveal CNV secondary to AMD, independent of lesion size or type (minimally/predominantly classic or occult), presence of submacular blood or pigment epithelium detachment. The subfoveal CNV lesions were not treatable by other modalities available at that time in our hospital (laser or PDT). Exclusion criterion for surgery was a visual acuity (VA) of ≥0.6 logMAR (20/80 Snellen acuity). The mean age was 79 years (range 59-93 years).

Preoperative examination included best-corrected ETDRS vision, dilated funduscopy and FA and/or ICG. During each postoperative visit, best-corrected ETDRS vision testing was performed. Color fundus pictures were taken 1, 2, and 3 years after surgery. Postoperative FA or ICG were performed in these 31 (out of 150) eyes because pathology was suspected in either eye (surgically treated or not).

The Institutional Review Board of the Rotterdam Eye Hospital approved this surgical trial, and all patients signed an informed consent form. Surgeries were performed between October 2001 and August 2005. The censoring date of this study was August 2006.

Postoperative images

FA, ICG, and fundus images were captured as digital images on a TOPCON TRC 50IA fundus camera attached to a MegaPlus II interline camera (Redlake, Tucson, AZ) and connected to an IMAGEnet 2000 system (TOPCON, Tokyo, Japan).

A reader (P.v.d.B.) masked to the pre-, intra-, and postoperative data of these patients evaluated the postoperative FAs, ICGs, and color fundus photographs. To study the perfusion of the graft, the early fluorescence in FA in the graft area was compared with the surrounding normal fundus and graded as present or absent. The early phase of the ICG was used to study the choroidal vessels in the graft area, and such vessels were graded as present or absent. If there were vessels present in the graft area, they were examined to see if they ran parallel to one another, forming a ladder-like pattern.

Early fluorescence in FA and/or a parallel orientation of the vascular pattern on ICG at the site of the graft were considered to be an indication of perfusion. Fundus images were compared with FA and ICG angiograms in each patient to assess the influence of graft pigmentation on the appearance of the angiograms.

The results of the evaluation of the FAs and ICGs were checked for consistency for each patient and correlated with postoperative best-corrected visual acuity (BCVA).

Results

Clinical Results

The mean preoperative BCVA of the 31 study patients was 0.94 logMAR (0.11 Snellen acuity). The postoperative BCVA was 0.77 (n = 31), 0.74 (n = 19), and 0.70 (n = 10) logMAR (Snellen acuity of respectively 0.17, 0.18, and 0.20) after 1, 2, and 3 years, respectively.

When removing the silicone oil, some degree of retinal puckering was present and peeled

in 22 patients. Retinal detachment developed in 2 patients (of 31); 1 due to proliferative vitreoretinopathy and 1 with a rhegmatogenous cause. In 9 patients, recurrent or persistent CNV membranes were detected (at 2-27 months after surgery). A nonischemic central retinal vein occlusion occurred in 1 patient.

Imaging

Postoperative angiograms were obtained in all 31 patients: in 17 patients, both FA and ICG were obtained; in 8, FA only; and in 6, ICG only. Indications for performing a postoperative angiogram were suspicion of a central retinal vein occlusion (n = 1), an unexplained loss of VA (n = 3), and subretinal fluid and/or blood to exclude a recurrent or persistent choroidal membrane (n = 18). In 9 patients, the angiograms were primarily made because of suspicion of pathology in the fellow eye. FA was performed in 25 of the 31 patients (range 1 week-36 months, median 6.5 months after surgery). Early fluorescence at the site of the graft was seen in 23 (92%) of the 25 patients (Fig. 1). Angiograms of 2 patients (8%), obtained at 1 week and 4 months after surgery respectively, did not show graft fluorescence (Fig. 1E).

ICG was performed in 23 of the 31 patients (range 1 week-27 months, median 6 months after surgery). In 20 (87%) patients, choroidal vessels could be observed in the area of the graft. In 3 (13%) patients, these vessels were absent. In 12 of the 20 patients with visible choroidal vessels, at least part of the vascular pattern in the graft was parallel and was therefore presumed to be a component of the graft (Fig. 1A-C; see Discussion). The vascular pattern of the other eight patients was not attributable to the graft (Fig. 1D). Thus, 12 grafts showed evidence of perfusion, and a total of 11 grafts had no signs of perfusion on ICG.

Of the 17 patients with both FA and ICG, 8 patients showed evidence of reperfusion in both types of angiography at 6.8 ± 5.6 (range 1-17) months after surgery. The two patients without signs of perfusion with FA also had none with ICG at 1 week and 4 months after surgery (Fig. 1E). In seven patients, a discrepancy existed, with early fluorescence of the graft being detectable by FA, but reperfusion not evident with ICG at 8.0 ± 5.7 (range 2-15) months after surgery (Fig. 1D).

From studying the angiograms and fundus pictures, some additional observations were made. The late phase of the ICGs showed staining of the graft in five patients. Twenty-seven grafts had features of folding on the fundus pictures, as indicated by fine hyperpigmented lines over the graft. Those lines were also obvious using FA, but difficult to discern on ICG. Irregular accumulations of hyperpigmentation over, along, or beside the graft could be seen in 22 patients, appearing at 3 months (mean 8) after surgery and increasing with time. Except for this hyperpigmentation, the overall appearance of the graft did not change during follow-up.

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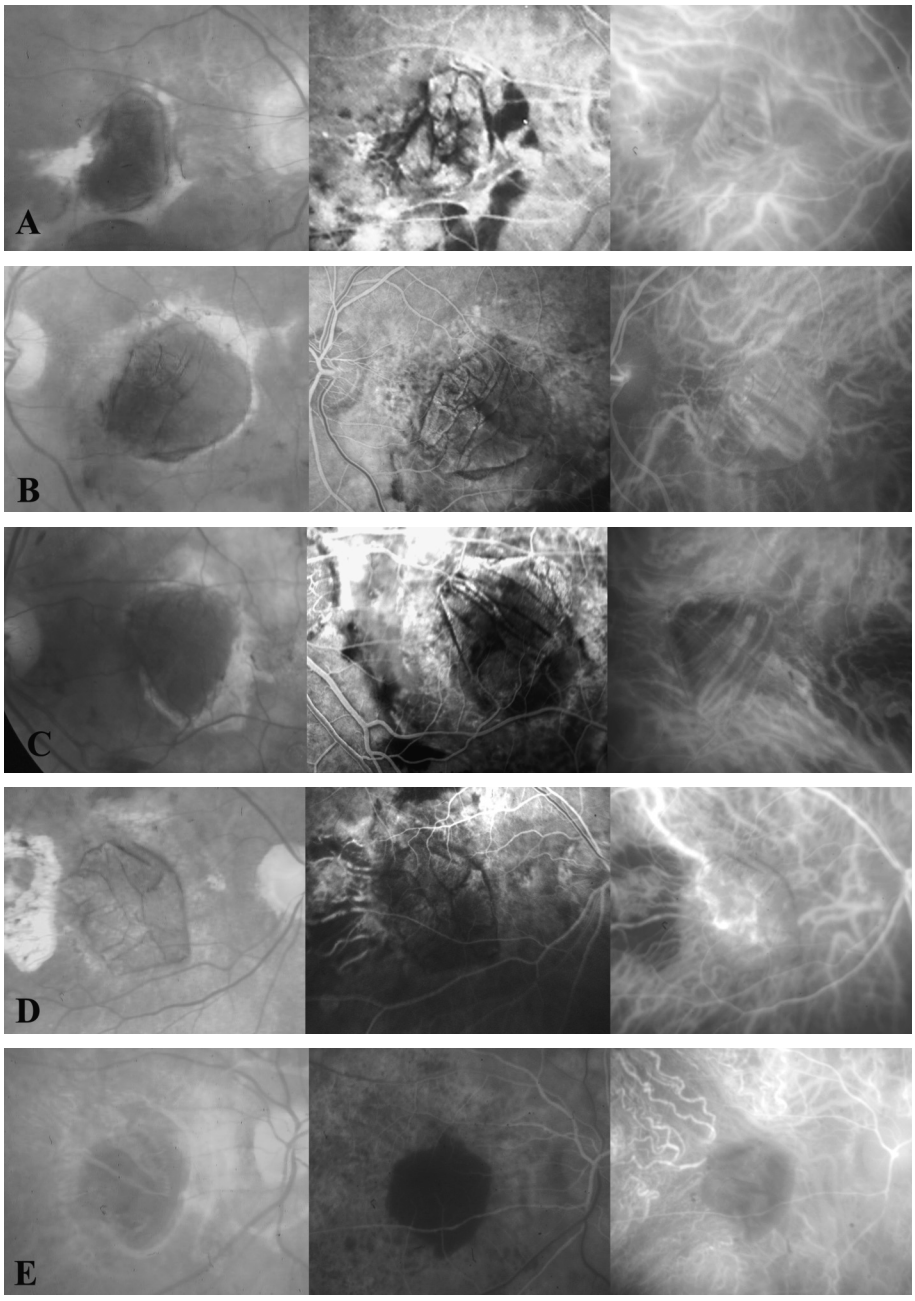

Figure 1. Color fundus photographs (left), early fluorescence angiograms (FA) (middle) and indocyanine green angiograms (ICG) (right) show retinal pigment epithelium-choroid grafts of five patients. (A, B, C) Three patients after 2 and 13 (n = 2) months after surgery with signs of perfusion of the graft by early fluorescence with FA and parallel oriented perfused graft vessels as observed with ICG. (D) A graft at 15 months after surgery which has signs of perfusion with FA, but the perfused vessels in the graft area as observed with ICG can not be attributed to the graft. (E) Nonperfused graft at 1 week after surgery. Color figure can be found in the appendix. See page 201.

Imaging and visual acuity

The 29 out of 31 patients with signs of perfusion of the graft with FA and/or ICG had a mean BCVA of 0.82 logMAR (0.15 Snellen acuity) at the time of imaging, compared to 1.40 and 1.80 logMAR (20/500 and 1/60 Snellen acuity) in the two patients without signs of perfusion. The VA of these two patients did not improve during follow-up.

Discussion

This study presents angiographic evidence for perfusion of autologous RPE-choroid grafts after CNV removal in patients with exudative AMD. Of the 31 patients in this study, 29 (94%) had signs of perfusion of the graft. The two patients whose grafts did not show early fluorescence on FA also showed no signs of perfusion using ICG (Fig. 1E). In these patients, no remnants of the original vascular bed were visible on ICG. The expected dysfunction of these two grafts correlated with the low BCVA of 1.4 and 1.8 logMAR (20/500 and 1/60 Snellen acuity) at the time of imaging.

The early phase of the FAs was used to assess perfusion of the graft. In this phase, almost all fluorescence originates from intravascular fluorescein in superficially located vessels.[13-17] Fluorescence from deeper layers is blocked by fluorescein and melanin in perfused grafts, and by melanin in non-perfused grafts.[13] One may conclude, therefore, that the graft is perfused if the fluorescence of the graft emerges simultaneously with the normal fundus outside the graft area.

In standard ICG, depth localization of vessels is not possible. However, since the parallel course of the vasculature of the choroid in the periphery, and thus in the graft, differs markedly from the radially arranged vascular system in the choroid in the macular area, recognition of the peripheral parallel vascular organization in the graft area is a sign of perfusion of the graft (Fig. 1A-C).[18,19]

In this study, seven patients showed evidence of graft perfusion by early fluorescence with FA and a satisfactory visual function, but an absence of signs of distinctly identifiable perfused graft vessels with ICG. This discrepancy might be due to the suboptimal resolution and contrast of our fundus camera (TOPCON TRC 50IA) as suggested by several studies.[20-24] Therefore, when standard fundus cameras are used, FA is probably the better technique for evaluating graft perfusion. Only the few institutes that have a high-speed camera will be able to study graft perfusion more precisely with ICG angiography.

The pigmentation of the graft on the fundus color photograph was evaluated, since

hypofluorescence with FA or ICG angiography can be caused by hyperpigmentation of the graft, and hypopigmented folds of the graft can be misinterpreted as blood vessels.

In five patients there was late, plaque-like staining of the graft seen by ICG resembling the appearance of choroidal neovascularization. This may be caused by immature or damaged endothelium in the vasculature of the graft that remains leaky for this albumen-bound dye.

Irregular accumulation of hyperpigmentation over, along, or beside the graft was observed in 22 patients and increased with time, which suggests ongoing remodeling of RPE.

Joussen et al, performing a similar grafting technique, reported reperfusion of the graft in 40 (95%) of 42 grafts. They presumed reperfusion of the graft when large choroidal vessels in the graft area were visible using ICG.[11] This definition is probably too broad, since these vessels can also belong to remnants of the original vascular bed.

MacLaren et al reported a 100% reperfusion rate in 4 patients, and looked for a "choriocapillary flush" with FA and ICG.[12] However, since the transit time from precapillary choroidal arterioles to postcapillary choroidal venules is very short, and the first bolus passage is relatively long in the choroid, this flush originates from choroidal arterioles, capillaries and venules alike. MacLaren's "choriocapillary flush" is, however, similar to our definition of early fluorescence in FA.

The mechanism of revascularization of free grafts has been studied in free skin transplants in patients and experimental models. After an early phase, in which plasma exudates from the recipient site, fills the lumina of the graft vessels, and supposedly supports vessel metabolism (imbibition phase), recirculation is achieved after 24-48 hours.[25-27] The initial revascularization is probably attributable to the early anastomosis (inosculation) between the preexisting graft and recipient vessels.[27] Neovascularization (vascular ingrowth from the recipient bed) and especially the replacement of the graft vasculature by endothelial and endothelial progenitor cells from the recipient bed are the other two processes involved in graft revascularization.[28]

In our study, 12 grafts had a vascular pattern that was similar to that of the choroidal periphery (Fig. 1A-C), suggesting that vessels of the recipient bed connected to existing graft vessels or that neovascular ingrowth may follow the choroidal lumina of the graft as described in free skin grafts. Determining the exact timing of revascularization/reperfusion of the RPE-choroid graft in our patients would require repeated postoperative angiographies, starting as early as 1 day after surgery. Such repeated angiograms, however, were not considered to be in the patients' best interest by our Institutional Review Board.

Revascularization of the RPE-choroid graft has been confirmed histologically in a pig model.[29] In this animal model, the graft was revascularized by vertically bridging vessels that connected the graft to the recipient bed. The pig model, however, proved surgically challenging and was

not suitable to study the sequence of revascularization timing.[29]

Revascularization and survival of RPE-choroid grafts is likely to depend on the presence of remnants of the recipient vascular bed underneath the graft. Thus, nonperfusion of two of the grafts could be explained by the complete removal of the original vascular bed during surgery, as shown with ICG. However, in seven grafts with no signs of remnants of the original vascular bed with ICG, the graft appeared perfused in all with FA, and perfusion of individual vessels of the graft was observed in six patients using ICG. This suggests that the graft in these cases was horizontally reconnected to the surrounding choroidal tissue and that this type of reconnection failed in the two nonperfused grafts.

Only a nonperfused graft with angiography predicts a poor visual outcome. However, one cannot predict the functional status of the graft when angiographic sings of graft perfusion are present, which is the case in the majority of the patients.

In conclusion, FA and ICG angiography demonstrated reperfusion of the RPE-choroid graft in 29 of the 31 patients (94%) when analyzed 1 week to 36 months after surgery. To study graft revascularization, analysis of the early phase of the FA is recommended.

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Chapter 2.6

Hyperfluorescence of the optic disc with indocyanine green angiography

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Eye 2008; in press

Abstract

Purpose

One fourth of the patients with exudative age-related macular degeneration (AMD) treated with an autologous retinal pigment epithelium (RPE)-choroid translocation had a hyperfluorescent optic disc with indocyanine green angiography (ICGA). This study aimed to identify whether indocyanine green (ICG)-assisted surgery was related to the hyperfluorescence of the optic disc with ICGA.

Methods

Retrospective observational case series of 31 AMD patients treated with an RPE-choroid translocation, and who had ICGA after surgery. The ICGAs were assessed for hypo/ iso/ hyperfluorescence of the optic disc, and fluoresence was related to the time interval between ICGA and the possible use of intravitreal ICG.

Results

The optic disc was hyperfluorescent in six patients, isofluorescent in one and hypofluorescent in 24 patients. All hyperfluorescent optic discs and seven of the 24 hypofluorescent optic discs were preceded with ICG-assisted surgery with a time interval of, respectively, 7 ± 3 weeks and 43 ± 12 weeks (P = 0.001, Student T-test). The other 17 hypofluorescent discs were not preceded by ICG-assisted surgery, and the one isofluorescent optic disc was observed 32 weeks after ICG-assisted ILM surgery.

Conclusion

There was a statistically significant correlation between intravitreal ICG use during surgery and a hyperfluorescent optic disc with ICGA in our patient group.

Introduction

One of the first principles one learns when interpreting indocyanine green angiography (ICGA) is that the optic disc is hypofluorescent in a normal angiogram. While evaluating postoperative angiograms of patients with exudative age-related macular degeneration (AMD) treated with an autologous retinal pigment epithelium (RPE)-choroid translocation, we encountered hyperfluorescent optic discs in one fourth of these patients (Fig. 1).

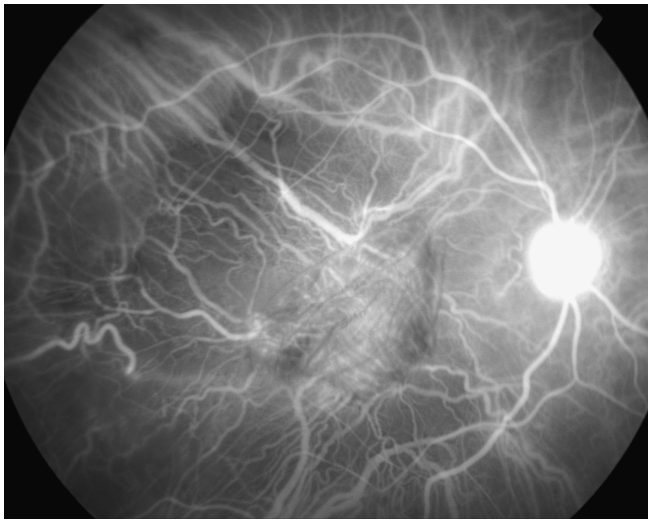

Figure 1. Indocyanine green angiography (ICGA) in patient with exudative age-related macular degeneration treated with an autologous retinal pigment epithelium-choroid translocation. ICGA shows hyperfluorescent optic disc at three months after intravitreal indocyanine green-assisted surgery.

We initially thought of an inflammatory cause, but this was highly unlikely as clinical signs for inflammation were absent, and the time interval between surgery and ICGA was too extended in most of the patients to be a plausible cause for postoperative inflammation. Another explanation could be the prolonged staining of the optic disc after intravitreal indocyanine green (ICG) administration during posterior segment surgery to visualize the inner limiting membrane (ILM). Weinberger et al. first reported this in 2001.[1] The diffusion of ICG in the optic tract was subsequently described and histological confirmed by Paques et al. in an animal model.[2]

This report aimed to identify whether ICG-assisted surgery was related to the hyperfluorescence of the optic disc in our patients, and to study its persistence.

Patients and Methods

Thirty-one patients with exudative AMD treated with an RPE-choroid graft translocation, and who had ICGA after surgery to exclude a possible recurrent neovascular membrane, were included in this retrospective observational case series. In this surgery, initial surgery with the autologous RPE-choroid graft translocation (surgery was performed as previously described by van Meurs et al.) is followed by silicone oil removal after 3-4 months, at which time ICG-assisted ILM removal is performed in selected patients.[3] In two patients the ICGAs were obtained before and in 29 after silicone oil removal. The ICGAs were assessed for hypo/ iso/ hyperfluorescence of the optic disc and related to the time interval between ICGA and the possible use of intravitreal ICG.

Results

The optic disc was hyperfluorescent in six patients, isofluorescent in one and hypofluorescent in 24 patients (Table 1). All ICGAs with hyperfluorescent and seven of the 24 hypofluorescent optic discs were preceded with ICG-assisted surgery with a time interval of respectively 7 ± 3 weeks and 43 ± 12 weeks (P = 0.001, Student T-test). The other 17 hypofluorescent discs were not preceded by ICG-assisted surgery and the one isofluorescent optic disc was observed 32 weeks after ICG-assisted ILM peeling (Table 1).

Of the six patients with hyperfluorescent optic discs, the optic discs of both the fellow eyes (available in three patients) and operated eyes before surgery (available in five patients) were hypofluorescent.

Table 1. Appearance of the optic disc in indocyanine green angiography (ICGA) related to indocyanine green (ICG)-assisted surgery in 31 AMD patients treated with a retinal pigment epithelium-choroid translocation.

ICG-assisted surgery before ICGA	**Optic disc staining with ICGA**	**Time (weeks) between ICGA and ICG-assisted surgery**
No (n = 18)	Hypofluorescent (n = 18)	Not applicable
Yes (n = 13)	Hyperfluorescent (n = 6)	7 ± 3 (range 4–12)
	Isofluorescent (n = 1)	32
	Hypofluorescent (n = 6)	43 ± 12 (range 28-62)

Discussion

A causal connection between intravitreal ICG use during surgery and a hyperfluorescent optic disc with ICGA in our patient group was suggested by the statistically significant correlation and the documented neurophilic staining properties of ICG.[2] Hyperfluorescence could be observed at least the first four months after surgery, and subsequently faded away till the normal hypofluorescence was returned after about ten months.

There are only a few articles reporting on the phenomenon of a hyperfluorescent optic disc after intravitreal ICG application, and all but one have a short-term follow-up. Shortly after ICG application hyperfluorescence of distinct areas of the retina was observed, which progressively migrated towards the optic disc with finally only the optic disc being hyperfluorescent. In these studies, hyperfluorescence of the optic disc also started to fade after about 3 to 6 months after surgery but could persist up to 6 to 12 months.[4-7] Only one study recently reported on the long-term follow-up (16-36 months after surgery) in 18 patients and their results suggested that ICG might even persist for years.[8] An explanation for this is that they used a high-contrast sensitivity scanning laser ophthalmoscope (HRA), which is more sensitive than ICG angiography with a conventional fundus camera used in this and several other studies because there is a lower level of light scatter.[8, 9]

In conclusion, since the introduction of intravitreal ICG-assisted surgery, a hyperfluorescent optic disc with ICGA may be observed more often in daily practice, a phenomenon we found to be unknown even under experienced medical retinal specialists. Beside inflammatory disorders of the eye, doctors should be aware that prior intravitreal use of ICG might be its cause.

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Chapter 2.7

SUBMACULAR SURGERY

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Ophthalmology 2006;113:1471.e1-2; author reply 1471-2

Dear Editor:

We read with interest MacLaren et al's article relating to the loss of function after long-term follow-up of patients with exudative age-related macular degeneration treated with autologous retinal pigment epithelium (RPE) and choroid transplantation.[1] The authors report, for 4 patients, on the loss of fixation over de RPE graft and loss of graft autofluorescence, which originally had been present during the first 2 years' follow-up. As optical coherence tomography images suggested the disappearance of photoreceptor outer segments, they hypothesize that this may be explained by chronic photoreceptor apoptosis, initiated by either surgery or the disease process itself. Few treatments are evaluated after 5 to 6 years, and for this reason alone, the study is very worthwhile. The authors justifiably conclude by cautioning against undue optimistic expectations from studies with a shorter follow-up.[2] We suggest, nevertheless, that several findings in their report might encourage continuation of the study of autologous RPE transplantation. First, fibrosis and recurrent choroidal membranes were not a major problem, whereas this has been reported to be a regular occurrence after most treatment options so far, such as laser photocoagulation, membrane extraction, and macular rotation surgery. Second, the suggestion that surgical trauma may damage the photoreceptors and that RPE dysfunction may be secondary to apoptosis of photoreceptors opens the possibility that less surgical damage might improve the long-term results. The authors' reference to cataract surgery brings another parallel to mind: endothelial damage (due to an imperfect technique) slowed down the introduction of phacoemulsification, but with an improved technique, phacoemulsification turned out to be useful after all.

The modification of the surgical technique of autologous RPE and choroid transplantation we introduced[2] (i.e., use of a free graft of RPE and choroid taken from the midperiphery, instead of from a juxtafoveal site as described by Stanga et al[3] and MacLaren et al) does reduce surgical manipulation to the submacular space, which might result in better photoreceptor survival.

At present, our first 18 patients have been observed for over 3 years.[4] Of the 7 patients who had an increase in vision after 1.5 years' follow up, 1 has died since, and 1 is not willing to return for further follow-up. Three patients have lost their previous gain- in 1, unexplained, despite preserved fundus autofluorescence and the suggestion of persistent perfusion of the graft on angiograpy, and in 2, by recurrent neovascularization. Two patients, however, continue to do well after 42 and 37 months, with 20/125 and 20/25 vision, respectively (Fig. 1, [available at http://aaojournal.org]).

The lack of a reproducable insertion technique requiring minimal manipulations remains the

major concern in our present surgery. We hypothesize that, with improved instrumentation, Peyman et al's[5] and Aylward's[3] concept of autologous RPE and choroid transplantation may prove clinically useful one day.

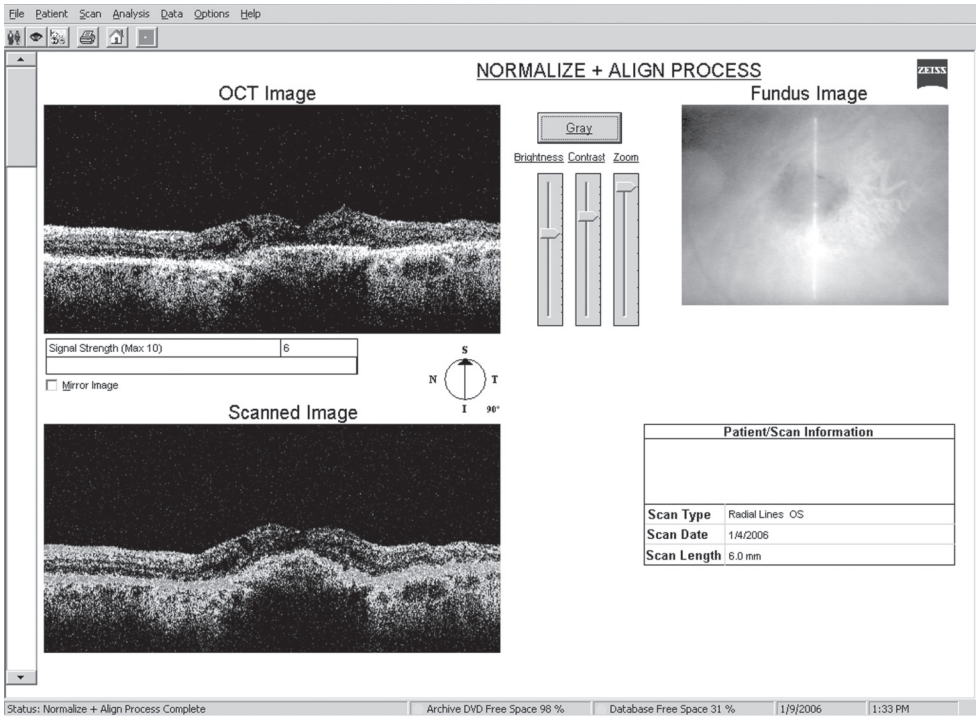

Figure 1. Optical coherence tomography (OCT) scan of a 78-year-old female, demonstrating fixation on the retinal pigment epithelium (RPE) graft and suggesting the presence of photoreceptors over the RPE graft, with 20/25 vision 37 months after the translocation of an autologous graft of RPE and choroid for exudative age-related macular degeneration with a fibrotic occult subfoveal membrane and preoperative vision of 20/160. I = inferior; N = nasal; OS = left eye; S = superior; T = temporal. Color figure can be found in the appendix. See page 202.

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Chapter 3.1

Histological evidence for revascularisation of an autologous retinal pigment epithelium-choroid graft in the pig

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Br J Ophthalmol 2007;91:546-50

Abstract

Background

Translocation of a free autologous graft consisting of retinal pigment epithelium (RPE), Bruch's membrane, choriocapillaris and choroid in patients with exudative age-related macular degeneration is currently being evaluated in clinical practice. Angiographic studies in these patients suggest that their grafts become revascularised.

Aim

To investigated the histological evidence of revascularisation of the graft in a porcine model.

Methods

In 11 pigs (11 eyes), an RPE-choroid graft was translocated from the mid-periphery to an intact or an intentionally damaged RPE and Bruch's membrane at the recipient site. The eyes were enucleated 1 week or 3 months after surgery. Tissue sections were evaluated using immunohistochemistry.

Results

Bridging vessels between recipient layer and graft were identified from 1 week to 3 months after surgery. This reconnection occurred regardless of whether the Bruch's membrane of the recipient site was left intact or intentionally damaged at the time of transplantation. The vasculature of the graft appeared open and perfused. Vessels with transcapillary pillars and conglomerates of small new vessels were present in the graft.

Conclusions

This study demonstrated histological evidence for revascularisation by angiogenesis of a free autologous RPE-choroid graft.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in elderly people in industrialised countries.[1] The exudative or neovascular form is responsible for the majority of patients with severe visual loss.[2,3] In exudative AMD, new choroidal blood vessels cross the Bruch's membrane, and invade the space underneath the retina pigment epithelium (RPE) and/or retina. This precedes the loss of macular photoreceptor cells and a decrease in visual acuity.[4,5] Laser coagulation or photodynamic therapy aims to occlude the choroidal neovascularisation (CNV), but just as in surgical removal of the CNV membrane,[6,7] invariably causes loss of RPE, with subsequent dysfunction of both choriocapillaris and overlying photoreceptors.[8-11] Therefore, recovery of RPE function is desirable. Even promising new therapies that neutralise growth factors such as vascular endothelial growth factor (VEGF) only affect active neovascularisation, and are unable to replace lost RPE cells.[12] Therefore, surgical strategies aim to reconstitute functioning RPE. Cell suspensions and sheets of homologous fetal RPE, suspensions of autologous iris pigment epithelium or RPE have failed to form a functional monolayer.[13-16]

Translocation of the macula to an adjacent healthy extra-macular area of RPE can preserve central vision and sometimes even improve macular function.[17-20] Drawbacks are its restriction to smaller CNVs, the challenging surgical technique, the confinement to second eyes, diplopia, and other complications, especially proliferative vitreoretinopathy.[21-22]

Transplantation of an autologous full-thickness graft consisting of RPE, Bruch's membrane, choriocapillaris and choroid was first suggested in 1991.[23-25] Later, the feasibility of an RPE-choroid graft taken from the mid-periphery was shown in a larger group of patients.[26-28] Angiographic data strongly suggest that revascularisation of the graft occurred with survival and function of the graft for >3 years.[29]

As there is currently no histological evidence for revascularisation, this study investigated the revascularisation of a free autologous RPE-choroid graft in a pig model.

Materials and Methods

Animals

This animal study was performed in accordance with the recommendations of the Association for Research in Vision and Ophthalmology and approved by the Bezirksregierung of Cologne (Number 50.203.2- K26 14/03), Germany.

In total, 11 pigs (11eyes), (6 Berlin minipigs and 5 domestic pigs aged 3-8 months) were pretreated with intramuscular azaperone (2mg/kg) and ketamine (15-20 mg/kg), and anaesthetised with an intravenous injection of propofol 1% (1-2 mg/kg). General anaesthesia was maintained by normal ventilation at physiologic blood gas values (partial pressure of carcon dioxide 40 mmHg) with isoflurane 3 %. Mydriasis was achieved using topical 0.5% tropicamide and 2.5% phenylepinephrine-HCl. Postoperatively, the pigs received buprenorphin (Temgesic; 0.02 mg/kg).

Surgery

A standard three-port vitrectomy including a posterior hyaloid detachment and lensectomy was performed. Subsequently, the anterior border venule and retinal vessels were coagulated. After a retinal detachment was created in the temporal quadrants by subretinal injection of balanced salt solution through a 42x21-gauge rigid microinjection cannula (Synergetics, St Louis, Missouri, USA), a 180^0 peripheral retinotomy was made. With the use of perfluorodecalin (Fluoron, Ulm, Germany), the temporal half of the retina was flapped over to the nasal periphery to allow direct access to the underlying RPE. To prepare the graft, a fluid-air exchange was performed and a mid-peripheral donor area of about 16 mm^2 was surrounded by laser or diathermy coagulation. The borders of the graft were cut with 20-gauge vitreous scissors (Geuder, Heidelberg, Germany). Subsequently, the full-thickness RPE-choroid graft (mean size 9 mm^2) was carefully separated from the sclera. In five pigs, RPE and Bruch's membrane of the recipient bed were intentionally damaged with a soft-tipped silicone loop to mimic the damage after CNV extraction in patients. The graft was translocated to the recipient bed in the macular area using retinal forceps or a specially designed aspiration-reflux spatula (Dutch Ophthalmic, Zuidland, the Netherlands). Care was taken to grasp the graft from the choroidal side to avoid damage to the RPE. The retina was repositioned and refixated by a reinjection of decalin, resulting in a graft covered by retina.

To complete the operation, decalin was exchanged for silicone oil (5000 Cst., Siluron, Fluoron).

Enucleation

The eyes were enucleated 1 week or 3 months after surgery. The pigs were sedated with azaperone (2mg/kg) and ketamine (20 mg/kg), and killed using pentobarbital sodium. The non-operated fellow eye served as a control.

Tissue preparation

The anterior segment of the eye was dissected posterior to the ora serrata, and the fundus was inspected for location and macroscopic features of the graft, retinal detachment or bleeding. The posterior cups were fixed with 4% buffered paraformaldehyde at 4°C overnight. Subsequently, the graft was cut out as a small block of tissue, including retina and sclera. For light microscopy, the fixed tissue was embedded in paraffin wax, processed for sectioning (4 µm thick sections), and stained with haematoxylin and eosin and periodic acid-Schiff (PAS).

Immunohistochemistry

The sections were dewaxed, rehydrated, and washed in three changes of phosphate-buffered saline (PBS). To eliminate endogenous peroxidase activity, specimens were incubated for 30 min in 3% hydrogen peroxide. After rinsing in water for 5 min and three changes of PBS, sections were pretreated for antigen retrieval with citrate buffer pH 6 for 15 min at 100°C. After three changes of PBS, incubation with the primary antibody (dilution 1:400) was performed at 4°C overnight. Primary antibodies used in the study were rabbit antihuman von Willebrand factor (vWF; DakoCytomation, Glostrup, Denmark) and rabbit antihuman antibody directed against VEGF receptor-1 (flt-1; Santa Cruz, California, USA).

After three washes with PBS-Tween 0.5%, specimens previously incubated with the anti-vWF were incubated with goat antirabbit Cy3 (1:400, 30 min at room temperature; Jacksons ImmunoResearch, Westgrove, Pennsylvania, USA) for immunofluorescent evaluation. For specimens incubated with flt-1, polyalkaline phosphatase-GAM /R IgG (ImmunoLogic, Duiven, The Netherlands) was applied for 30 min, followed by washes with PBS and TRIS/HCl. New Fuchsin was used to develop the alkaline phosphatase-chromogen. All specimens were counterstained with haematoxylin.

Results

Complications of surgery

In most pigs, complications were encountered during surgery, mainly consisting of choroidal and retinal haemorrhage (n = 8). Despite an uncomplicated operation, pig 3 had to be killed 18 h after surgery because of postoperative lung oedema.

Revascularisation of the graft

All RPE-choroid grafts had continuous or partial contact with the macular recipient bed (Fig. 1 A1-C1). In 6 of 11 pigs, the graft vasculature appeared open and perfused (presence of erythrocytes) 1 week and 3 months after transplantation. Moreover, in these six grafts, vertical bridging vessels between recipient area and graft were found after 1 week (pigs 1, 8 and 10) and 3 months (pigs 5, 7 and 11; Fig. 1 A2-C2). On serial sectioning, no obvious differences in the number of bridging vessels were seen between 1 week and 3 months after surgery. This vessel infiltration into the graft was detected irrespective of whether the recipient Bruch's membrane was intentionally damaged or left intact as much as possible at the time of transplantation, and irrespective whether intraoperative haemorrhages had occurred or not.

In addition, bridging vessels were spread equally over graft and recipient interface.

Owing to impaired visualisation, the graft was accidentally placed upside down in two animals (pigs 8 and 11) as shown by histological evaluation; however, bridging vessels were found centrally between the recipient and graft and not at the edges.

Furthermore, vessels with transcapillary pillars and conglomerulates of small vessels in the graft were observed (pig 6). In all grafts, the large majority of vessels stained positive for flt-1 (VEGF receptor-1) and vWF, as did the choroid of the recipient bed. There was no difference in flt-1 or vWF staining between the early and late postoperative course.

Tissue responses

The graft consisted of choroid, choriocapillaris, Bruch's membrane and RPE cells. Macroscopically, the graft had a dark grey appearance. Numerous macrophages were morphologically identified on haematoxylin and eosin-stainedsections in the graft after 1 week and 3 months. In the fellow eye, macrophages were not observed.

In the grafts harvested 18 h (n = 1) and 1 week (n = 4) after surgery, large areas of haemorrhages were macroscopically apparent, which were microscopically noted subretinally, and between the graft and recipient tissue. Organisation of these haemorrhages had started at 1 week with fibroblastic and capillary ingrowth. At 3 months, a fibrovascular scar underneath the retina (pigs 2 and 6) or graft (pigs 5 and 11) was present (Fig. 1 A_1). Bridging vessels crossed the fibrovascular layer connecting the recipient and graft (Fig 1 A_2).

Proliferative vitreoretinopathy (PVR) was present in six pigs as observed macroscopically and microscopically from 1 week after surgery.

In three grafts, giant cells and histiocytes surrounded intraretinal or intrachoroidal vacuoles filled with silicone oil (Fig. 1 C_1).

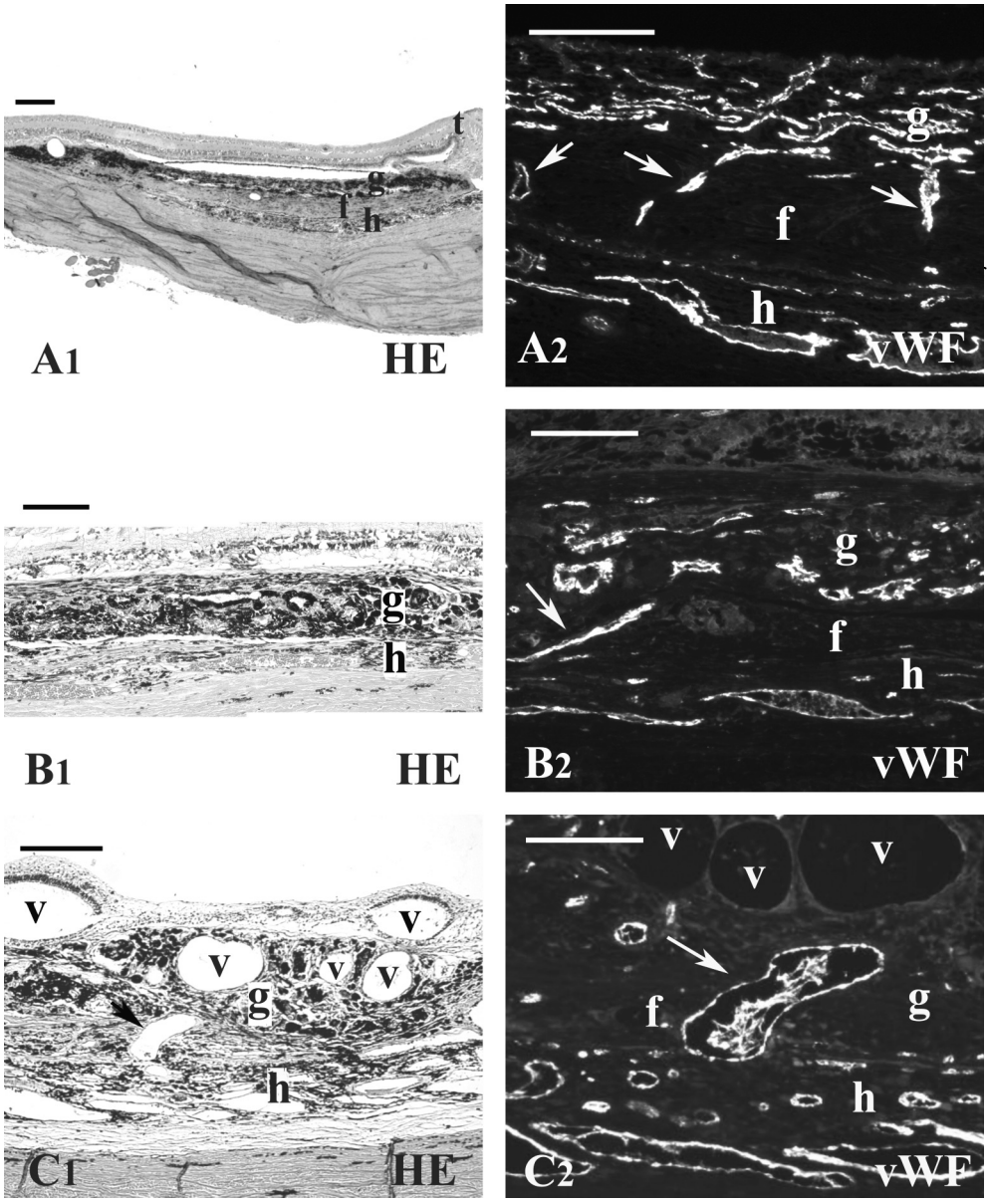

Figure 1. (A1-C1) Haematoxylin and eosin (HE) staining with accompanying von Willebrand factor (vWF) staining (A2-C2) of three retinal pigment epithelium-choroid grafts: vertically bridging vessels between recipient and graft. Arrows indicate bridging vessels; g, graft; f, fibrovascular layer; h, host choroid; t, transition healthy-degenerated retina; v, intraretinal or intrachoroidal vacuoles filled with silicone oil. Scale bars: A1-C1 200 µm and A2-C2 100 µm.
Color figure can be found in the appendix. See page 203

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Retinal pigment epithelium

RPE cells covered the graft up to 3 months after surgery (Fig. 1). In pigs with traumatic surgery, stretches of graft RPE were degenerated or absent.

The RPE cells of the recipient bed underneath the graft were scarcely present or had degenerated, both in pigs where RPE was intentionally removed or was left intact as much as possible.

Bruch's membrane

PAS staining for basement membrane proteins showed the presence of the recipient Bruch's membrane underneath the graft 18 h after surgery (pig 3). In contrast, 1 week and 3 months after surgery, the recipient Bruch's membrane had largely disappeared underneath the graft. This was restricted to the transplantation area and occurred regardless of whether the recipient bed was intentionally damaged during surgery or not.

Retina

There was an obvious transition from relatively healthy retina, which had remained attached during the operation, to degenerated retina, which had been detached during the 180^0 retinotomy (Fig. 1 A_1).

The presence of subretinal haemorrhage in some grafts prevented RPE-retina contact, with loss of photoreceptors. In pigs (pigs 1, 4 and 5) where stretches of RPE monolayer covered the graft with a direct contact between the retina and graft, normal photoreceptors were observed up to 3 months after surgery.

Discussion

This study shows histological evidence for revascularisation of a free autologous RPE-choroid graft, which was apparent as early as 1 week and persisted up to at least 3 months after surgery. Vertically bridging vessels between the macular recipient bed and graft could be observed, suggesting revascularisation by vessel invasion from the underlying choroid. The vasculature of the graft appeared open, perfused and viable, as shown by intravascular blood cells and VEGF receptor-1 expression. There was evidence of angiogenesis in the graft, as indicated by vessels with transcapillary pillars and conglomerates of small vessels.

Revascularisation of free grafts is well known in skin transplants. Survival of a free skin graft is initially accomplished by plasmatic imibibition until definitive recirculation is achieved by

anastomosis of host and graft vasculature (after 24-48 h) by neovascular ingrowth.[30,31] In our study, the exact timing of revascularisation by localised choroidal neovascular ingrowth of the RPE-choroid graft was not determined. This might be established by repeated postoperative angiography in patients, starting as early as 1 day after surgery.

Interestingly, histologically, revascularisation of the graft did not result in the formation of subretinal neovascularisation, but was restricted to bridging vessels and vessel formation in the graft.

Several distinct processes seem to be involved in revascularisation of the graft. Potential stimuli include hypoxia, an inflammatory response and a break down of Bruch's membrane. Although we can only speculate as to whether relative hypoxia in the transplanted graft might be a stimulation for revascularisation, a striking finding was the ubiquitous presence of macrophages. These macrophages are either derived from the circulation or are migrated and dedifferentiated RPE cells from the recipient site. Macrophages have a central role in the normal chronic cellular inflammation response in wound healing. They release many growth factors that promote angiogenesis.[32-34] The macrophage response was seen as early as 1 week and persisted during the 3 months follow-up.

The macrophage invasion probably occurs in the context of an immune reaction to tissue damage after surgical trauma (as proposed by Matzinger[35] in the Danger Model) and may contribute to neovascularisation, leading to revascularisation.

In patients, and in animal models of CNV, ruptures in Bruch's membrane are a prerequisite for the development of CNV.[36-38] Therefore, it was initially hypothesised that an intact Bruch's membrane at the recipient site would act as a natural barrier, preventing infiltration of vessels into the graft. However, vascularisation of the graft was observed, irrespective of whether the recipient Bruch's membrane was intentionally damaged or left intact at the time of transplantation and even when the graft was placed upside down. PAS staining showed that the recipients Bruch's membrane had largely disappeared underneath the graft, whether or not the recipient site was kept intact. This suggests that Bruch's membrane degenerates underneath the graft in the pig model. The macrophage reaction discussed above may contribute to this process. Joussen et al.[28] recently reported angiographic data on the revascularisation of an RPE-choroid graft in patients with atrophic AMD, which supports the finding that vessels can reconnect despite an initially intact Bruch's membrane.

The investigations were carried out in pigs, as the morphology of the porcine choroid is very similar to that in humans.[39,40] However, surgery in the pig proved to be much more difficult than in patients, mainly because a wide-angle viewing system could not be used and the illumination was suboptimal. Moreover, porcine retina differs from human retina by the

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presence of a large anterior circumferential border venule and superficially located retinal vessels. Severe haemorrhages from these vessels complicated the 180^0 retinomy in some cases, prevented reattachment of the retina over the graft, with consequent loss of photoreceptors, and may have contributed to an excessive fibrovascular response under the graft. A small paramacular retinotomy in the temporal raphe could have improved the retinal reattachment rate over the graft, as in patients this approach is associated with fewer complications during and after surgery.[27,28]

Additionally, the 180^0 retinotomy with fluid-air exchange caused mechanical retinal trauma.[41,42] There was a sharp border between relatively healthy retina (remained attached during the operation) and the degenerated retina, which had been in contact with air. This border was found over or just nasal to the graft, and should not be mistaken for degeneration caused by the graft itself. Moreover, the pig retina is not supplied by a central retinal artery, but by retinal arteries directly originating from the ciliary arteries with lower perfusion pressure, and therefore more vulnerable to intraoperative ischaemia during folding and a high bottle pressure.[39,40] For these reasons, this animal model proved to be unsuitable to verify the clinical experience that RPE and retina can survive after a choroid-RPE graft transplantation.

This study, however, did serve to demonstrate histological evidence of revascularisation of a free autologous graft of RPE and choroid.

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Chapter 4.1

An attempt to induce revascularization of a retinal pigment epithelium–choroid graft in a perfusion tissue culture

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In preparation

Abstract

Purpose

To investigate the feasibility to induce revascularization of a retinal pigment epithelium (RPE)-choroid graft placed upon an RPE-choroid recipient in a perfusion tissue culture.

Methods

RPE-choroid grafts and retina-RPE-choroid recipients were prepared from freshly enucleated porcine eyes. After intentionally damaging the Bruch's membrane and RPE of the recipient, the graft was located upon the recipient RPE, covered with retina and fixed in a holding ring. The specimen was transferred to a perfusion tissue culture. To stimulate angiogenesis, the culture conditions were varied with respect to culture duration, and supply of oxygen, growth factors and nutrients.

Results

We performed 15 separate perfusion tissue cultures of which 24 specimens were histologically evaluated. In one specimen, a vessel breaking through the choriocapillaris of the recipient towards the graft was observed, which was suggestive for vessel growth. This specimen had been cultured for 15 days under normoxia without adding growth factors, and was exposed to hypoxia for the initial three hours of culture. All specimens cultured under continuous hypoxic condition were autolytic.

Conclusion

It remains uncertain whether a perfusion tissue culture might be a feasible model to study revascularization of the graft. Further study is needed of cultures with shorter durations of hypoxia, and additional immunohistochemical staining is required to determine whether the vessel suspected for angiogenesis was newly formed during culture or was pre-existing.

Introduction

The translocation of an autologous retinal pigment epithelium (RPE)–choroid graft is used as a last treatment option in patients with exudative age-related macular degeneration (AMD). [1-8]

The RPE-choroid graft (approximately 2 x 2 mm in size) is translocated from the midperiphery to the subfoveal area to restore the Bruch's membrane and RPE layer after removal of the choroidal neovascularization (CNV). Subsequent revascularization is required for survival and functioning of the graft. Previous studies already showed reperfusion of the graft by fluorescein and indocyanine green angiography in patients.[5,7-9] The histological evidence of revascularization of the graft was demonstrated by an in vivo study in pigs.[10]

However, more detailed information about the revascularization process itself could not be obtained from these studies; it was difficult to obtain post-mortem eyes in the patients and the translocation procedure in porcine eyes was complicated, time-consuming and expensive.

Therefore, in the present study, experiments were carried out to investigate the feasibility to induce revascularization of an RPE-choroid graft in a perfusion tissue culture. This would offer an ex vivo model to study new vessel in growth in a graft with the potential to study variables for such growth.

Materials and Methods

Preparation of the specimens

The eyes were obtained from domestic pigs (3 months of age) that were used for cardiovascular open-heart experiments. The anesthetized animals were killed using pentobarbital sodium and a short 9-volt electric pulse from a battery directly to the heart.

The eyes were kept in a physiologic salt solution (PSS) on ice, and after immersion in 70% alcohol for 5 seconds, they were prepared under sterile conditions for tissue culture within 20 to 60 minutes after death.

After a dissection was made posterior to the ora serrata, the anterior segment together with the corpus vitreum was removed. The posterior segment was cut in a larger and smaller part. The larger part would be used to prepare the recipient retina-RPE-choroid and the smaller part to prepare the RPE-choroid graft. Both parts were frequently kept moisture with DMEM (Dulbecco's modified Eagle's medium; Invitrogen, Breda, the Netherlands) to prevent drying of the tissues. With the use of a dissecting microscope, the retina together

with the RPE and choroid were carefully separated from the sclera of the larger halve. This procedure was facilitated by first flattening the tissue by making small cuts at the edges of the sclera and fixation of the sclera to the table with needles.

One part of the tissue holder ring (diameter of 13 mm) (Minucells and Minutissue, Bad Abbach, Germany) was placed under the choroid of the recipient. Subsequently, the graft was prepared from the smaller tissue part of the eye cup; the retina was removed and the graft (size 2x2 to 5x5mm), consisting of RPE and choroid, was separated from the sclera. The graft was placed on top of the recipient RPE-choroid by a retinotomy, or after folding back the retina and replacing the retina over the graft again. The RPE and Bruch's membrane (BM) of the recipient were intentionally damaged to induce or facilitate vascular growth. Finally, the second part of the tissue holder ring was placed upon the retina and pressed together with the other ring underneath the recipient choroid. The specimen was now fixated in the tissue holder ring and transferred to the perfusion container (Figs. 1 and 2).

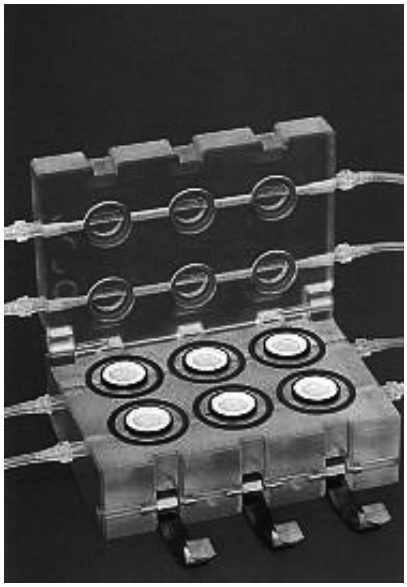

Figure 1. The perfusion tissue container. In this example, the container is opened and six holding rings (diameter of 13 mm) are present. Both sides of the specimens fixed in the rings can be separately perfused with different media. Color figure can be found in the appendix. See page 203.

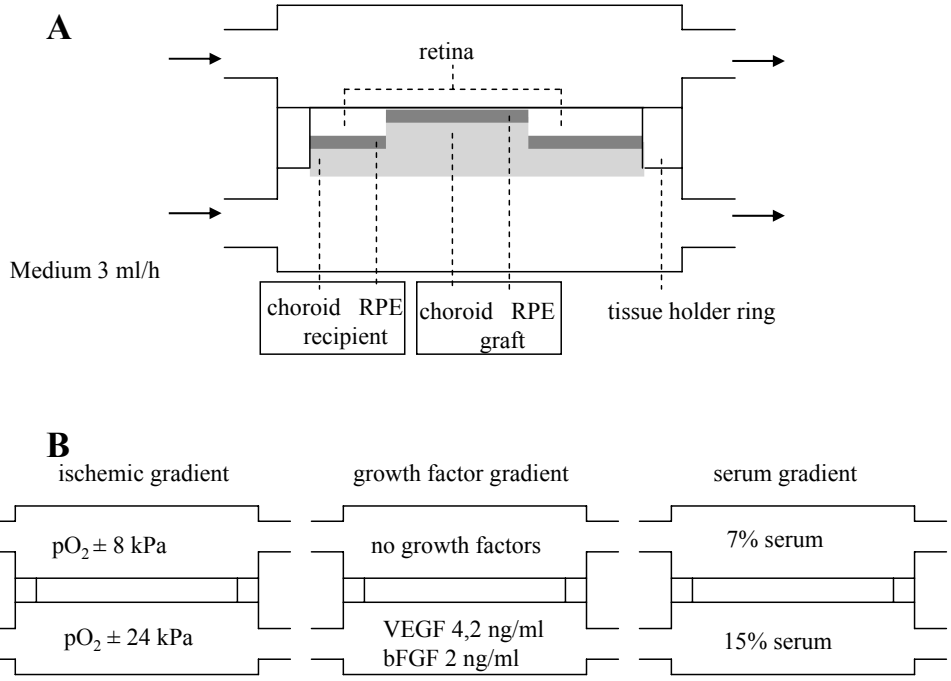

Figure 2. Schematic illustration of the perfusion tissue container. (A) The specimen, consisting of graft and recipient, is fixed in the holding ring. This specimen divides the compartment in an upper and lower part. Each part is connected to a supplying and draining perfusion line. (B) Varies culture conditions were applied. The separate medium flow enables to create a gradient over the specimen, resulting in different culture environments for the graft and recipient.

Perfusion tissue culture

The preparation of the tissue and the perfusion tissue culture (with exception of the RPE-choroid graft translocation procedure and variations in culture conditions) used in the present study were based on previous studies with retina-RPE-choroid in perfusion culture.[11,12]

The perfusion tissue culture (Minucells and Minutissue, Bad Abbach, Germany) consists of a container with six compartments (consisting of two sets of 3 compartments in serie)(Fig. 1). By placing the ring in the container each compartment is divided in an upper and lower part, with each part connected to a supplying and draining perfusion line (Fig. 2). This enables to use two different kinds of mediums at the graft and recipient side of the specimen.

One set of three compartments share the same perfusion flow. To prevent a specimen from being too much influenced by receiving metabolites from the specimens upstream in the perfusion flow, one or maximally two rings per serie were placed. Rings holding a Thermanox plastic coverslip (Nunc, Wiesbaden, Germany) were used in the empty compartments to

keep the upper and lower flow separated. The medium was continuously perfused at a rate of 3 ml/h with an IPC N-8 peristaltic pump (Ismatic, Wertheim-Mondfeld, Germany).

Perfusion culture conditions

The standard medium used was DMEM with 4500 mg/L D-Glucose and 4 mM L-glutamine (high energy sources) added with 25 mM HEPES and 1% penicillin-streptomycin. The medium was refreshed every 4 days. Some additions varied between cultures (Table 1, Fig. 2B): 1) Porcine serum concentrations used were 15% on both sides of the specimen, or 7% on the graft side and 15% at the recipient side; 2) Without or with the addition of growth factors on both sides of the specimen, or only at the recipient side, i.e. the growth factor gradient. We used 4,2 ng/mL human $VEGF_{165}$ (Vascular Endothelial Growth Factor; PeproTech, London, UK) and 2 ng/mL human FGF-basic (Fibroblast Growth Factor-basic; PeproTech, London, UK).

The experiments were performed under normoxic or hypoxic conditions or with an ischemic gradient applied (Table 1, Fig. 2B). In the ischemic gradient experiments the perfusion fluid at the graft side had a pO2 of ± 8 kPa and at the recipient side ± 27 kPa. Twenty-seven kPa was defined as "normoxic" as this was the oxygen pressure measured within the medium exposed to normal air.

In the experiments performed under normoxic conditions the container was placed on a 38^0C heating plate (Broedmij/Therbo, Oosterhesselen, the Netherlands). For the hypoxic conditions, the perfusion container was placed in a CO_2 incubator at 37^0C. The percentage of oxygen in the incubator could be decreased from 21% (normal air) to 7.2%. The bottles containing the medium and the bottles for the drained medium stood outside the incubator on ice and were connected to the culture container by perfusion lines passing a small hole in the incubator.

The medium perfused to the tissue culture container became hypoxic by gas exchange in the permeable perfusion lines (about 1 meter) inside the CO_2 incubator. An ischemic gradient was reached by thoroughly taping the line perfusing the recipient side, which prevented gas exchange inside the incubator (medium kept a pO_2 of ± 27 kPa). In one experiment, hypoxia on both sides of the specimen was only applied for 3 hours followed by normoxia during 15 days (Table 1).

Oxygen tension and pH measurements of the medium supplying and leaving the perfusion container were made every three days in the first two experiments (18 days and 21 days in culture) under continuous hypoxic conditions. Measurements were made with a blood gas analyzer (type ABL825, Radiometer Nederland B.V., Zoetermeer, the Netherlands).

The incubation time was gradually increased to three weeks after verifying the morphology of the tissue after the first experiments with shorter incubation times under normoxic conditions.

Table 1. Varied conditions used for the perfusion organ culture. In total 24 specimens were evaluated.
In some perfusions gradients were applied, i.e. a different concentration of a substance was created between the two flows of medium superior and inferior of the specimen (Fig. 2B).

pO_2 medium	porcine serum gradient	growth factors	days in culture	number of grafts
Normoxic	no	no	4	1
			5	4
			6	5
			10	1
			18	3
			21*	1
3 hours of hypoxia	no	no	15	1
hypoxic	yes	yes	11*[1]	2
			18	1
			21	3
Ischemic gradient	yes	gradient	18	2

** Culture with fungus growth. *[1] Untimely termination of culture due to fungus.*

Histology

After different incubation times, the specimens (within the holding rings) were fixed with 4% buffered paraformaldehyde. Subsequently, the tissue was removed from the holding ring and placed between a fine folded gauze to prevent graft detachment during the paraffin wax-embedding procedure. The tissue was then processed for sectioning (4-µm thick sections) and, for light microscopy, stained with haematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

For immunohistochemistry, sections were incubated with the following primary antibodies: rabbit antihuman von Willebrand factor (vWF; DakoCytomation, Glostrup, Denmark, dilution 1:400 overnight), rabbit antihuman antibody directed against VEGF-receptor-1 (flt-1; Santa Cruz, California, USA, dilution 1:400 overnight), and rabbit antihuman antibody directed against proliferating cells (Ki-67, clone MIB-1; DakoCytomation, Glostrup, Denmark, dilution 1:10 for 30 minutes).

Before applying the primary antibody, the sections were dewaxed, rehydrated and washed in 3 changes of phosphate-buffered saline (PBS). To eliminate endogenous peroxidase activity, specimens were incubated for 30 min in 3% hydrogen peroxide. After rinsing in water for

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5 min and 3 changes of PBS, the sections for vWF and flt-1 staining were pretreated for antigen retrieval with citrate buffer pH 6.0 for 15 min at 100°C. After three changes of PBS, incubation with the primary antibody (vWF or flt-1) was performed at 4°C overnight. Following three washes with PBS-tween 0.5%, specimens previously incubated with the anti-vWF, were incubated with goat antirabbit Cy3 (1:400, 30 min at RT) (Jacksons ImmunoResearch, Westgrove, USA) for immunofluorescence evaluation. For Flt-1 incubated specimens, poly-alkaline phosphatase-GAM /R IgG (ImmunoLogic, Duiven, Netherlands) was applied for 30 min, followed by washes with PBS and tris/HCl. New Fuchsin was used to develop the AP-chromogen and the specimens were counterstained with haematoxylin.

For staining with Ki-67 the steps after incubation in hydrogen peroxide included: pretreatment with pronase for 20 min at 37^0C, two changes of cold PBS (4^0C) and incubation with the primary antibody. The sections were then incubated in poly-alkaline phosphatase-GAM /R IgG for 30 min, washed with PBS and tris/HCl. The reaction was developed with levamisol for 30 min in a dark environment. After rinsing in distilled water the specimens were counterstained with haematoxylin.

Results

General course

In total 15 perfusion tissue cultures under different culture conditions were performed with in total 39 specimens consisting of an RPE-choroid recipient with an RPE-choroid graft upon it covered by retina (Fig. 2, Table 1). Success of the culture was defined as a graft kept in contact with the recipient at the end of the tissue culture and fixation process. In total 24 grafts remained attached to the recipient. Reasons for loss were: fungus growth (n = 3), graft lost during culture (n = 6), and graft detached from recipient while manipulating the graft (n = 6). Manipulations were required to remove the specimen from the holding ring and prepare it for the paraffin wax-embedding procedure.

Twice a culture had been contaminated with a fungus in the CO_2 incubator (Table 1). In one culture, one of the two specimens was destructed with loss of the graft. This was noticed at time of removal of the specimens from the culture container after 21 days of culture. Another culture had to be prematurely stopped after 11 days because of fungus growth. In this culture, two of the four specimens (both placed in one serie) were unsuitable for evaluation (Table 1).

The macroscopical morphology of the retina was often already poor because of manipulations

to translocate the graft, but became even poorer after culturing under hypoxic conditions. The retina was often lost while placing the specimen in the paraformaldehyde or after removing the specimen from the tissue holder ring.

In the two first two experiments performed under hypoxia, the medium in the medium bottle and in the (taped) perfusion line just before entering the culture container had a pO_2 of 26.7 ± 1.6 kPa and a pH of 7.1 ± 0.1. The permeable ("hypoxic") perfusion line supplied medium with pO_2 of 8.2 ± 0.6 kPa and pH of 7.1 ± 0.1. The drained hypoxic medium had a pO_2 of 7.8 ± 1.3 kPa and a pH of 7.3 ± 0.05.

Histology

HE and PAS staining

HE and PAS staining showed that under normoxic conditions the recipient and graft RPE and choroid remained well preserved in 9 out of the 15 specimens up to 18 days in culture; normal endothelial cells lined the open vessels and the RPE remained a monolayer up to 10 days of culture (Fig. 3). After 18 and 21 days of culture, stretches with RPE monolayer alternated with RPE multilayers. The RPE on top of the graft had often disappeared.

From 6 days onwards in normoxic culture pycnotic cells could be observed. The specimen cultured for 21 days was autolytic due to fungus growth (Table 1).

Both the specimen with only 3 hours of hypoxia of the total 15 days, as well as the 11 to 21 days cultured specimens under hypoxic conditions had an autolytic appearance with loss of nuclei in almost all cells (Fig. 4). In only one specimen, cultured for 11 days under hypoxia, some normal endothelial cells could be observed. Remnants of an RPE monolayer were present, as well as multilayered RPE and many stretches without RPE. The retina was lost in al of these specimens.

Ki-67 and flt-1 staining

With Ki-67 staining, proliferating RPE cells appeared to be migrating from the recipient onto the graft under normoxic conditions (Fig. 3). No proliferating endothelial cells were observed in all of these specimens. Nor did we observe increased color intensity in any vessel when staining with flt-1. Ki-67 and flt-1 staining of the hypoxic cultured specimens were not available at time of printing this thesis.

VWF staining

One specimen was very suggestive for vessel growth between the graft and recipient (Fig. 4). Sections revealed a vessel breaking through the choriocapillaris of the recipient towards the

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graft. At a next 4 µm-section, the same vessel was located outside the recipient near the graft (Fig.4). This specimen was cultured for 15 days with only the first three hours of hypoxia and without growth factors added.

Eighteen out of the 24 specimens were cultured with an edge of the recipient or graft folded. This resulted in areas with RPE-to-RPE or choroid-to-choroid contact. No signs of newly formed vessels were present between these interfaces.

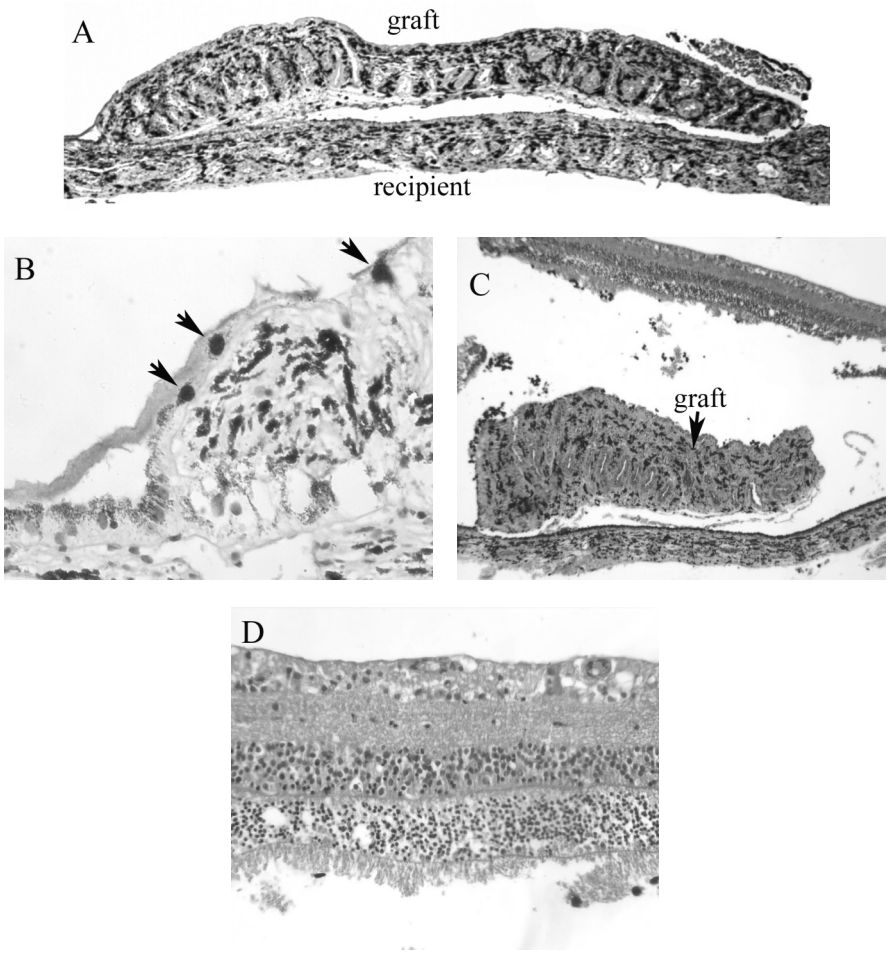

Figure 3. Light micrographs of two specimens cultured for four (Fig. 3A-B) and ten (fig. 3c-d) days under normoxia. (A) Overview of a graft. The graft has lost its retinal pigment epithelium (RPE) and is attached by one side to the recipient. (B) KI-staining of the same graft reveals proliferating RPE cells (arrows) at the border of graft and recipient. (C) Recipient with RPE monolayer. RPE has almost totally disappeared from the graft due to manipulation of the tissue. The detached retina is an artifact caused by sectioning. (D) Retina reveals a good morphology. Color figure can be found in the appendix. See page 204.

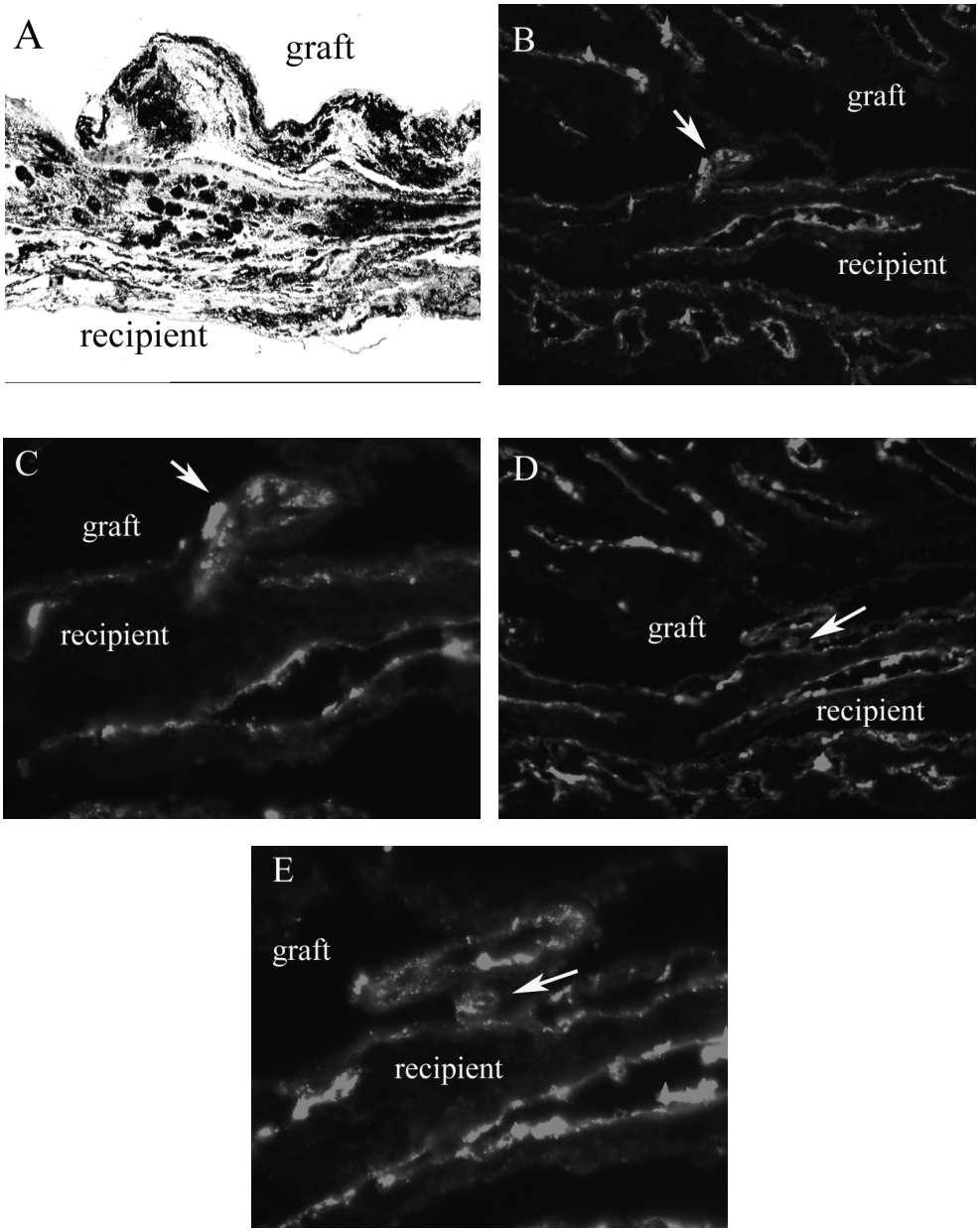

Figure 4. Haematoxylin and eosin (HE) and von Willebrand factor (vWF) staining of graft initially cultured for three hours under hypoxia followed by 15 days under normoxia. (A) Overview of autolytic graft and recipient. (B-E) Vessel breaking through the choriocapillaris of the recipient towards the graft (arrow). There is 4 μm in between sections B-C and D-E. Color figure can be found in the appendix. See page 204.

Discussion

Several culture conditions were used to study the feasibility to induce revascularization of an RPE-choroid graft placed upon a recipient consisting of RPE and choroid in a perfusion tissue culture. Of 24 specimens, only one was strongly suggestive for vessel growth between the graft and recipient. This specimen had been cultured for 15 days with only the first three hours of hypoxia. The endothelial cells were stained with vWF. To determine whether this vessel was newly formed or pre-existing, specific staining of markers exclusively expressed during angiogenesis are required, like tissue factor on endothelial cells or fibronectin and colligin 2 in the extracellular matrix (ECM).[13-17] These immunohistochemical stainings will be performed as a next step in a future study.

Based on the results of the perfusion cultures under normoxic conditions made us decide to add stimuli for angiogenesis to the culture: growth factors (VEGF and bFGF), hypoxia and a decrease of serum concentration (Table 1). Moreover, we tried to establish gradients of these stimuli over the specimen by creating a normoxic environment with normal serum concentrations and enough growth factors for the recipient, and with a graft in need for oxygen, nutrients and vessel growth (Fig. 2).

In contrast to the specimens cultured under normoxic conditions, the morphology of the specimens cultured for 15 to 21 days and supplied with hypoxic medium was very poor with autolysis of almost all cells. The specimen cultured for 11 days under hypoxia could not be assessed for the influence of hypoxia due to fungus growth. It would have been interesting to obtain data from hypoxic cultures with shorter culture durations to estimate the timing of autolysis in these specimens. This is important as vessel growth needs to occur before the autolysis starts. The strong autolytic effect on the tissue by hypoxia was surprising as the pO2 of 8.2 ± 0.6 kPa of the hypoxic medium does not differ much from the pO2 of 9.3-12 kPa assessed in vivo in the RPE in pigs.[18] However, it is likely that the O_2 diffusion from the medium to the cells is suboptimal, with an actual lower pO2 of the RPE and choroid.

In skin grafts neovascular in growth is achieved after one to two days.[19,20] In organ cultures with embryonic quail hearts or vena cava or aorta ring assays, vessel growth in an artificial gel starts after two to three days.[21-23] However, these data were considered not to be a guarantee for vascular growth in tissue ex vivo. One concern was the absence of macrophages in our experiments. Macrophages are important in all phases of angiogenesis by secreting growth factors and allowing migration of endothelial cells by the destruction of the basement membrane and local degradation of the ECM.[24] These latter two functions are not required for angiogenesis in a gel in vitro, but probably are in our ex vivo tissue culture. As in the

present study only one specimen out of 24 showed a vessel suggestive for angiogenesis, more stimulation might be needed by adding macrophages and substances required for breakdown of the ECM like proteinases. However, in the only study reported on microvascular development in tissue ex vivo no macrophages were added.[25] The same perfusion tissue culture model as in our present study was used for kidney cortex explants. After 13 days in a normoxic culture and supplementation with VEGF proliferation of endothelial cells and vascular network development were observed.[25] Moreover, in our model, the damaged (intentionally or by our manipulation) RPE of the recipient site could have been activated with subsequent secretion of proteinases like metalloproteinase, decreasing the need for adding these substances.[26]

The RPE remained a monolayer up to 10 days in culture and had stretches of multilayered RPE from 18 days onwards. These results are similar to other studies on culturing retina-RPE-choroid in the same perfusion culture system.[11,12] Del Priore et al. even reported on a monolayer up to four weeks in a static tissue culture.[27]

In the present study several culture conditions were applied to induce vessel growth. Especially hypoxia turned out to be devastating to the tissue with autolysis of cells. Only in one specimen cultured for 15 days under normoxia (only the first three hours hypoxic) a vessel suggestive for angiogenesis was found. Supplementation with growth factors did not result in vascular growth in the presence of hypoxic autolysis.

As we performed our histological studies after the conclusion of the experiment, it turned out that we had tried out too many stimulations at once. The results of this study may help to design better staged further studies.

In conclusion, the presence of one vessel suggestive for angiogenesis just leaves the door open for further studies to confirm or reject the feasibility to induce revascularization of an RPE-choroid graft in a perfusion tissue culture.

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Chapter 5.1

Threshold amplitude and frequency for ocular tissue release from a vibrating instrument: an experimental study

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Abstract

Purpose

During retinal pigment epithelium (RPE) and choroid graft translocation in the treatment of patients with exudative age-related macular degeneration, the adhesion of the graft to the translocation instrument complicated its submacular release. Vibration of the instrument improved the release of the graft. This study was conducted to validate the effectiveness of the principle of vibration and to determine the threshold amplitude and frequency required for development of an optimized instrument.

Methods

An experimental in vitro model with fresh porcine RPE-choroid grafts was used. Release of the graft was studied by a masked observer for amplitudes in the range of 0.05 to 1.2 mm and frequencies in the range of 25 to 200 Hz in the horizontal plane.

Results. The minimum threshold amplitude required to release the graft was approximately 0.15 mm from a frequency of 100 Hz and higher.

Conclusions

This study confirmed the clinical experience that vibration of an instrument induces the release of the RPE-choroid graft. The minimum threshold amplitude and frequency needed for optimum tissue release were estimated.

Introduction

A retinal pigment epithelium (RPE)-choroid graft translocation is used as a last treatment option in patients with exudative age-related macular degeneration (AMD).[1,2] During this surgery, the neovascular membrane is removed from the subretinal space through a paramacular retinotomy in the temporal raphe. This procedure is preceded by the induction of a posterior vitreous detachment and a complete vitrectomy. After circular heavy diathermia in the midperiphery at the 12 o'clock position, a full-thickness graft of retina, RPE and choroid of approximately 2 x 3 mm is cut. The graft is grasped from the choroidal side, and the neurosensory retina is removed just before the graft is repositioned under the macula through the existing paramacular retinotomy. The midperipheral donor site is surrounded with laser coagulation followed by a silicone oil tamponade. [1,2]

The most critical step during this surgery was the submacular release of the graft, which was complicated by the adhesion of the graft to the translocation instrument.

Two kinds of translocation instruments were used - the aspiration-reflux spatula and the fine forceps currently used (both from the Dutch Ophthalmic Research Center [DORC], Zuidland, the Netherlands; Fig. 1)- but both presented the problem of persistent adhesion. These instruments hold the graft from the choroidal side (by suction and grasping, respectively) to avoid damage to the RPE.

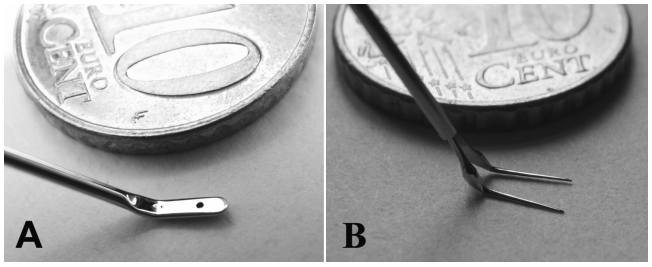

Figure 1. The aspiration-reflux spatula (A) and the translocation forceps (B). Diameter of 10-eurocent coin is 19.75 mm.

The injection of perfluorocarbon liquid (PFCL) to hold the graft in place and facilitate release of the graft when retracting the instrument slightly improved the release. However, real improvement was achieved by having the instrument vibrate during the submacular release of the graft. Vibration was transmitted to the translocation instrument by attaching a mobile phone vibration device (which had a frequency of 140 Hz) to the handle of the translocation instrument.

The rationale for vibrating the instrument was to exceed the maximum friction force between the graft and the instrument by accelerating the instrument. The amplitude and frequency of the vibration of the instrument determine the acceleration.

Therefore, the present study was performed to validate the principle of tissue release by vibration and to determine the minimum threshold amplitude and frequency needed for optimum tissue release from a vibrating translocation instrument.

Materials and Methods

Preparation of the graft

Freshly enucleated porcine eyes were prepared (within 12 hours after death). First, the anterior segment was removed together with the vitreous. The posterior segment was dissected into two parts, and the neural retina was removed.

Small grafts (~ 4 mm^2) consisting of the RPE and choroid were cut and separated from the sclera. These grafts were kept in physiologic salt solution (PSS) until use in the experiments.

Loading of the graft onto the instrument

For every experiment, the graft was placed on an aspiration-reflux spatula (DORC), which was connected to a 5-mL syringe by a 10-cm long polyurethane tube (lumen diameter, 2.3 mm) and filled with PSS. Gentle suction was applied by retracting the syringe plunger for 1 mL, as used during the RPE-choroid translocation surgery in patients. Subsequently, the suction was terminated by a slow reflux, and finally the syringe was disconnected from the spatula. This procedure resulted in a graft adhering to the spatula in a manner that mimics the clinical situation. A new graft was used for every measurement.

Maximum friction force of the graft

First, an experiment was designed to determine the maximum friction force between the graft and the aspiration-reflux spatula (Fig. 2). The results of this experiment were to be used as input for the mathematical model.

On one platform of a fine balance, we secured an aspiration spatula connected by a 10-cm long polyurethane tube (lumen diameter, 2.3 mm) to a 10-mL syringe. The RPE-side of a graft was fixated by applying strong suction (exerted by retracting the plunger of a 10-mL syringe for 8 mL) to the aspiration spatula, while the choroidal side of the same graft was aspirated with another aspiration-reflux spatula by gentle 1-mL suction and slow reflux. This

aspiration-reflux spatula was fixated into an instrument holder. Weights were added to the other side of the balance until tissue release was achieved from the aspiration-reflux spatula by the choroidal side of the graft (Fig. 2). The maximum friction force was calculated from the mass and position of the weights. The experiment was repeated eight times.

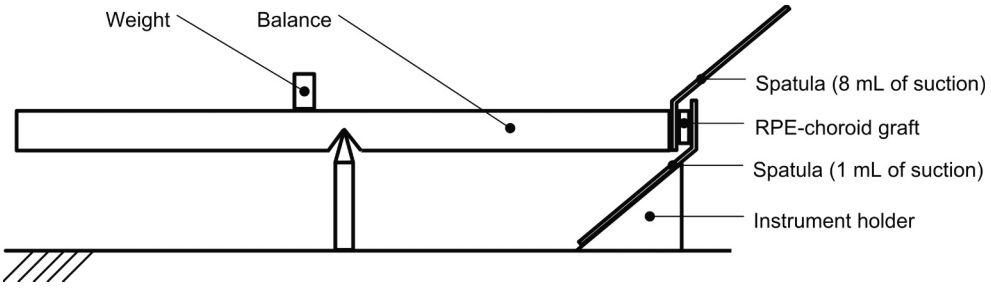

Figure 2. The experimental setup to determine the maximum friction force between the graft and the aspiration-reflux spatula. An RPE-choroid graft was attached on the RPE-side to a spatula attached to the tip of the balance (suction, 8 mL) and on the choroidal side to another spatula (suction and reflux, 1 mL). Weights were added to the other side of the balance until tissue release from the choroidal side was achieved.

Mathematical model

A straightforward mathematical model was derived. The model assumed release of the graft when its inertial force exceeded the friction force between the graft and the instrument.

The graft was modeled as a rigid body, and the friction force was assumed to be acting on the graft's center of mass. No assumptions were made regarding the type of friction between the graft and the spatula. Interactions between the surrounding PSS and the graft were not included in the model.

We estimated the mass of the graft at 0.8 mg by multiplying the dimensions of the graft by its density (2 mm x 2 mm x 0.2 mm x 1000 kg/m^3).

The x-direction for the adhered graft lies in the plane of the blade of the spatula, parallel to the lateral edges (Fig. 3). The equation of motion in the x-direction for the graft was defined as

$$F(t) = m\frac{d^2}{dt^2}\left(x(t)\right) \qquad (1)$$

In this equation, $F(t)$ is the friction force acting on the graft, m is the mass of the graft, and $x(t)$ is the position of the instrument (Fig. 3) and can be written as

$$x(t) = \frac{A}{1000}\sin(2\pi ft) \qquad (2)$$

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where A is the amplitude of the vibration (in millimeters) and f is the frequency (in hertz).

Acceleration of the graft can then be expressed as

$$\frac{d^2}{dt^2}\left(x(t)\right)=\frac{d^2}{dt^2}\left(\frac{A}{1000}\sin\left(2\pi ft\right)\right)=-\frac{A}{1000}\left(2\pi f\right)^2\sin\left(2\pi ft\right) \qquad (3)$$

The absolute maximum value of the friction force throughout time is given by

$$\max\left(\left|F(t)\right|\right)=m\cdot\frac{A}{1000}\left(2\pi f\right)^2=\frac{\pi^2 mAf^2}{250} \qquad (4)$$

If this value exceeds the maximum friction force (F_{max}), the model predicts tissue release. The threshold frequency and amplitude are therefore given by

$$F_{max}=\frac{\pi^2 mAf^2}{250} \qquad (5)$$

Experimental setup

An experimental setup was used to validate the results of the mathematical model (Fig. 3). The experimental setup consisted of an aspiration-reflux spatula fixated into a linear slide. The linear slide consisted of two leaf springs that allowed motion of the instrument holder in the horizontal plane exclusively (i.e., with the spatula moving backward and forward). The tip of the spatula was placed horizontally in a Petri dish filled with PSS to simulate the conditions during surgery (Fig. 3).

To make the instrument vibrate, the linear slide was physically attached to a loudspeaker operating as a linear motor. The loudspeaker was connected to one channel of a stereo amplifier. The amplifier was connected to the sound card of a computer. Vibration of the instrument was achieved by supplying a sinusoidal input signal generated by the computer.

The position of the instrument (x(t)) was measured at 1000 Hz throughout the experiment by a laser displacement sensor (optoNCDT ILD1401-20, Micro-Epsilon Messtechnik GmbH & Co., Ortenburg, Germany). The data were transmitted to the computer by means of a data-acquisition device (LabJack UE9, LabJack Corp., Lakewood, CO).

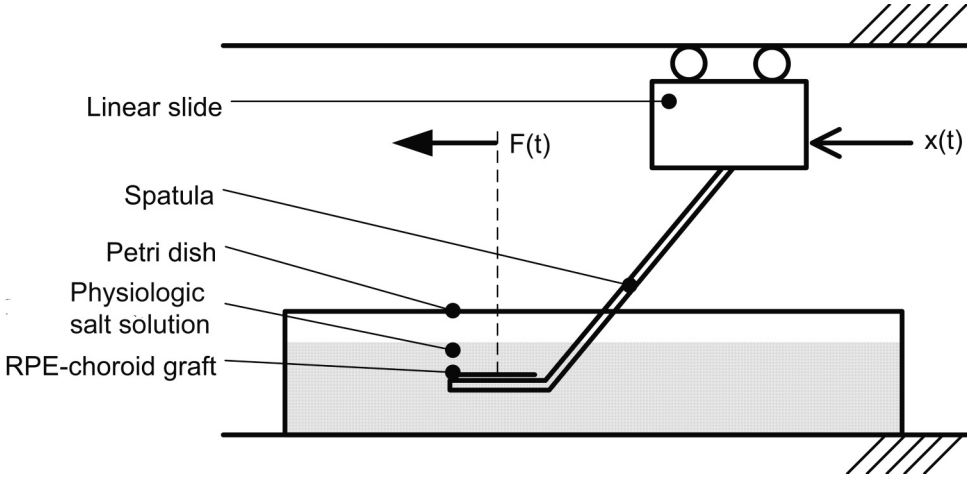

Figure 3. Experimental setup to determine the threshold amplitudes and frequencies for sinusoidal vibrations that induce tissue release from an instrument.

Experimental method

Five seconds after release of the suction, the instrument was vibrated for 1 second. The vibration signal consisted of a sinusoidal waveform starting with a smooth increase to the maximum amplitude (within 250 ms) and ending with smooth decrease to resting position. Special care was taken to have a fast but controlled increase of the amplitude of the input signal to the desired value to avoid an initial peak amplitude overshoot as observed in pilot measurements.

All experiments were monitored on video and assessed after the experiments by an examiner masked to frequency and amplitude. Release of the graft was defined as a complete loss of contact between graft and spatula. Recordings of a sham procedure were made for each combination of frequency and amplitude.

To identify the threshold amplitude and frequency, the experiment was performed at eight frequencies and at four amplitudes per frequency. Each combination of frequency and amplitude was tested five times. If the graft was released three out of five times or more, the graft was said to be released at that combination of frequency and amplitude.

For each frequency, the first amplitude was selected on the basis of the computer simulation model. The sequential amplitudes were chosen with the bisection method: If the graft had been released at the last amplitude, the next amplitude was set to the average of the last amplitude and the highest amplitude at which the graft had not been released and vice versa.

The data were saved and analyzed and the experimental setup controlled by computer (MatLab; The MathWorks, Natick, MA).

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Measurements of the mobile phone vibration device

The amplitude and the movement directions of the tip of the vibrating forceps currently used during surgery were determined.

The instrument was fixated between silicon rubber pads to mimic the surgeon's hand. Measurements were performed at a frequency of 70 and 140 Hz and were recorded in two planes: (1) straight superior of the instrument (observing the forward-backward and side-to-side movement) and (2) from the side of the instrument (observing the upward and downward movement). Recordings (1250 frames per second) were made with a high-speed camera system (Motion Pro 10000 and associated MiDAS software; Redlake Imaging, Tucson, AZ) attached to a microscope (SZ-PT SZ-40; Olympus, Tokyo, Japan).

Results

Maximum friction force of the graft

The maximum friction force between the graft and the instrument was 0.32 ± 0.11 mN (mean ± SD).

Mathematical model

The value of the maximum friction force, as estimated in the balance model, was used in equation 5 of the mathematical model to predict the threshold amplitudes for each vibration frequency (Fig. 4).

The predicted threshold amplitudes were higher than the amplitudes determined with the experimental setup.

Experimental results

The release of the RPE-choroid graft from the instrument could easily be observed. Contact between graft and instrument was lost immediately after onset of the vibration. The graft remained adhered to the instrument during the sham procedures.

The modes of the experimental results are shown in Figure 4. With increasing frequency, the threshold amplitudes remain approximately constant (± 0.15 mm) from about 100 Hz and higher.

The initial amplitude that was tested at 200 Hz did not induce tissue release, and this necessitated a break from the original experimental protocol. The initial amplitude was increased to 0.15 mm, and the protocol was executed again. In addition, experiments were

performed at amplitudes below the initial amplitude to determine whether the results of the experiments at the initial amplitude were accurate.

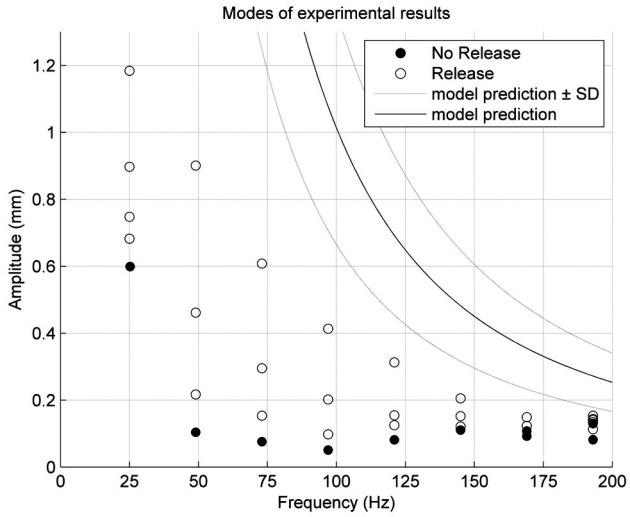

Figure 4. Frequencies and amplitudes to release the graft as predicted with the mathematical model and as estimated with the experiment. The mathematical model curve divides the graph in grafts released (above the line) and grafts not released (below the line).

Measurements of mobile phone vibration device

The amplitude (in millimeters) of the tip of the vibrating forceps in different movement directions (i.e., in different planes), is shown in Table 1. The movements were ellipse–shaped, reflecting the direction of vibration forces caused by the rotating unbalanced motor in the mobile phone vibration device.

Table 1. The amplitude of the ellipse-shaped movements of the tip of the forceps attached to a mobile phone vibration device.

	Movement (mm)		
Frequency (Hz)	**Forward-Backward**	**Side-to-Side**	**Up-Down**
70	0.22	0.29	0.45
140	0.18	0.19	0.40

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Discussion

To improve the predictability of submacular release of the RPE-choroid graft during translocation surgery, we empirically had the translocation instrument vibrate. The vibration was achieved by attaching a mobile phone vibration device (frequency, 140 Hz) to the handle of the instrument. The translocation instrument tip moved approximately less than a quarter of the diameter of a 20-gauge cannula, when viewed with standard video camera recordings edited with video software (Pinnacle Studio ver. 9, Pinnacle Systems, Mountain View, CA).

Vibration of the translocation instrument improved the submacular release of the graft. Because the vibrating instrument was in contact with the choroidal side of the graft, damage to the RPE and retina was unlikely. Potential damage is probably balanced by the advantage gained by having a predictable release with a subsequent decrease of submacular manipulations. Further studies are necessary to exclude an increased release of RPE cells by having the instrument vibrate. However, a smooth graft insertion and less manipulation correlated with better visual outcome.[3]

In this study, a minimum amplitude of ~ 0.15 mm was needed to release the graft. This minimum threshold amplitude was effective from a frequency of 100 Hz and higher. At lower frequencies, a higher amplitude was needed for the release.

High-speed camera observation of the tip of the instrument attached to the mobile phone vibration device revealed that the amplitude in the horizontal plane (forward and backward and side-to-side movement) was already just above the threshold amplitude at a frequency of 140 Hz, as estimated in this study. The vertical amplitude, however, was approximately 0.40 mm. It is uncertain whether release of the graft in the clinical setting was achieved by the amplitude in the horizontal or vertical plane. It is likely, however, that movement in the horizontal plane achieves the safest and most effective instrument tip acceleration to overcome the friction between instrument and tissue.

For the experimental setup, an aspiration-reflux spatula was used instead of the fine forceps currently used during surgery. The rationale was that the suction force could be very accurately reproduced in all measurements, whereas it would have been difficult to achieve an identical grasping force with the forceps or to grasp an identical amount of tissue for each graft.

The mathematical model identified the upper boundary for the theoretical threshold curve. The model predicted higher threshold values than found in our experimental model. This result may be explained by (1) not taking the influence of fluid flow in account; and (2) the estimate of the measured maximum friction force between the graft and the aspiration-reflux spatula was too high in the experimental model, because it was measured in air. However,

the shape of the curve of the mathematical model is almost identical, as estimated with the experimental mode, which indicates that the variables used in the mathematical model were correct.

Backward retraction of the instrument as occurs during surgery was not performed. In our experimental study, the graft was released immediately after onset of the vibration. It is likely that the shearing force caused by vibration is greater than the shearing force of a slowly retracting instrument would be. Therefore, additional retraction may not have influenced the results.

The present study confirmed the clinical impression that having an instrument vibrate helped the release of the RPE-choroid graft. The principle of vibration-induced release may also be valuable for other surgical techniques in ophthalmology. The threshold amplitudes and frequencies for tissue release as well as the instrument tip movements were determined, to be better able to develop an optimized instrument.

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Chapter 6.1

De Nederlandse versie van de Radner leeskaart voor het beoordelen van de functionele visus

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C Meulendijks
W Radner
JC van Meurs
CB Hoyng

Ned Tijdschr Geneeskd 2007;151:2494-6

Samenvatting

- Een kaart voor het meten van de leesvisus is een betere functionele test dan de traditioneel gemeten afstandsvisus met enkele optotypen.
- Eigenschappen voor een goede leeskaart zijn: logaritmische afname van lettergrootte, de mogelijkheid tot het simultaan meten van leesvisus en -snelheid en tot het berekenen van één score waarbij de leesvisus is gecorrigeerd voor het aantal leesfouten.
- De oorspronkelijk Duitstalige Radner leeskaart voldoet aan al deze eigenschappen, maar Radner benadrukt als enige bovendien het principe van de 'zinoptotypen'; dit zijn hooggestandaardiseerde zinnen om de variatie in lexicale moeilijkheid en de invloed daarvan op leesprestatie te minimaliseren.
- Nederlandstalige zinoptotypen zijn ontwikkeld volgens de strikte principes van Radner en deze zijn getest op leessnelheid en aantal leesfouten per zin. De meest overeenkomende zinoptotypen werden geselecteerd voor de introductie en eerste druk van de Nederlandstalige Radner leeskaart.
- De Nederlandse Radner leeskaart is nauwkeurig en praktisch en daarom geschikt voor zowel onderzoeksdoeleinden als de dagelijkse praktijk.

Introductie

In het rapport van het project 'VISION 2020 Netherlands' van de Wereldgezondheidsorganisatie wordt het aantal mensen met een visuele beperking in Nederland in het jaar 2000 geschat op 231.000. Deze groep bestaat uit 168.000 slechtzienden en 63.000 blinden.[1] De verwachting is dat in Nederland het aantal mensen met een visuele beperking aan beide ogen tussen 2000-2020 met 35% zal toenemen. Dit is vooral het gevolg van het toenemende aantal mensen boven de leeftijd van 60 jaar, met leeftijdsgebonden maculadegeneratie als belangrijkste oorzaak.[1]

Beperking van de visusmeting op afstand

Voor veel mensen met een verminderde visus hebben problemen met lezen de grootste invloed op het dagelijkse leven en functioneren.[2-4] De routinematige visusmeting op afstand middels het gebruik van enkele optotypen, zoals die van Snellen, heeft weinig voorspellende

waarde voor het werkelijke leesvermogen en daarmee voor de functionele visus. Dit geldt des te meer als er een afwijking is van de macula (gele vlek), die men nodig heeft voor het centrale zien.[5-7] De verklaring hiervoor is dat een gebrek aan centraal zien bij het op afstand bekijken van enkele optotypen vaak gecompenseerd kan worden doordat men met een meer perifeer gelegen deel van het netvlies gaat benutten: het excentrische kijken. Bij testen op korte afstand en lezen kan de patiënt minder gebruik maken van dit compensatiemechanisme. Ook bij andere afwijkingen, bijvoorbeeld aan het voorsegment van het oog of bij amblyopie, wordt met een leestest gevoeliger gemeten doordat bij het lezen complexere eisen aan het oog worden gesteld en daardoor gebreken eerder naar voren komen.[8-10]

Voordelen van een leeskaart voor het meten van de functionele visus

Een kaart voor het meten van de leesvisus verschaft gedetailleerder informatie over de visuele beperking en deze staat een evaluatie van de werkelijke leesfunctie toe. De kaart moet toepasbaar zijn bij zowel patiënten met een normale tot lage visus als patiënten die visueel beperkt zijn door andere visusvariabelen zoals een verminderd gezichtsveld, verminderde kleur- of contrastgevoeligheid, verminderde licht-donker adaptatie of de aanwezigheid van scotomen.[7,11-12]

Kenmerken van een goede leeskaart

In onze zoektocht naar een leeskaart die geschikt zou zijn voor wetenschappelijk onderzoek en voor de dagelijkse praktijk kwamen wij tot een aantal voorwaarden:

- een logaritmische schaal: de afname van de lettergrootte per zin is uniform (elke zin is een factor 1.2589 kleiner dan de voorgaande), hetgeen de berekening van de leesvisus op verschillende testafstanden mogelijk maakt.[13,14]
- de mogelijkheid om de leessnelheid in woorden/min simultaan met de leesvisus te kunnen meten. Dit is met name bij nauwkeurige metingen voor onderzoeksdoeleinden van belang, omdat de leessnelheid de gevoeligste parameter is en het sterkst correleert met de functionele visus, nog beter zelfs dan de leesvisus.[12, 15]
- de bereikte leesvisus is zowel af te lezen in logMAR- als snellen eenheden (de logMAR-eenheid is de logaritme van de mininale hoek van oplossend vermogen).

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- de mogelijkheid om een score te berekenen waarbij de bereikte leesvisus wordt gecorrigeerd voor het aantal leesfouten, hetgeen vergelijken van opeenvolgende metingen eenvoudig maakt.

De Radner leeskaart

Zowel de Engelstalige 'Minnesota reading chart' (MNREAD) als de Duitstalige Radner leeskaart voldoet aan de genoemde voorwaarden en wordt internationaal veel toegepast .[16,17] De Radner leeskaart heeft echter één extra voordeel: de MNREAD gebruikt zinnen die alleen gelijk zijn in aantal regels en letters, maar niet in grootte en positie van woorden. Radner benadrukt echter het belang van zinstandaardisatie, omdat zincomplexiteit ook de leessnelheid beïnvloedt: bij het afnemen van de leestest mag alleen de afname van lettergrootte invloed hebben op de leesprestatie en niet een verschil in grammaticale moeilijkheid tussen de zinnen. Hij ontwikkelde daarom gestandaardiseerde en zeer vergelijkbare zinnen wat betreft lexicale moeilijkheidsgraad, syntaxis, woordgrootte, aantal lettergrepen en woordpositie (Tabel 1).[17] Deze zogenoemde 'zinoptotypen' resulteerden in een minimale variatie in leesmoeilijkheid tussen de zinnen. De zinoptotypen bleken een hoge betrouwbaarheid en validiteit te hebben bij het testen van het leesvermogen bij personen met een normale en verminderde visus. De validiteit was getest door de leessnelheid van de Radner leeskaart te vergelijken met die van een gestandaardiseerde langere tekst van 261 woorden en de correlatie tussen beide methoden was hoog ($r = 0.9$). Bovendien zijn de Radner leeskaarten onderling getest - er bestaan 3 versies, om een leereffect te voorkomen - en met een tijdsinterval van 3 weken ertussen bij groepen proefpersonen met verschillende afstandsvisus. Zowel leesvisus als leessnelheid correleerde goed in alle groepen ($r > 0.9$).[7,17-19]

Ontwikkeling van een Nederlandstalige Radner leeskaart

Methode

Volgens dezelfde strikte zinsconstructie van de Duitstalige Radner leeskaart zijn 32 Nederlandstalige zinoptotypen gemaakt (zie de Tabel 1, Fig. 1). Het enige kleine verschil is dat de eerste woorden van de 1e en 2e regel uit 2 letters bestaan in plaats van 3, zoals in de Duitstalige versie. De zinnen zijn grammaticaal gecontroleerd door de Faculteit der Letteren van de Universiteit van Utrecht.

Tabel 1. De strikte regels voor de zinconstructie voor de Radner leeskaart;[13] iedere zin bestaat uit 3 regels van 14 woorden, 22-24 lettergrepen en 82-84 aanslagen.

	aantal		**woord**					**opmerking**
	aanslagen	**letter-grepen**	**1e**	**2e**	**3e**	**4e**	**5e**	
regel 1	27-29	7-8		znw†	znw†			≥ 2 woorden van 2 lettergrepen, waarvan er 1 een znw mag zijn
aantal lettergrepen			1					
aantal letters			2					
regel 2	27-29	7-8		znw				2e woord wordt gevolgd door een komma en een bijzin
aantal lettergrepen			1	3	1	1	1	
aantal letters			2	10				
regel 3	27-29	7-9		znw	ww	ww		
aantal lettergrepen			2	2	3	2		

Znw = zelfstandig naamwoord; ww = werkwoord; † ofwel het 2e ofwel het 3e woord is een znw.

De jongens waren zes maanden
op wereldreis, toen zij ons
deze foto opgestuurd hadden

Na schooltijd gingen ze naar
de dierentuin, waar net twee
bruine wolven geboren waren

De jonge pastoor zei tijdens
de avondpreek, dat hij graag
alle dagen voetballen wilde

Figuur 1. Voorbeeld van 3 zogenaamde zinoptotypen opgesteld volgens de principes van zinstandaardisatie van de Radner leeskaart, voor het beoordelen van de leesfunctie. De lettergrootte is 12 punten, lettertype Arial.

Om de meest op elkaar lijkende zinnen te selecteren, op basis van leessnelheid (woorden/min) en aantal leesfouten, werden 109 studenten met Nederlands als moedertaal getest. Inclusiecriterium was een minimale visus van 6/6 voor beide ogen. Exclusiecriteria waren aanwezigheid van een ziekte en gebruik van medicatie die het reactievermogen kunnen beïnvloeden, en daarmee het leesvermogen. Er werden 3 versies met een verschillende

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zinsvolgorde getest om een eventueel leereffect te spreiden en iedere student las 1 van de 3 versies. De testen werden binoculair afgenomen op 40 cm testafstand en een verlichting van 80-90 cd/m^2. De lettergrootte was 12 punten en het gebruikte lettertype was Arial, een schreefloze letter. Dit is een lettergrootte boven de drempelwaarde zodat het oog binnen fysiologische grenzen werkt, waardoor de resultaten niet door andere condities werden beïnvloed. De metingen werden uitgevoerd door 2 examinatoren en op video opgenomen om de nauwkeurigheid van de metingen te verifiëren indien deze niet overeen zouden komen. Om de meest vergelijkbare zinnen te selecteren werden zinnen uitgesloten als ze minimaal aan 1 van de volgende 2 voorwaarden voldeden: als de leessnelheid van een zin meer dan 1 SD afweek in meer dan één versie, of als in een zin meer dan gemiddeld studenten leesfouten maakten (gemiddeld ± 1 SD). Op basis van deze criteria werden uiteindelijk 8 zinnen uitgesloten. De interne consistentie tussen de 24 overgebleven meest overeenkomende zinnen op basis van leessnelheid liet een α-coëfficiënt van Cronbach van 0.98 zien. In alle 3 de versies met een verschillende zinvolgorde bleek geen leereffect te zijn opgetreden. De metingen van de 2 examinatoren van de 32 zinnen hadden een hoge correlatie: 0.99 (t-toets).

Beschouwing

De geselecteerde 24 zinoptotypen die een hoge interne consistentie bleken te hebben, zijn gebruikt voor het drukken van de Nederlandstalige Radner leeskaart. Nu deze logaritmisch geschaalde Radner leeskaart voorhanden is, is een goede volgende stap het testen van de betrouwbaarheid bij oudere mensen met normale tot verminderde visus. Bij het selecteren van de meest overeenkomende zinnen was namelijk daarentegen bewust gekozen voor proefpersonen met een visus van 6/6 om te voorkomen dat de resultaten werden beïnvloed door andere factoren. Bij het testen van slechtzienden zou de spreiding in meetresultaten te groot worden, wat de selectie van de meest overeenkomende zinnen had belemmerd.

Voor het testen van de 32 zinnen volstond lettergrootte 12 punten Arial, want het doel was niet om de leesvisus of -snelheid voor deze specifieke lettertype en deze lettergrootte op deze leesafstand of deze specifieke zinnen te testen, maar om de Nederlandstalige zinoptotypen te testen op hun gelijkheid, op basis van leessnelheid en aantal leesfouten.

Een beperking is dat de zinnen alleen bij universitaire studenten getest zijn. Dit is een homogene groep, hetgeen resulteert in een lage standaarddeviatie en een hoge vergelijkbaarheid in gemeten leessnelheid van de overgebleven 24 zinnen: de cronbach-α-coëfficiënt was 0.98. Er zijn geen lageropgeleiden getest, wat wel gedaan is bij de originele Duitstalige Radner

leeskaart.[18] Die studie liet zien dat, hoewel de lageropgeleiden significant langzamer lazen met meer leesfouten, de cronbach-ï-coëffi ciënt (van studenten en lageropgeleiden samen) van hun 24 overgebleven zinnen 0.98 was.[19] We veronderstellen daarom dat het bestuderen van een meer heterogene groep dezelfde uitkomst zou geven.

Conclusie

Deze Nederlandstalige Radner leeskaart is primair ontwikkeld voor wetenschappelijk onderzoek. Omdat de Radner leeskaart in toenemende mate wordt overgezet in andere talen en daarmee in steeds meer internationale studies wordt gebruikt, maakt een Nederlandstalige versie het mogelijk om aan internationale studies mee te doen of resultaten beter te vergelijken. Echter, aangezien de Radner leeskaart erg gebruiksvriendelijk is en het gebruik ervan niet meer tijd kost dan andere beschikbare leeskaarten in Nederland kan deze ook in de dagelijkse praktijk gebruikt worden voor de simpele bepaling van de leesvisus.

Dankbetuiging

Deze studie werd gefinancierd door de SWOO- Flieringa stichting (Stichting Wetenschappelijk Onderzoek Oogziekenhuis Rotterdam) aan Maaijwee en de Gelderse Blindenvereniging aan Meulendijks. De auteurs bedanken Leo Lentz, Faculteit der Letteren, Universiteit van Utrecht, voor de grammaticale controle van de Radner leeskaart zinnen.

De Radner leeskaarten zijn te bestellen bij Maaijwee (zie correspondentie adres). De kosten zijn 90€ exclusief BTW. De opbrengst komt geheel ten goede aan de SWOO- Flieringa stichting (Stichting Wetenschappelijk Onderzoek Oogziekenhuis Rotterdam).

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Abstract

The Dutch version of the Radner Reading Chart for assessing vision function

- A reading chart for measuring visual acuity is a better functional test than the routine distance visual acuity testing with single optotypes.
- The characteristics of a good reading chart are: a logarithmically diminishing print size, simultaneous measurement of reading acuity and reading speed, and the calculation of one score for reading acuity corrected for the number of reading errors.
- The original German-language Radner Reading Chart meets all these requirements, and above all emphasizes the principle of "sentence optotypes" i.e. highly standardized sentences, because sentence complexity also influences reading performance.
- Sentence optotypes were created in the Dutch language according to Radner's strict principles and tested. The most equally matched sentence optotypes in terms of reading speed and number of reading errors were selected for the introduction and printing of the first Dutch version of the Radner Reading Chart.
- The Dutch Radner Reading Chart is precise and practical and therefore useful for research and daily practice.

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Chapter 6.2

Reliability testing of the Dutch version of the Radner Reading Charts

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P Mulder
W Radner
JC van Meurs

Optom Vis Sci 2008; in press

Abstract

Purpose

To statistically analyze the test-retest and inter-chart reliability of the newly developed actual logarithmically scaled Dutch Radner Reading Charts in an older population affected by macular disease. These Dutch Radner Reading Charts are developed according to the strict principles of sentence construction of the originally German language Radner Reading Charts.

Methods

Thirty-six subjects aged 50 years or older and affected with a macular disease monocularly read the three charts of the Radner Reading Charts in a randomized order twice with one month in between. The subjects were divided into three groups according to their distance logMAR visual acuity (group 1, ≥ 0.1; group 2, 0.12–0.4; and group 3, 0.42–0.8). Reading acuity (logRAD, the reading equivalent of logMAR), logRAD score, maximum reading speed, critical print size and logRAD/logMAR ratio were measured. Variance component analysis was used to determine the sources of variability.

Results

The test-retest and inter-chart reliability was high for all visual acuity groups and variables. For all groups together the chart accounted for maximal 5 % of the total variability for all measurements. The individual subject did have the largest influence on the measurements (88 – 98% of the variability).

Conclusions

The Dutch version of the Radner Reading Charts provided high reliable test-retest and inter-chart measurements of reading performance in a heterogeneous group of subjects with subnormal to low vision. In addition, this study showed that the strict principles of sentence construction of the originally German Radner Reading Charts may also be successfully used for other languages.

Introduction

For many persons dealing with visual impairment, reading difficulty has the largest impact factor on their daily life and functioning.[1-3] Routine single optotype distance visual acuity (VA) tests have been shown to be poor predictors for reading performance and cannot elucidate the full functional impairment of several ophthalmic diseases.[4-6] Reading performance tests can provide more detailed information about visual impairment and allow the clinical evaluation of reading function. This is useful and suitable in low vision patients as well as in patients with almost normal distance vision, who are, nevertheless, visually handicapped. The latter group involves patients with a (near to) normal distance acuity threshold, but with a decrease in other vision variables that influence daily life and functioning like e.g., a decrease in visual field, color or contrast sensitivity, and light-dark adaptation.[6-8]

In order to create a well-standardized and reproducible Dutch reading performance test for daily practice as well as scientific research, various designs of (inter) national reading charts were studied. Both the Minnesota Reading (MNRead) chart as well as the Radner Reading Charts meet the requirements of a logarithmically progressing print size from one sentence to another, and the possibility to acquire reading speed (in words/minute) and reading acuity simultaneously. [9-11] The Radner Reading Charts had one more advantage over the MNRead; the MNRead used sentences that were only similar in number of lines and number of characters, but not in length and position of words. However, Radner et al. emphasize the importance of sentence standardization, because sentence complexity also influences reading speed. He therefore developed and standardized highly comparable sentences in terms of lexical difficulty, syntactical complexity, word length, number of syllables, and position of words.[11] These so called 'sentence optotypes' result in a minimal variation between the test items in terms of reading difficulty and optimal constant geometric proportions, as the sentences are almost equal in length. These sentences were shown to be of almost equal reliability in reading performance, and with a high validity in subjects with normal and impaired visual acuity.[6,11-13] Therefore, in a previous study, we developed 32 sentence optotypes in the Dutch language according to the strict principles of Radner and we tested the sentences in 109 university students with a VA of 20/20, which is a very homogenous group of subjects. The 24 sentences most comparable in reading speed and reading errors were selected for the introduction of the Dutch version of the Radner Reading Charts.[14]

The purpose of the present study is to test the logarithmically scaled (logRAD) Dutch Radner Reading Charts in an older population affected by macular disease to analyze statistically the reliability of the newly developed reading chart in a heterogeneous group of patients.

Methods

Subjects

The 36 subjects selected were all patients with macular disease in the tested eye and aged 71 ± 11 (mean ± SD) years (range 50-92). The pathology consisted of drusen maculopathy (n = 9), atrophic age-related macular degeneration (AMD) (n = 10), exudative AMD (n = 11) and diabetic retinopathy (n = 6). The patients were eligible if their disease had been unchanged in the last three months and progression within the next month was unlikely. Exclusion criteria included the inability to read Dutch and a non-ocular disease or use of medication or drugs that could influence their cognitive skills. The patients were divided in three groups of 12 subjects (6 females and 6 males each) according to their distance logMAR VA (group 1, ≥ 0.1; group 2, 0.12–0.4; and group 3, 0.42–0.8).

The Institutional Review Board of the Rotterdam Eye Hospital approved the study; informed consent was obtained from all patients in accordance with the ethical standards laid down in the Declaration of Helsinki.

Testing procedure of the subjects

The Radner Reading Charts consists of three charts with 14 sentences each with a print size ranging from 1.2-0.2 logRAD (reading equivalent of logMAR) (equivalent to 6.4-0.25 M) when viewed from a reading distance of 40 cm. An example of three sentences is shown in Figure 1. These 14 sentences are selected from a pool of 24 sentences and are arranged such that patients with a VA of < 0.1 logRAD do not come across one sentence twice from one chart to another.

We wished to test the inter-chart reliability (measured within one testing procedure) and test-retest reliability (with one month in between two measurements). To minimize the learning effect the sequence of the three reading charts were randomly assigned according to the orthogonal Latin square design.

The testing procedure was performed monocularly with the subject's optimal refractive correction after a full objective and subjective refraction. At the second testing procedure the same refractive corrections were used. First, the distance VA was determined at each testing session using the Early Treatment Diabetic Retinopathy (ETDRS) chart. If distance VA had changed after one month the subject was withdrawn from the study.

The reading test was performed at a distance of 28 cm with a luminance of 80-90 cd/m^2. To ensure a constant viewing distance within and between the testing sessions, a special

experimental set-up was designed. This construction consisted of a headrest for the forehead attached to a chart holder. The sentences were covered with a piece of paper, and the subjects were asked to uncover sentence after sentence, reading each one aloud as quickly and accurately as possible, and to read the sentence to the end without correcting any reading errors. The subjects continued reading until no words could be read in a sentence.

All testing procedures were monitored with a video and audio recording. These recordings were used to later measure the reading speed and number of syllables of the missed, mispronounced or repeated words. One examiner (KM) performed both the testing procedures and measurements of the recordings. The examiner did not give instructions during either testing procedures influencing the patients' reading performance. At time of analyzing the recordings the examiner was masked to the results of the first testing session per subject.

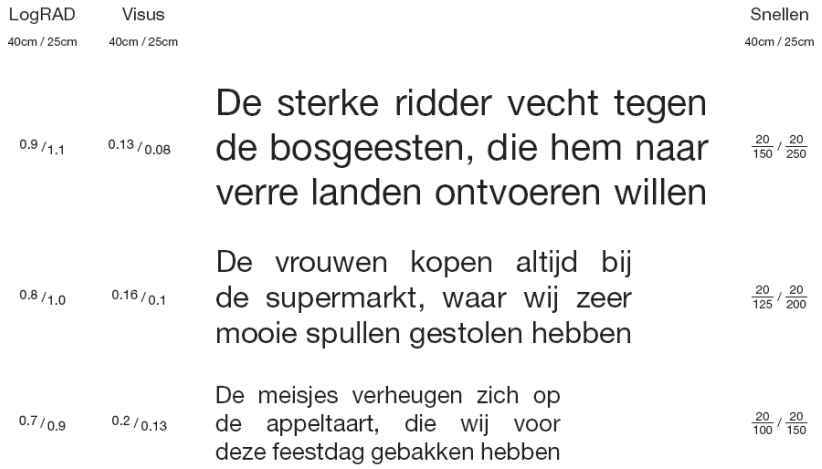

Figure 1. Example of Dutch sentences tested in this study. The sentences were constructed according the strict principles of the Radner Reading Charts.

Analysis of the measurements

The variables determined were the reading acuity (logRAD), reading speed per sentence in words per minute (wpm), the maximum reading speed (wpm), the critical print size (CPS) and the logRAD/logMAR ratio (%).

If a subject started to make more reading errors with decreasing print size, the criterion for reading acuity was defined as read more than 80% of the words correctly.[15] The exact viewing distance was 28 cm (instead of the initial desired 32 cm). A correction value of 0.15 logRAD had therefore to be added to the reading acuity.

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The number of syllables of the incorrectly read words, even when immediately corrected, of the last sentence was counted. Each syllable is given a value of 0.005 logRAD, as most sentences have 20 syllables and one sentence represents an increase of 0.1 logRAD in print size.[11] To correct the reading acuity for the number of reading errors a logRAD score was calculated (logRAD score = logRAD + 0.005 x syllables of incorrectly read words).

As the distance acuity and reading acuity can differ significantly in some diseases, the logRAD/logMAR ratio was calculated (1 – logRAD divided by 1 – logMAR times 100).[6,16-18]

The reading time per sentence was measured twice with a stopwatch. If more than 0.2 seconds difference existed between the measurements within one sentence an extra measurement was made. The reading speed (wpm) was calculated based on the number of words in a sentence and the time needed to read the sentence (14 words x 60 sec divided by the reading time).

For determination of the maximum reading speed the reading speed of the first two sentences (1.35 and 1.25 logRAD) were excluded as it is known that reading speed decreases from optimum reading speed for both small and very large print sizes.[19,20] The mean reading speed of the subsequent sentences up to the CPS sentence was defined as maximum reading speed.

The CPS is the print size at which reading speed starts to deteriorate; after calculating the reading speed per sentence (wpm) this was plotted on a graph and the print size where the line suddenly dropped was defined as the CPS.

Statistical analysis

A variance component analysis was used to identify which part of the total variability could be attributed to which source. The sources of variation were: subject (36 subjects), session (two sessions with one month in between), chart (three chart types per session), and residual error. The within-session order effect was considered a fixed effect and a proxy for the learning effect. The contribution of each source to the total variability was calculated as a percentage. The variance components were determined for the variables reading acuity (logRAD), logRAD score, maximum reading speed (wpm), CPS and logRAD/logMAR ratio.

The analyses were performed for each group separately (i.e. the three groups of subjects divided according to their distance VA) and for all 36 subjects together. The relative contribution of subject in the total variance is a measure of the reliability of the measurement, known as the intra class correlation coefficient (ICC). The standard error of measurement (SEM) was defined as the square root of the sum of the within-subject variance components due to session, chart and residual error. The reproducibility was determined as $1.96\sqrt{2} = 2.77$ times the SEM, representing the maximum absolute difference between two measurements within

the same subject that may be due to chance with 95 % probability.

To enable comparing results of this study with repeatability studies, the SEM without session component and the repeatability, defined as reproducibility with the exclusion of the session component, were also determined.

The SEM, reproducibility and repeatability have the same dimension as the variable considered, while the ICC is a dimensionless number. The reproducibility can be considered the proper generalization of the limits of agreement introduced for duplicate measurements by Bland and Altman.[21] As a further generalization of the Bland and Altman method we investigated the level-dependency of the measurement error by considering the rank correlation between the within-subject standard deviations and the within-subject averages across all subjects. Finally, a mixed model ANOVA was done to investigate the dependency of reading speed (wpm) on sentence, and the possible modification of this fixed effect by group and chart through entering and testing the appropriate interaction terms as fixed effects in the model. In this analysis subject, session and chart (unless its interaction with sentence was significant) remained as random effects.

Statistical analyses were performed using statistical software SAS Release 8.2 (SAS Institute, Cary, N.C., USA).

Results

In total 45 subjects were included. Nine of these subjects were withdrawn from the study as three of them failed to return, five had an increase or decrease in distance VA at the second testing session and one patient was influenced by alcohol abuses at the second testing session.

The distance logMAR visual acuities (mean ± SD) for the three subgroups were 0.05 ± 0.05 (group 1, range: –0.1 - 0), 0.15 ± 0.06 (group 2, range: 0.1 - 0.21) and 0.55 ± 0.18 (group 3, range: 0.4 – 0.92). The reading logRAD visual acuities (mean ± SD) were 0.16 ± 0.11 (group 1, range 0 - 0.55), 0.37 ± 0.17 (group 2, range 0.11 - 0.65), and 0.66 ± 0.21 (group 3, range 0.45 – 1.25).

In all 36 subjects together the chart (chart 1, 2 or 3) accounted for maximally 3% of the variance (Table 1). This concerns the between-charts within-session within-subject component. The between-sessions within-subject component had a maximum of 5 %. The individual subject (between-subjects component) contributed the major part to the variance (88-98% in the total group of subjects), hence the ICC ranged from 0.88 to 0.98 in the total group. The

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maximum reading speed had the highest between-charts and between-sessions components. The residual error or "unidentified within-subject sources" had the highest within-subject contribution to the variance, be it maximally only 5 % (Table 1). The scatter plots of the within-subject standard deviations against the within-subject averages across all subjects in the total group showed rank correlations ranging from –0.31 to 0.11 with p-values ranging from 0.07 to 0.81 for the five variables considered (results not shown). Hence, there was no clear suspicion of any variable having a level-dependent measurement error.

The relation between reading speed (wpm) and sentence was significantly influenced by group ($P < 0.0001$), but not significantly so by chart ($P = 0.075$).

Discussion

The special quality of the original German language Radner Reading Charts that distinguishes this chart from other reading charts like the MNRead, is the principle of sentence optotypes. These are highly comparable sentences constructed according to very strict criteria resulting in a minimum variation in sentence complexity.[11] The purpose of the present study was to investigate whether the use of these strict principles to construct sentence optotypes in another language (Dutch) would also result in the same high reliability as already observed with the German Radner Reading Charts.[13] In a previous study, we developed Dutch sentence optotypes. The finally selected 24 sentence optotypes were very similar in terms of reading speed and number of reading errors (Cronbach's alpha coefficient of 0.97).[14] However, these data were obtained in a very homogenous group of university students, and all sentences had the same print size. Therefore, we decided to test the reliability of the actual logarithmic progressing Radner Reading Charts in a more heterogeneous group of patients aged 50 years or older and with a macular disease. The variance component analysis of this study showed a high test-retest and inter-chart reliability for all visual acuity groups and variables. The three charts of the Dutch Radner Reading Charts caused minimum variability (accounting only up to 5% of the variance). This also means that there were no substantial learning or fatigue effects, as already reported for other reading charts.[10,22] Moreover, in daily practice only one chart of the three will be normally used minimizing the learning effect even more.

The individual subject explained the largest part of the variance, which indicates that the Radner Reading Charts accurately reflects the personal performance of each tested individual. The logRAD score (reading acuity corrected for the number of reading errors) had exactly the same influence on the variance as the reading acuity alone.

Table 1. Results of the statistical analysis: 1. total standard deviation (SD); 2. the variability (% of total variance) being introduced by each parameter: the individual subject, the reading chart (three charts per session), session (two sessions with one month in between), and unidentified sources (error); 3. the standard error measurement (SEM) equals the within-patient SD, defined as the square root of the sum of the within-patient variance components due to session, chart and error; 4. the reproducibility is defined as $1.96\sqrt{2}$ = 2.77 times SEM, which is the maximum absolute within-patient difference due to chance ("limits of agreement"); 5. the SEM without session component; and 6. the repeatability is defined as reproducibility with the exclusion of the session component.

Variable	Group	SD	% subject (95 % CI)	% Session	% Chart	% Error	SEM	Reproducibility.	SEM without session component	Repeatability
VA (logRAD)	1	0.121	77.3 (61.7 – 91.9)	12.2	0.8	9.7	0.057	0.159	0.039	0.108
	2	0.178	95.7 (91.8 – 98.5)	0	1.0	3.2	0.037	0.101	0.037	0.101
	3	0.222	96.3 (92.9 – 98.7)	1.4	0	2.3	0.043	0.118	0.034	0.094
	1+2+3	0.266	97.0 (95.5 – 98.2)	1.1	0.2	1.7	0.046	0.127	0.036	0.101
LogRAD-score	1	0.121	77.3 (61.6 – 91.9)	12.2	0.8	9.7	0.057	0.159	0.039	0.108
	2	0.177	95.8 (91.8 – 98.5)	0	1.1	3.1	0.037	0.101	0.037	0.101
	3	0.224	96.4 (93.0 – 98.7)	1.3	0	2.3	0.043	0.118	0.034	0.094
	1+2+3	0.266	97.0 (95.5 – 98.2)	1.1	0.2	1.7	0.046	0.127	0.036	0.101
Maximal reading speed (wpm)	1	21.4	90.9 (83.2 – 96.7)	0	3.4	5.7	6.5	17.9	6.5	17.9
	2	20.0	71.8 (54.2 – 89.8)	11.6	3.1	13.6	10.6	29.5	8.2	22.6
	3	32.3	84.1 (71.8 – 94.4)	6.8	4.4	4.7	12.9	35.7	9.7	27.0
	1+2+3	29.3	87.7 (82.1 – 92.6)	4.5	2.8	5.0	10.3	28.5	8.2	22.7
CPS (logRAD)	1	0.180	79.1 (64.3 – 92.6)	8.6	6.4	5.9	0.082	0.228	0.063	0.174
	2	0.166	85.9 (75.0 – 94.9)	1.3	2.6	10.2	0.062	0.172	0.06	0.164
	3	0.189	89.5 (80.8 – 96.3)	3.7	0.9	5.9	0.061	0.170	0.049	0.136
	1+2+3	0.233	91.2 (87.0 – 94.7)	2.7	1.8	4.3	0.069	0.192	0.058	0.159
LogRAD/ logMAR-ratio (%)	1	0.118	77.4 (61.8 – 92.0)	12.3	0.9	9.5	0.056	0.155	0.038	0.105
	2	0.234	96.6 (93.4 – 98.8)	0	0.9	2.5	0.043	0.119	0.043	0.119
	3	0.951	98.7 (97.4 – 99.6)	0.3	0	1.0	0.108	0.300	0.093	0.258
	1+2+3	0.555	98.2 (97.3 – 99.0)	0.5	0	1.3	0.074	0.204	0.063	0.174

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This is explained by the fact that the "weight" of misread syllables is that low (0.005 logRAD per incorrect read syllable) compared to the reading acuity (logRAD), that is does not significantly influence the total logRAD score. However, for studies interested in detecting minimum visual changes in reading acuity per subject the use of the logRAD score remains recommended.

Compared to the study by Stifter et al. where the same variance component analysis was performed for the original Radner Reading Charts, the present study is in line with theirs with respect to the minimum variability being introduced by the reading chart itself and test replication ("chart" respectively "session" in Table 1). However, the current study showed a smaller error component for each of the variables assessed, especially concerning the CPS.[13] An explanation might be that Stifter et al. performed the stopwatch measurements immediately at the testing session and assessed the CPS at that moment. In our study, all measurements were made using video and audio recordings. The reading time per sentence was measured twice and if more than 0.2 seconds difference existed between the measurements within one sentence an extra measurement was made. Subsequently, the reading speed was calculated (wpm) and plotted on a graph to estimate the CPS. This procedure is much less prone to incorrect measurements and subjectivity of the examiner and might explain the difference in unidentified sources (error) observed between the studies of Stifter and ours.

The test-retest repeatability of two MNRead charts was assessed with only a short break for the subjects (n = 30) in between two measurements.[23] To compare their repeatability outcomes with our reproducibility outcomes (testing sessions with one month in between), we had to omit the session component in the analysis (Table 1). The results of the present study compare favorably with those of the MNRead, even though they tested 30 normal young adults (mean age of 23 years) compared to our subjects aged 50 years or older affected by a macular disease; their coefficient of repeatability was 0.05 logMAR for reading acuity, 8.6 wpm for reading speed and 0.12 logMAR for CPS, compared to our results of 0.036 logRAD (equivalent of logMAR), 8.2 wpm and 0.06 logRAD, respectively.[24] These small differences are probably related to the principle of sentence optotypes used for the development of the Radner Reading Charts. Another explanation might be the use of the Ariel font (a sans serif font) for the Radner Reading Charts and Times Roman font (a serif font) for the MNRead charts, as sans serif fonts are read significantly better (however, with only a minimal difference) than serif font in subjects with low vision.[24] This difference was not observed in subjects with a normal vision.[25]

As expected, the mean reading acuity was worse than the mean distance acuity as the subjects in this study were all affected by a macular disease. Different macular diseases as well as

their stages influence the reading variables (reading acuity, maximum reading speed, CPS) differently.[6,7] In the present study all reading variables accounted for almost the same degree of variance for both the subjects, chart, time-point of measurement (session) and residual effects (Table 1). We could neither observe an increase of the individuals contribution to the variance with decreasing distance VA, which is normally specifically true for the maximum reading speed.[5,6,13, 20] These different findings might be explained by the variety of macular diseases of the subjects included in this study.

We initially intended to test the subjects at a testing distance of 32 cm like Stifter et al. did with the originally German language Radner Reading Charts.[13] However, the special designed headrest attached to the chart holder resulted in a distance of 28 cm between the subject's eye and the Radner Reading Charts. As we only discovered this later on in the study we decided to continue at this distance.

The testing conditions as described in this study are not representative of daily practice, but were performed this way to estimate precisely the variability of all sentences. The estimated high reliability of the sentences reflects that the principle of sentence standardization minimizes variation between test items. That means that in clinical practice, where it is first not necessary and secondly often not possible to perform the measurements that precisely as described in the present study, there will inevitably be a higher variability and consequently lower reliability of the measurements. However, the present study served to show that the degree of variability influenced by the chart itself is minimal (5% of the variance).

The Dutch language was the first foreign language the Radner Reading Charts were converted to. At the moment the Radner Reading Charts are being developed in several more languages (e.g. Swedish, English, French and Spanish). The Dutch and German languages share many features in idiom and syntax. However, the German sentences could not literally be translated into Dutch and new sentence optotypes had to be composed. For this purpose, the original Radner criteria could almost be identically followed. This may not be possible for other less related languages. Other sentence criteria have to be developed reflecting the typical characteristics of that specific language regarding lexical difficulty, syntactical complexity, word length, number of syllables, and position of words. Therefore, it is important for every single language to test the reliability of the Radner Reading Charts.

The current study showed the high inter-chart and test-retest reliability of the Dutch Radner Reading Charts. This makes the chart suitable for scientific research. Soon the Radner Reading charts will be available in more languages enabling participation in multicenter trials evaluating reading vision with one reading chart. Moreover, the Radner Reading Charts are also very convenient to use, which makes them also appropriate for daily practice.

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Chapter 7

General discussion

This thesis was set up to evaluate the new surgical technique of autologous full-thickness retinal pigment epithelium (RPE)-choroid translocation in the treatment of patients with exudative age-relate macular degeneration (AMD).

Topics of interest of the clinical evaluation were visual outcome after surgery, intraoperative and postoperative complications, influence of preoperative and intraoperative variables on visual outcome after surgery, and to study whether the RPE-choroid graft would be a suitable treatment for patients with an RPE tear secondary to AMD.

In addition, a variety of aspects was studied:

- the revascularization of the RPE-choroid graft with the use of angiography in patients, and with histological analysis after in vivo and ex vivo experiments;
- the effectiveness of the principle of a vibration to improve the submacular release of the RPE-choroid graft, and the estimation of the minimum threshold amplitude and frequency needed for optimum tissue release in an experimental setup;
- a new Dutch reading chart was developed and tested for its reliability to accurately obtain the functional vision in patients treated with an RPE-choroid graft translocation before and after surgery.

7.1 Clinical results

An autologous RPE-choroid translocation was performed in 84 patients with late stage AMD, a group in which the subfoveal serum or blood leakage and progressive scarring might already have been harmful to the overlying neurosensory retina, even though the inclusion criteria included a visual loss of less than 6 months.[1] Some patients had been previously treated with laser, photodynamic therapy (PDT), or intravitreal bevacizumab (Avastin, available March 2006 in our hospital). Despite these poor prognostic factors and with a mean preoperative visual acuity (VA) of 20/200, an RPE-choroid translocation resulted in stabilization and even slight improvement of the mean VA in these patients up to four years after surgery.[1] One fourth of the patients reached a VA of 20/80 at one year after surgery.[1]

A separate group of subjects studied concerned six patients with an RPE tear secondary to AMD for which up till today no treatment was available. The mean preoperative VA improved from 20/160 to 20/80 at 14 ± 6 months (mean ± SD) after surgery.[2]

However, as our series were non-controlled, one might argue that the extraction of the choroidal neovascular (CNV) membrane and blood alone was responsible for visual improvement. In comparison, the Submacular Surgery Trials (SST) showed that CNV-removal alone resulted

in a VA of 20/400 at two years after study entry (VA of 20/100 at baseline) and 4 out of 214 (2%) patients reached a VA of 20/80 at one year after surgery.[3] This comparison strongly suggests that the RPE-choroid graft was indeed responsible for functional improvement.

The inclusion criteria in the macular translocation (MTS) studies were comparable, and the patients had the same mean preoperative VA of 20/200.[4-7] Their one and two-year outcomes revealed improvement or stabilization (< 3 ETDRS-lines loss) in half of the patients compared to 68% after the RPE-choroid translocation.[1,4-7] The intraoperative course of MTS is more complex with consequently more intraoperative manipulations. This is reflected by a higher postoperative complications rate, like retinal detachment secondary to proliferative vitreoretinopathy (PVR) in 17-26% of the patients with MTS, compared to 8% with RPE-choroid translocation. A study in this thesis confirmed this relation between intraoperative course and postoperative results, i.e. the worse the intraoperative course, the worse the postoperative visual outcome.[8]

In 2006, two randomized controlled trials (ANCHOR and MARINA study) showed the effectiveness of therapy for subfoveal exudative AMD with intravitreal injection of vascular endothelial growth factor (VEGF) inhibitors (anti-VEGF).[9-11] VEGF inhibitors (Lucentis, Avastin) are currently the treatment of choice with their promising visual outcome and low risks.

However, the patients included in the anti-VEGF studies were not treated before and had (much) less advanced AMD at baseline, i.e. a mean VA of 20/50 - 20/63 (mean 54 - 46 letters on the ETDRS VA chart), a lesion size of less than 12 disc area's (DAs), and subretinal hemorrhage of either ≤50% of the total lesion or ≤1 DA in size.[9-11] Therefore, the comparison of the clinical outcomes in terms of VA between anti-VEGF randomized controlled trials and the current RPE-choroid translocation series is flawed. However, anti-VEGF is certainly associated with much less complications than surgery.

The next step currently being considered is a randomized controlled trial in which the RPE-choroid graft translocation is compared to bevacizumab (Avastin). Patients with AMD are eligible if they have a CNV with a pigment epithelium detachment, or with a hemorrhage involving more than 50% of the lesion, i.e. those patients excluded from the anti-VEGF trials.

7.2 Revascularization of the graft

Revascularization of the RPE-choroid graft is likely to be required for survival and therefore function of both the graft and the overlying neurosensory retina. The principle of RPE-choroid graft translocation is comparable with skin graft transplantation. Vessel growth by angiogenesis into the skin graft has been first reported in the 50's by Converse et al. and have ever since been extensively investigated in general surgery.[12] The revascularization of a transplant in the eye had not been previously studied.

We wished to obtain post-mortem eyes for histological evaluation from patients treated with an RPE-choroid graft. Complex legislation made us abandon this initiative.

In patients, daily repeated angiograms after surgery would have been necessary to exactly determine the moment of reperfusion of the RPE-choroid graft. However, in our institution, such repeated angiograms were not considered to be in the patients' best interest. Therefore, angiography was only performed if pathology of the treated or fellow eye was suspected. The data of the latter group were used to angiographically study the perfusion of the graft by comparing fluorescein angiography (FA) and indocyanine green angiography (ICGA). This resulted in a recommendation to use the early phase of the FA to assess the graft perfusion.[13] More detailed information about the feeder vessels and filling pattern of the graft can be obtained from high-speed angiography, but this device is not commonly available in most institutes.

MacLaren et al. reported on high-speed ICGA in four patients with AMD treated with an RPE-choroid graft. Angiography showed that the graft was not perfused from a short posterior ciliary artery underneath the graft, but was horizontally perfused from the residual adjacent choroid.[14] This is in contrast with the histologically observed vertically bridging vessels between the recipient and graft in the experimental in vivo study in pigs, as described in this thesis.[15] However, in the pigs only the Bruch's membrane of recipient layer directly underneath the graft was intentionally damaged. The graft placed upon this recipient site is only in contact with the underlying choroid and does not contact the choroid from the surrounding recipient. Horizontal bridging vessels are therefore highly unlikely in this experimental setup. In patients, the removal of the CNV results in a "gap" in the recipient site. In this situation, the graft is in direct contact with the underlying residual choroid and possibly with the surrounding extrafoveal choroid. MacLaren et al. hypothesized that the subfoveal area would consist of fibrotic avascular scar tissue secondary to AMD, which would require horizontal perfusion. However, postoperative ICG angiography revealed that 87% (20 out of 23) of our patients had a vascular pattern attributable to the residual choroidal

vessels underneath the graft.[13] Therefore, in our opinion, both horizontally and vertically bridging vessels may reperfuse the graft in patients.

Leaving the choroid and choriocapillaris attached to the Bruch's membrane and RPE (enabling vertical and horizontal perfusion) of the graft may be essential for long time survival and we therefore would not consider the use of an excimer laser to remove the choroidal tissue of the graft before translocation. This was proposed by Holz et al. to obtain physiological subretinal dimensions.[16] However, having a slight elevated plateau in the macular area due to the subfoveal RPE-choroid graft, as observed with optical coherence tomography (OCT), did not result in an increase in metamorphopsia in the patients. Moreover, fewer intraoperative manipulations are related to a better postoperative visual outcome.[8]

In a series of patients in Cologne, angiography showed that the graft may also become revascularized when used in the treatment of patients with atrophic AMD. It was necessary to intentionally damage and rupture the Bruch's membrane of the recipient to allow vessel ingrowth. Visual gain may be achieved, but the mean VA deteriorated in this patient group.[17] Nevertheless, in atrophic AMD there is no treatment available at all. If an optimal selection of patients and timing of surgery would improve results, RPE-choroid translocation surgery would have a highly interesting application.

The Danger model (proposed by Matzinger in 2002) suggests that the immune system is more concerned with damage than with foreignness.[18] Thus, the autologous and therefore antigenically similar (histocompatible) RPE-choroid graft is no guarantee for tolerance. The Danger model is supported by the findings that a dedicated and typical immune reaction (such as activation of antigen presenting cells) can be elicited by distressed or injured cells. For example, breakdown products of hyaluron (produced when vessels are damaged) or ischemic damage are endogenous, non-foreign alarm signals activating the immune reaction.[18] These alarm signals may also be induced by the extraction of the CNV membrane in patients with AMD, the ischemic damage of the RPE-choroid graft, and during reperfusion of the graft.

The RPE-choroid grafts were shown to be functional up to four years after surgery. It is known from organs, like kidneys, that a lower extent of damage can lead to longer acceptance, e.g. kidneys from living donors are accepted more easily than from cadavers.[19] This is promising for the RPE-choroid graft translocation technique, as the graft is taken from healthy tissue and the time between harvesting and transplantation is only a few minutes, minimizing the damage. Probably, the small size of the graft also minimizes the immune response.

It remains a biological miracle that free RPE-choroid grafts maintain a functional balance in complex processes, such as on one hand neovascularization leading to revascularization, without on the other hand recurrent and persistent neovascularization and progressive

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fibrosis. It is hard to discern, however, whether an unfavorable visual outcome is attributable to an immuno-mediated rejection, ischemia of the graft, surgical damage to RPE and retina, failure of revascularization or pre-existing damage to the overlying retina.

7.3 Imaging techniques

The function of the neurosensory retina over the graft was estimated with visual acuity measurement, and with fixation testing. Fixation testing can be accurately performed by biomicroscopy with the slit lamp, or even more detailed by micro-perimetry. Fixation on the graft was achieved in 74% of the patients up to their last examination (follow-up of 1 to 4 years), and loss of fixation was associated with a marked decrease in mean visual acuity.[1]
Imaging techniques used in this thesis to study the morphology of the graft were color fundus photography, OCT, FA and ICGA.
OCT by 6 mm radial scans was systematically performed before surgery to roughly estimate the condition of the retina, like the presence of a layered retinal structure or foveal depression, cystoid edema, RPE detachment or RPE tear. However, in many patients the OCT was of poor quality due to cloudy optical media, large subretinal hemorrhage, and difficulty of focusing caused by the elevated irregular macular area. This made us decide not to evaluate the OCT as predictive variable for postoperative visual outcome.
Preoperative functional testing of the neurosensory macular retina with multifocal electroretinography (mfERG) might provide useful information to predict whether AMD patients would benefit of an RPE-choroid translocation. However, mfERG was not available in our institute.
Nor did we systematically obtain fundus autofluorescence, as it was only available in our hospital from November 2006 onwards. Studies revealed that autofluorescence of the RPE-choroid graft is difficult to interpret as the degree of autofluoresence did not correlate with visual function nor vascularization of the graft.[17,20,21] Only an absence of autofluoresence may correspond to lost foveal fixation and a poor visual acuity.[22]

7.3 Surgical technique

Our hypothesis that less surgical damage might improve long-term results was supported by the results of the study in which the intraoperative course adjusted for preoperative variables

proved to have a statistically significant correlation to postoperative visual outcome.[8,23]

The two major problems encountered during RPE-choroid translocation were:

1) curling up of the graft after excision from the donor site, that necessitates additional corrective manipulations to flatten the graft underneath the fovea;

2) adhesion of the graft to the translocation instrument complicating its submacular release.

The surgical technique was refined to deal with the curling graft in different aspects: the initially used aspiration-reflux spatula was replaced for fine forceps to have more graft control. Moreover, instead of directly removing the retina within the diathermy marks at the donor site before cutting out the graft, the retina was removed from the graft just before the submacular release. The attached retina prevents folding of the graft. Recently, the addition to use a round graft instead of a square graft subjectively appeared to decrease submacular folding of the edges of the graft (verbal communication, 2007, Roider).

To overcome the problem of adhesion, Holland (Department of Experimental Medical Instrumentation, Erasmus University Medical Center, Rotterdam, the Netherlands) suggested to use vibration of the instrument during the submacular release. Subjectively, improvement was observed. This was objectified by an experimental in vitro model.[24] The minimum threshold amplitude and frequency needed for optimum tissue release were estimated.[24]

The visual outcomes of the patients treated with these improved techniques were not reported on in this thesis. Currently, more than 240 patients with have been treated. One might expect better outcomes compared with the long-term results of the first 84 patients treated with an RPE-choroid translocation, as described in this thesis. On the other hand, with the introduction of anti-VEGF only more desperate cases are now being treated.

Future research in the instrumental development involves the principle of micron scale heat induced attachment and detachment (HIAD).[25] In collaboration with the Delft University of Technology a first prototype was built and tested by Knulst et al.[25] The HIAD consists of an electrically heatable wire that heats the instrument-tissue interface indirectly. Adhesion occurs at a temperature of about 73^{0}C and detachment at 100^{0}C.[26-28] The first results are promising. Further development and investigation are needed to make this instrument useful, safe and available for graft translocation during surgery.

Further improvement may be achieved by combining graft surgery with advances in the rapidly expanding field of molecular biology and genetics: the use of vectors to genetically modify the RPE graft preceding translocation. Adenovirus vectors did not produce serious adverse events when injected into human ocular tissues.[29,30]

It would be interesting to transfect the RPE with genes that express substances that have a neuronal rescue effect, like transfection with the brain-derived neurotrophic factor (BDNF),

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or with genes coding for complement factor H, the major so far identified genetic factor related to AMD.[31,32]

This could be performed within one operation procedure as an incubation for 20 minutes was sufficient to transduce RPE in a principle of proof experiment using an enhanced green fluorescent protein.[14]

Additional to gene transfer, which aims to rescue the RPE or retina, retinal repair by cell transplantation with the graft acting as a carrier would be another strategy. In a mouse model, donor rod photoreceptor precursor cells integrated into the outer nuclear layer in adult or degenerated retina if they were taken from a developing retina at a time coincident with the peak of rod genesis.[33] Or autologous precursor cells collected from the peripheral retina could be used.[34]

Therefore, techniques applied during RPE-choroid translocation surgery may remain part of future developments.

7.4 Organ culture

We would like to have an ex vivo model to study the revascularization of the graft with the potential to study variables for such growth. As static cultures do not preserve the typical cellular composition of tissue for two weeks, we decided to use perfusion culture, which would result in a higher specimen viability, retention of cellular diversity and tissue composition.[35] Moreover, this decision to investigate whether angiogenesis could be initiated in porcine ocular tissue in the setting of an RPE-choroid translocation was supported by the finding that the vascular network of neonatal rabbit kidney explants continued to growth in perfusion culture with stimulation of VEGF and basic fibroblast growth factor (bFGF).[35,36]

Although we varied culture conditions with respect to the culture duration and supply of oxygen, growth factors (VEGF and bFGF) and nutrients, it remained uncertain whether a perfusion tissue culture might be a useful model to study revascularization of the graft. The inability to induce vessel growth on a large scale in our model may be due to our using mature tissue (pigs of three months of age), instead of the immature tissue of the kidney explants who were already in the development phase of angiogenesis.

Because we failed to verify the effect of each angiogenesis stimulating factor step by step, further studies would be necessary to confirm or reject whether it would be possible at all to induce revascularization of an RPE-choroid graft in a perfusion tissue culture. The present study may help to design a better staged experiment.

7.5 Radner Reading Chart

AMD primarily affects the central vision and particularly the reading ability. As routine single optotype distance visual acuity tests have been shown to be poor predictors for reading performance, reading performance tests can provide more detailed information about visual function in this elderly population.[37-39]

For an international controlled study for RPE-choroid graft surgery in patients with AMD (in preparation), we needed a reading chart that was reliable, but would also have its validated counterparts in German and English.

Obviously, a reading chart is language specific, whereas single optotypes charts are not. To estimate the reading performance in a well-standardized and reproducible manner we created a new Dutch reading performance test for daily practice as well as scientific research.

The principles of the originally German language Radner Reading Charts were used as a template.[40] Subsequently, the reliability of the chart was tested in university students as well as subjects aged ≥50 years and affected with a macular disease. The test-retest and inter-chart reliability was high for all visual acuity groups and variables. For all groups together the chart accounted for maximal 5 % of the total variability for all measurements.[41]

The Dutch Radner Reading Charts has a logarithmic progressing print size, like the ETDRS-chart, which has become the scientific measure of choice for distance visual acuity tests. We encourage to use the Radner Reading chart for scientific research as it is accurate and reliable, and the logarithmic scale enables to compare reading acuities across studies.

Recently initiated research in the evaluation of the Dutch Radner reading chart focuses on the reliability testing in low vision patients with or without magnifier aids (low vision is defined as visual acuity of < 0.33, but ≥ 0.05, or a corresponding visual field loss of < 20 degrees in the better eye with best possible correction).

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Chapter 8

Summary
Nederlandse Samenvatting

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Summary

Age-related macular degeneration (AMD) is the most important cause of irreversible legal blindness in elderly persons in industrialized countries. AMD has two forms: atrophic (dry) and exudative (wet). In the wet form, abnormal blood vessels, arising from the choriocapillaris (choroidal neovascularization, CNV) underneath the macula, grow through ruptures in the Bruch's membrane, into the sub-retinal pigment epithelium (RPE) space or into the sub-retinal space, or a combination of both. Blood and serum leakage from the CNV below the RPE and/or below and within the retina are harmful and finally causes irreversible damage to the photoreceptors of the overlying macula which results in a central scotoma.

Since October 2006, the most effective proven therapy for subfoveal exudative AMD is an intraocular injection of an inhibitor of vascular endothelial growth factor (VEGF). A second choice for predominantly classic lesions is photodynamic therapy with verteporfine. However, there are patients not responding to or not eligible for these therapies. These are mostly patients with advanced stages of exudative AMD. For this patient group, a surgical approach may be used as a last resort option.

This thesis presents the data of the translocation of a free autologous full-thickness RPE-choroid graft after removal of the CNV in patients with exudative AMD. To minimize trauma to the macular area, the graft is cut out from the midperiphery.
The main objective of this thesis was to evaluate whether the RPE-choroid graft translocation would be a useful and safe technique in the treatment of patients with exudative AMD.

In **chapter 2.1 and 2.2** we reported on the long-term clinical results of the RPE-choroid graft translocation in 83 AMD patients (84 eyes). The mean visual acuity improved slightly up to 4 years after surgery, and more patients had a postoperative VA better than 20/80 (about 20%) at their last examination, than there were before surgery (6%). The retina overlying the graft was able to function, as fixation was located on the graft in 62 (74 %) eyes up to the last examination. Complications during surgery occurred in 17% (14 out of 84) of the eyes. The major postoperative complications consisted of retinal detachment (n = 7), a recurrent or persisting CNV (n = 11), and postoperative hemorrhages (n = 9).
ANCOVA analysis revealed that the preoperative variables predominantly classic and occult lesions had a statistically significant better visual outcome at one year after surgery than minimally classic or hemorrhagic (≥ 50% blood) lesions. However, the intraoperative course

might have acted as a confounder in this study. Therefore, in **chapter 2.3** we evaluated four critical surgical steps, and the intraoperative course was graded from 0 (uncomplicated surgery) tot 5 (most complicated surgery). The intraoperative course (graded from 0 to 5) adjusted for preoperative variables had a statistically significant influence on postoperative visual outcomes. This confirmed our hypothesis that the intraoperative course had acted as an independent variable, and was a better predictor for visual outcome than preoperative angiographic characteristics. Moreover, this result supported our hypothesis that refining surgery could improve results.

In **chapter 2.4,** we investigated whether an RPE-choroid graft translocation might be a treatment option for patients with an RPE tear secondary to exudative AMD. This is a population for which no other treatment was available till present. The mean VA (n = 6 patients) improved from 20/160 at baseline to 20/80 at 13 ± 7 (mean ± SD) months after surgery. This is favorable compared with the natural course (mean VA of 20/200 or worse), and showed the potential of the RPE-choroid translocation in the treatment of patients with an RPE tear.

In **chapter 2.5**, the graft reperfusion was studied in 31 patients with AMD who had fluorescein angiography (FA) and/or indocyanine green angiography (ICGA) performed after an RPE-choroid graft translocation. Fluorescence of the graft in the early phase in FA and/or a parallel orientation of the vascular pattern in the macular area with ICGA were considered to be an indication of perfusion.

Perfusion of the graft did not correlate to postoperative visual acuity and may therefore not be used as predictor for the functional status of the graft. Only a nonperfused graft predicted a poor visual outcome. Moreover, for assessing reperfusion of the graft, analysis of the early phase of the FA turned out to be more reliable than ICGA.

While examining the ICGAs, hyperfluorescent optics discs were observed in one fourth of the patients treated with an RPE-choroid graft. In **chapter 2.6,** we described a study in which we aimed to identify whether ICG-assisted surgery was related to this hyperfluorescence of the optic disc. We studied the ICGAs of 31 patients who had ICGA after surgery, and assessed the hypo/iso/hyperfluorescence of the optic disc and the time interval between ICGA and possible use of intravitreal ICG. There was a statistically significant correlation between ICG use during surgery and a hyperfluorescent disc. The optic disc lost its hyperfluorescence at about 43 ± 12 (mean ± SD) weeks after ICG-assisted surgery.

In **chapter 2.7,** we emphasized the positive side of the hypothesis by MacLaren et al. that the loss of photoreceptor outer segments may be explained by chronic photoreceptor apoptosis, initiated by either surgery or the disease process itself. We argued that this hypothesis

supports the attempt to minimize surgical manipulations, especially in the submacular space, to achieve a better photoreceptor survival and therefore improve functional outcome.

In **chapter 3.1,** we have assessed the histological evidence for revascularization of the RPE-choroid graft in an in vivo study. In this study the RPE-choroid translocation was performed in 11 pigs (11 eyes). The eyes were enucleated at one week or three months after surgery. Immunohistochemistry of the tissue sections showed bridging vessels between the graft and recipient layer and the vasculature of the graft appeared open and perfused. Vessels with transcapillary pillars and conglomerates of small new vessels were present in the graft. All these signs confirmed the revascularization of the graft by angiogenesis.

As the in vivo experiments in pigs were complicated, time-consuming and expensive, attempts to induce revascularization of an RPE-choroid graft placed on a RPE-choroid recipient layer in a perfusion tissue culture were made and described in **chapter 4.1.** The culture conditions varied with respect to culture duration (in days) and supply of oxygen, growth factors and nutrients. In total 15 cultures were performed of which 24 specimens were histologically evaluated. In one specimen we observed a vessel breaking through the choriocapillaris of the recipient towards the graft, which was suggestive for vessel growth. This specimen had been cultured for 15 days under normoxia without adding growth factors and was exposed to hypoxia for the initial three hours of culture.

We recommended to perform additional immunohistological staining to determine whether the vessel suspected for angiogenesis was newly formed or pre-existing. Moreover, as we explored the possibilities and restrictions of the RPE-choroid graft in tissue perfusion culture, further better designed perfusion cultures will be performed to confirm or reject whether it is possible to induce revascularization of an RPE-choroid graft in an ex vivo perfusion culture.

During the RPE-choroid graft translocation, the adhesion of the graft to the translocation instrument complicated its submacular release. Vibration of the instrument by attachment of a mobile phone vibration device (which had a frequency of 140 Hz) to the handle of the instrument improved the release. In **chapter 5.1,** we describe how we confirmed the effectiveness of the principle of vibration with the use of a mathematical and experimental model. The minimum threshold amplitude required to release the graft was approximately 0.15 mm from a frequency of 100 Hz and higher. We also determined the instrument tip movements induced by the mobile phone vibration device with the use of a high-speed

camera. These data will enable us to develop an optimized instrument, but might also be valuable for other surgical techniques in ophthalmology.

Reading vision is an important functional evaluation tool in the treatment of the AMD patients. For an international controlled study for RPE-choroid graft surgery in patients with AMD (in preparation), we needed a reading chart that was reliable, but would also be available in different languages. In **chapter 6.1,** we first described the requirements for a good reading chart. We explained the reason to choose for the originally German language Radner Reading Charts: this reading chart met all our requirements, but moreover, is the only reading chart that emphasizes the principle of 'sentence optotypes', i.e. these are highly standardized sentences, because sentence complexity also influences reading performance. Subsequently, 32 Dutch sentence optotypes were created according to the strict principles of Radner and tested in 109 university students. The most equally matched sentence optotypes (n = 24) in terms of reading speed and number of reading errors were selected for the introduction and printing of the first Dutch Radner Reading Charts. Each Radner Reading Chart consists of three charts.
In the study presented in **chapter 6.2,** we statistically analyzed the test-retest and inter-chart reliability of the logarithmically scaled Dutch Radner Reading Charts in 36 patients affected by a macular disease and aged 50 years or older. The patients were divided in three groups according to their distance logMAR visual acuity. The patients read the three Radner Reading Charts in randomized order twice with one month in between. Outcome measures were reading acuity, logRAD score (reading acuity adjusted for number of reading errors), maximum reading speed, critical print size and reading VA/ distance VA ratio. For all patients together the chart accounted for maximal 5% of the total variability for all measurements. The individual patient did have the largest influence in the measurements (88-98% of the variability). These data indicate the that the Dutch version of the Radner Reading Charts provide high reliability test-retest and inter-chart measurements of reading performance in a heterogeneous group of subjects with subnormal to low vision. Moreover, it is useful for international studies as it has its validated counterparts in German and English.

In summary, we believe that the RPE-choroid translocation is a realistic option for patients with exudative AMD not treatable or no longer responding to other less invasive treatments.
An RPE-choroid graft may stabilize or even improve visual acuity in these patients, with patients with an RPE tear appearing to have the best prognosis.

Nederlandse Samenvatting

Leeftijdsgebonden macula degeneratie (LMD) is de belangrijkste oorzaak van blindheid bij ouderen in de Westerse wereld. LMD bestaat in twee vormen: de atrofische (droge) en de exsudatieve (natte) vorm. In de natte vorm ontstaan abnormale bloedvaten (choroideale neovascularisatie, CNV) vanuit het vaatbed (de choriocapillaris) onder de macula (gele vlek). Deze vaten groeien vervolgens door kleine openingen in het Bruch's membraan naar de ruimte onder het retina pigment epitheel (RPE) en/of in de ruimte onder de retina (netvlies).
Lekkage van bloed en serum vanuit deze CNV onder het RPE en/of onder en in de retina is schadelijk en leidt uiteindelijk tot onomkeerbare schade aan de fotoreceptoren van de overliggende macula. Dit resulteert in een centraal scotoom in het gezichtsveld.

Sinds oktober 2006 is een intra-oculaire injectie met een remmer van de vasculaire endotheliale groeifactor (VEGF) de meest effectieve bewezen therapie voor subfoveale exsudatieve LMD. De tweede keus van behandeling is fotodynamische therapie met verteporfine, maar deze is alleen geschikt voor laesies die voor meer dan 50% uit de klassieke component van het CNV bestaan. Er zijn echter patiënten die niet meer reageren op of niet in aanmerking komen voor deze therapieën. Dit betreft vooral patiënten met vergevorderde exsudatieve LMD. Voor deze patiëntengroep zou een chirurgisch ingreep een laatste optie kunnen zijn.

Dit proefschrift gaat over de translocatie van een vrij autoloog RPE-choroidea transplantaat dat vanuit de midperiferie naar het subfoveale gebied (meest centrale plek van de macula) wordt geplaatst, nadat de CNV onder de fovea is verwijderd.
Het belangrijkste doel van dit proefschrift was om na te gaan of een RPE-choroidea translocatie een effectieve en veilige techniek zou zijn in de behandeling van patiënten met exsudatieve LMD.

In **hoofdstuk 2.1 en 2.2** werden de lange-termijn resultaten besproken van de RPE-choroidea translocatie in 83 LMD patiënten (84 ogen). De gemiddelde gezichtsscherpte verbeterde licht tot 4 jaar na de operatie. Er was een toename van patiënten met een gezichtvermogen van ≥20/80 op hun laatste controle na de operatie (ongeveer 20%) vergeleken met voor de operatie (6%). De retina dat over het transplantaat ligt toonde functie, gezien fixatie op het transplantaat werd waargenomen in 62 (74%) ogen tot aan de laatste controle. Complicaties tijdens de operatie gebeurde in 17% (14 van de 84) van de ogen. De belangrijkste postoperatieve complicaties bestonden uit netvliesloslatingen (n = 7), een recidiverende of

persisterende CNV (n = 11), en postoperatieve bloedingen (n = 9).
ANOVA analyse liet zien dat de preoperatieve variabelen "predominant" klassieke en occulte laesies een statistisch significante betere functionele uitkomst hadden na de operatie dan minimaal klassieke of hemorrhagische (≥ 50% bloed) laesies. Echter, het peroperatieve beloop was niet in deze analyse meegenomen en zou als "confounder" hebben kunnen opgetreden.
Daarom werden in **hoofdstuk 2.3** de vier belangrijkste stappen tijdens de operatie geëvalueerd. Het peroperatieve beloop werd gegradeerd van 0 (ongecompliceerde chirurgie) tot 5 (meest gecompliceerde chirurgie). Het peroperatieve beloop (score 0 tot 5), gecorrigeerd voor de preoperatieve variabelen, bleek een statistisch significante invloed te hebben op de gezichtsscherpte na de operatie. Dit bevestigde onze hypothese dat het peroperatieve beloop als een onafhankelijke waarde had moeten worden meegenomen in de voorgaande studie, hetgeen de waarneming teniet doet dat bepaalde angiografische eigenschappen van het CNV de uitkomst na de operatie significant beïnvloeden. Bovendien ondersteunden deze resultaten de veronderstelling dat het verbeteren van de chirurgische techniek de postoperatieve resultaten kan verbeteren.
In **hoofdstuk 2.4** hadden we onderzocht of de RPE-choroidea translocatie ook een behandelingsoptie kan zijn voor patiënten met een RPE scheur ten gevolge van exsudatieve macula degeneratie. Dit betreft een groep patiënten waarvoor tot op heden nog geen behandeling bestond. De gemiddelde gezichtsscherpte van de 6 patiënten verbeterde van 20/160 van voor de operatie tot 20/80 op 13 ± 7 (gemiddelde ± SD) maanden na de operatie. Dit is gunstiger vergeleken met het natuurlijke verloop (gemiddelde van 20/200 of slechter) en laat de potentie zien van de RPE-choroidea translocatie bij patiënten met een RPE-scheur.
In **hoofdstuk 2.5** wordt de reperfusie van het transplantaat angiografisch onderzocht middels de studie van 31 LMD patiënten die een fluorescentie angiogram (FA) en/of indocyanine groen angiogram (ICGA) hadden gehad na een RPE-choroidea translocatie. Fluorescentie van het transplantaat in de vroege fase van het FA, of parallel georiënteerde vaten ter plaatse van het transplantaat met het ICGA werden als indicatie voor reperfusie gezien.
Perfusie van het transplantaat correleerde niet met de gezichtsscherpte na de operatie en mag daarom niet worden gebruikt als predictor van de functionele status van het transplantaat. Alleen een niet-geperfundeerd transplantaat voorspelde een slechte gezichtsscherpte. Analyse van de vroege fase van het FA bleek meer betrouwbaar te zijn voor het vaststellen van reperfusie dan het opzoeken van het parallel georiënteerde vasculaire patroon in het ICGA.
Tijdens het bestuderen van de ICGAs zagen we een hyperfluorescente papil in een vierde van de patiënten met een RPE-choroidea transplantaat. In **hoofdstuk 2.6** beschrijven we een studie waarin we probeerden na te gaan of ICG gebruik tijdens de operatie was gerelateerd

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aan deze hyperfluorescentie van de papil. We bestudeerden de postoperatieve ICGAs van 31 patiënten en bepaalden de hypo/iso/hyperfluorescentie van de papil en het tijdsinterval tussen de ICGA en het eventuele gebruik van intravitreaal ICG. Er was een statistisch significante relatie tussen ICG gebruik tijdens de operatie en een hyperfluorescente papil. De hyperfluorescentie van de papil verdween na ongeveer 43 ± 12 weken (gemiddeld ± SD) na ICG-geassisteerde chirurgie.
In **hoofdstuk 2.7** benadrukten we de positieve zijde van de hypothese van MacLaren en medeauteurs, waarin ze stelden dat het verlies van de buitenste segmenten van de fotoreceptoren verklaard zou kunnen worden door chronische apoptose van de fotoreceptoren, welke geïnitieerd zou kunnen zijn door de operatie of door het ziekteproces zelf. We beargumenteerden dat deze hypothese onze initiatieven ondersteunt om de chirurgische manipulaties te minimaliseren, vooral in de submaculaire ruimte, om minder schade aan de fotoreceptoren te veroorzaken met daarmee een betere functionele uitkomst.

In **hoofdstuk 3.1** leverden we het histologische bewijs voor de revascularisatie van het RPE-choroidea transplantaat in een in vivo studie. In deze studie hadden we een RPE-choroidea translocatie uitgevoerd in 11 varkens (11 ogen). De ogen werden 1 week of 3 maanden na de operatie uitgenomen. Immunohistologisch onderzoek van de weefselsnedes liet vaatverbindingen zien tussen het transplantaat en ontvangende laag. De vaten van het transplantaat zagen er open en geperfundeerd uit. Ook waren er vaten met tekenen van actieve deling en conglomerulaten van kleine nieuwe vaten aanwezig. Al deze bevindingen bevestigden de revascularisatie van het transplantaat door middel van angiogenese.

De in vivo experimenten in de varkens verliepen moeizaam, waren tijdsintensief en duur. Daarom werd een poging ondernomen om revascularisatie van het RPE-choroidea transplantaat geplaatst op een RPE-choroidea laag (de ontvangende laag) te induceren in een perfusie weefsel cultuur. Dit werd beschreven in hoofdstuk 4.1. De cultuur condities werden gevarieerd op basis van duur (in dagen), zuurstofvoorziening, groeifactoren en voedingsstoffen. In totaal werden 15 weefselculturen uitgevoerd waarvan 24 transplantaten histologisch werden geanalyseerd. In één transplantaat werd een vat gezien dat door de choriocapillaris van de ontvangende laag heen brak in de richting van het transplantaat, wat suggestief is voor vaatgroei. Dit transplantaat had 15 dagen in cultuur gestaan onder normoxische omstandigheden zonder toevoeging van groeifactoren en was alleen de eerste drie uren blootgesteld aan hypoxie.
We besloten dit hoofdstuk met het advies om aanvullende immunohistologische

weefselkleuringen te doen om te zien of dit vat suspect voor angiogenese een nieuwgevormd of al langer bestaand vat was. Gebaseerd op de mogelijkheden en beperkingen tegengekomen in deze eerste experimenten zullen nu beter opgezette weefsel perfusie culturen uitgevoerd kunnen worden, die de haalbaarheid van het induceren van revascularisatie van het transplantaat in een ex vivo perfusie cultuur gaan bevestigen of verwerpen.

Het vast blijven plakken (adhesie) van het RPE-choroidea transplantaat aan het instrument bemoeilijkte het achterlaten van het transplantaat onder de macula tijdens de operaties in patiënten. Trilling van het instrument verbeterde het loslaten van het transplantaat subjectief. Deze trilling werd opgewekt door het vastmaken van het trilapparaat van een mobiele telefoon (welke een frequentie had van 140 Hz) aan het handvat van het instrument. In **hoofdstuk 5.1** beschrijven we hoe we de effectiviteit van het trilprincipe hebben bewezen door gebruik te maken van een wiskundig en experimenteel model. De minimale amplitude nodig om het transplantaat los te laten gaan van het instrument was ongeveer 0.15 mm vanaf een frequentie van 150 Hz en hoger. We bepaalden ook de bewegingen van het uiteinde van het instrument dat bij patiënten tijdens de operatie werd gebruikt met een hoge-snelheids camera. Deze gegevens zullen bijdragen aan het verbeteren van het instrument, maar kunnen eventueel ook nuttig zijn voor andere chirurgische technieken in de oogheelkunde.

Bij patiënten met macula afwijkingen is met name de leesvisus een belangrijke maat. Voor een gecontroleerde, internationale studie van RPE-choroidea transplantatie (in voorbereiding) zou een betrouwbare en in verschillende talen gevalideerde leeskaart nodig zijn. In **hoofdstuk 6.1** beschreven we eerst de vereisten voor een goede leeskaart. We beargumenteren de reden waarom we gekozen hebben voor de oorspronkelijk Duitstalige Radner leeskaarten: deze voldeed én aan onze vereisten, maar was bovendien de enige leeskaart dat het principe van de “zinoptotypen” benadrukt. Zinoptotypen zijn hoog gestandaardiseerde zinnen, om de invloed van zincomplexiteit op de leesprestatie te minimaliseren. Vervolgens hebben we 32 Nederlandstalige zinoptotypen gemaakt volgens de strikte principes van Radner en deze werden getest op 109 universitaire studenten. Op basis van leessnelheid en aantal leesfouten werden de 24 meest identieke zinnen geselecteerd voor de introductie en druk van de Nederlandstalig Radner leeskaart. Iedere Radner leeskaart bevat 3 leeskaarten.
In de studie zoals gepresenteerd in **hoofdstuk 6.2** analyseerden we statistisch de betrouwbaarheid van de logaritmisch geschaalde Nederlandstalige Radner leeskaarten in 36 patiënten van 50 jaar en ouder met een macula aandoening. De patiënten werden opgedeeld in 3 groepen naar gelang hun gezichtsscherpte. De patiënten lazen de drie leeskaarten twee

keer op in een willekeurige volgorde met een maand tussen beide metingen. Uitkomstmaten waren leesvisus, logRAD score (leesvisus gecorrigeerd voor aantal leesfouten), maximale leessnelheid, de kritische lettergrootte (lettergrootte waarbij iemand langzamer gaat lezen) en de leesvisus/afstandsvisus ratio. Voor alle patiënten samen zorgde de Radner leeskaarten maar voor 5% van de totale variabiliteit in metingen. De individuele patiënt had de grootste invloed op de metingen (88-98% van de variabiliteit). Deze uitkomsten lieten zien dat de Nederlandstalige Radner leeskaarten een zeer betrouwbare meting van het leesvermogen geven in een heterogene groep van patiënten met een subnormale tot lage gezichtsscherpte.

Samenvattend zijn wij van mening dat de RPE-choroidea translocatie bij uitbehandelde patiënten met exsudatieve LMD een reële optie is om de gezichtsscherpte te stabiliseren tot verbeteren. Patiënten met een RPE scheur lijken het meeste baat bij deze operatie te hebben.

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Mijn waarde collega's arts-assistenten van het Oogziekenhuis Rotterdam: jullie zijn een hechte en warme groep, en het is alleen al heerlijk om de schoonheid van de oogheelkunde niet aan elkaar te hoeven uitleggen.

Ik heb een sterk Vlaams team aan paranimfen achter mij staan. Allereerst mijn schoonpapa Valeer Eckelmans. Zelf geoloog en scheikundige en in deze laatste ook gepromoveerd. Ik heb mij dikwijls afgevraagd hoe mijn promotie eruit zou hebben gezien in een tijdperk zonder computers en internet. Dat maakt mijn eerbied voor uw doctorstitel nog groter!
De gedachte dat Tom Missotten aan mijn andere zijde zal staan is een hele geruststelling. Tom, ik bewonder je hoeveelheid klinische ervaring en wetenschappelijke kennis die je op jouw jonge leeftijd al hebt vergaard. Probeer wel je sleutels minder vaak te vergeten, want de tijd die het je telkens kost om weer terug te komen om ze op te halen, zou je op jaarbasis de tijd leveren om minstens weer een extra publicatie op je naam te zetten.

Maarten, Simone, Richard en Alexander alias het "sick brother" gezelschap: onze vele weekenden waar we ons helemaal laten gaan (het is beter hier niet in detail te treden) zijn altijd hilarisch. Onze vriendschap stamt uit de prille basisschooltijd en het is mooi om met jullie alle levensfases mee te maken.

Joline: jij staat symbool voor onze windsurf en mountainbike tijd. Ook al heb ik dat tijden niet meer gedaan, toch voel ik me telkens weer sportief als ik jou heb ontmoet. En het heerlijke lachen met en om elkaar is onveranderd sinds de middelbare school.

Iris, Dapne, Merel, Janneke, Susanne en Pam: ik vind jullie stuk voor stuk stoere meiden! We hebben alle zeven een anders medisch specialisme gekozen en het is mooi om ieders passie

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voor het eigen vak te zien. We zien elkaar nu minder vaak, maar dat doet geen afbreuk aan onze vriendschap.

Kees, Ineke, Linda, Roy, Sander en Esther, lieve buren. Jullie hebben ons zuiderlingen meteen met open armen ontvangen en wij zijn ons zeer bewust van deze rijkdom. We danken jullie niet alleen voor de oneindig vele malen te hulp schieten (variërend van gesprongen riool, het vangen van mollen, tot het liefdevol oppassen op Lore), maar vooral ook voor de mooie vriendschap.

Schoonpapa Valeer c.q. bompa, schoonmama Janske, Ingrid en Wim, jullie zijn een warme familie en ik heb geluk daar nu al weer 11 jaar bij te horen. Het zou inderdaad veel makkelijker zijn geweest als het Oogziekenhuis gewoon in Maastricht had gelegen (een kleine naamsverandering is dan op zijn plaats), maar de afstand Rotterdam-Tongeren wordt nu telkens beloond met heerlijke weekenden samenzijn.

Nel Hulsenboom-van Amen (geboren 1910), onze mater familias, u heeft een geweldige positieve en optimistische levensinstelling, die u gelukkig als dominant gen aan de hele familie heeft doorgegeven. U moest eens weten hoe die van pas is gekomen tijdens mijn 4 jaar promotieonderzoek! Leeftijdsgebonden macula degeneratie heeft u vast nog niet, want u overruled de umpire nog geregeld bij het tennis kijken.

Lieve papa, mama, en grote zus Mignon. Jullie hebben mij geleerd nooit te drentelen, maar de koe altijd meteen bij de horens te nemen en dat tegenslagen altijd prima te relativeren zijn. Dit zijn eigenlijk de ingrediënten om een promotietraject goed door te komen.
Het is super hoe jullie altijd paraat staan. Neem alleen al de oneindig vele keren dat jullie naar Rotterdam zijn gekomen om ons te helpen met het verbouwen van ons huis of om op Lore te passen, als het onderzoek weer eens voorrang kreeg. Dit boekje is dan ook voor jullie!!

Liefste Wouter, het waren vier intense maar mooie jaren: mijn onderzoek met een jaar verblijf in Keulen, jij met de start van je eigen bedrijf, het totaal renoveren (deels eigenhandig) van ons huis, en de komst van ons kleine mooie meisje Lore in januari 2006. Je bent een ontzettende stimulans in mijn leven en ik ben mij echt iedere dag bewust hoe ik het met je getroffen heb. Dank je mijn liefste.

Curriculum vitae

Kristel JM Maaijwee werd 10 maart 1977 geboren en groeide op in Drunen (Noord-Brabant), alwaar zij in 1995 haar VWO diploma behaalde aan het d'Oultremontcollege.

De studie Geneeskunde volgde zij aan de Universiteit Maastricht. Als student-assistent heeft ze aan meerdere onderzoeksprojecten meegewerkt. Bij de vakgroep Fysiologie (Cardiovascular Research Institute Maastricht, CARIM) werkte ze twee jaar in het gebied van de microcirculatie op het onderwerp "leukocyt-vaatwand interacties: de rol van L- en E-selectines" onder leiding van Dr. M.G.A. Oude Egbrink en Dr. S.C Tromp (prof. dr. R.S. Reneman, afdelingshoofd). In het kader van dit onderzoek heeft zij in 1998 gedurende twee maanden een wetenschapstage gedaan op de afdeling Pathologie (prof. dr. U.H. von Andrian, afdelingshoofd), the Center for Blood Research, Harvard Medical School in Boston, Verenigde Staten van Amerika. Daarnaast was ze een jaar werkzaam bij de vakgroep Interne Geneeskunde (CARIM) op het onderwerp "Diagnostische waarde van 24-uurs ambulante bloeddrukmeting in relatie tot linker ventrikel hypertrofie" (prof. dr. P. de Leeuw, afdelingshoofd).

Nadat ze in februari 2002 haar arts examen had afgelegd, heeft ze een jaar als arts-assistent heelkunde in het Reinier de Graaf Gasthuis te Delft gewerkt.

Vanaf 2003 verrichtte ze promotie onderzoek aan het Oogziekenhuis Rotterdam onder leiding van prof. dr. J.C. Van Meurs, zoals beschreven staat in dit proefschrift. Het eerste jaar heeft zij haar experimenten uitgevoerd aan de Cell Center Cologne aan de Universiteit van Keulen, Duitsland, mede onder supervisie van prof. dr. B. Kirchhof en prof. dr. A.M. Joussen. In Rotterdam heeft zij in de daarop volgende drie jaren haar experimentele onderzoek voortgezet in het Laboratorium van Metabole Ziekten (Dr. F. de Rooij, afdelingshoofd) en de afdeling Pathologie (Dr. C.M. Mooij, supervisor), beide in het Erasmus Medisch Centrum te Rotterdam.

Vanaf december 2007 is zij in opleiding tot oogarts in het Oogziekenhuis Rotterdam (opleider prof. dr. J.C. van Meurs).

Kristel heeft samen met haar partner Wouter een dochter van twee jaar oud.

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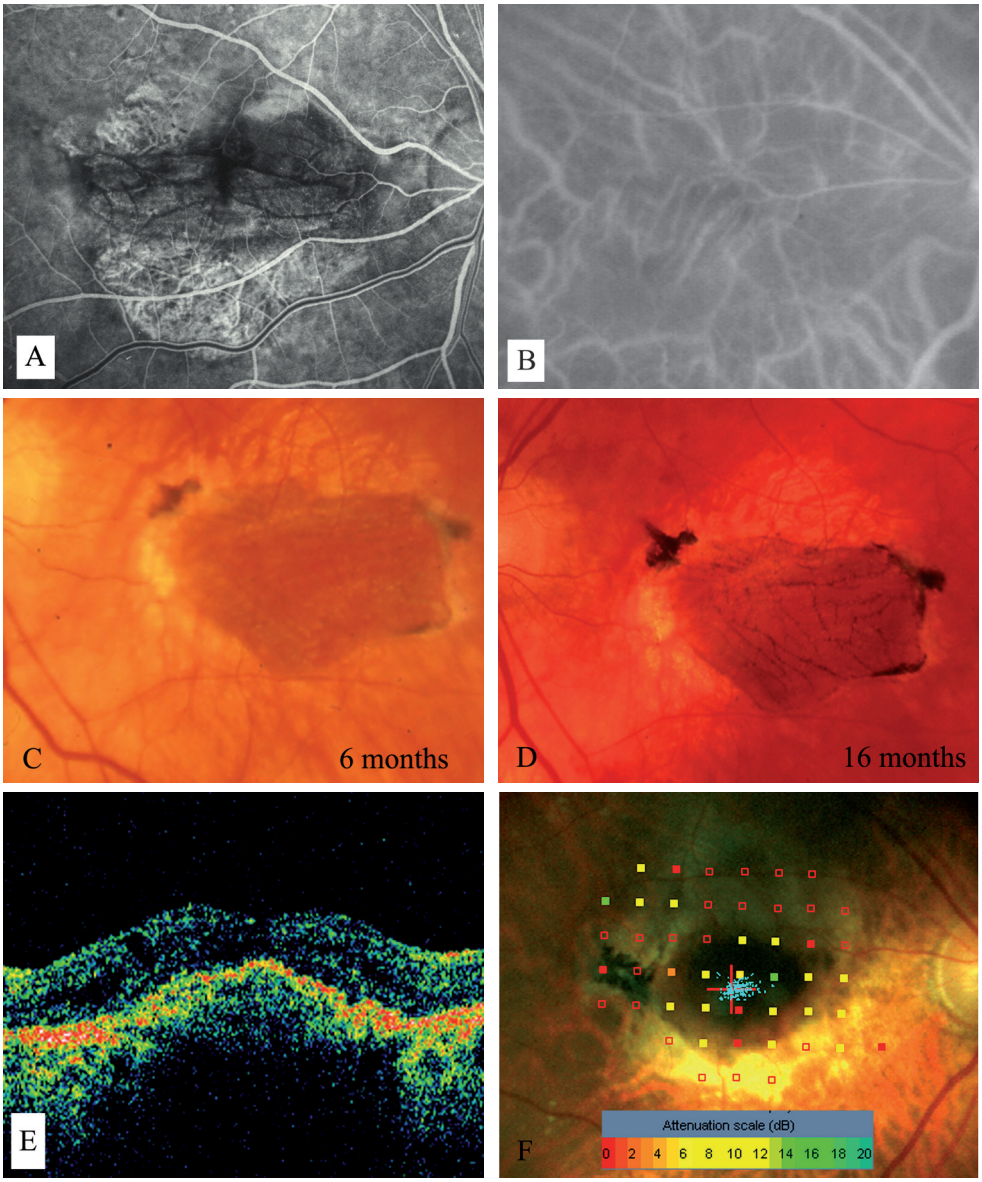
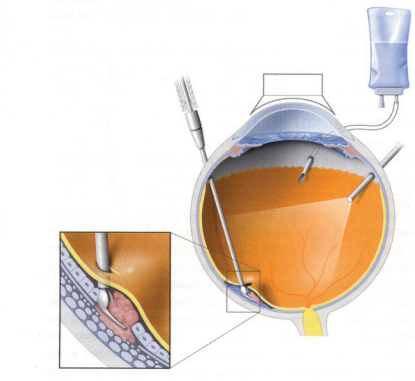
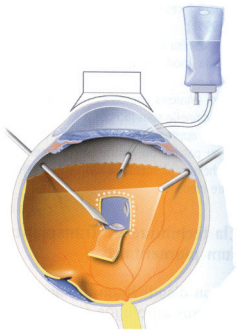
Appendix



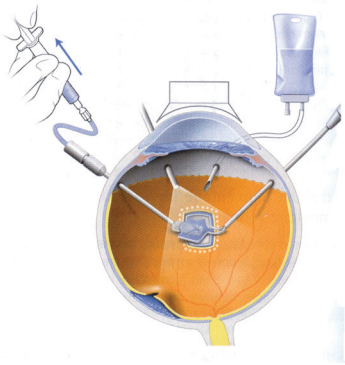
Chapter 2.1, Figure 4



Chapter 2.2, Figure 1



Chapter 2.2, Figure 2



Chapter 2.2, Figure 3

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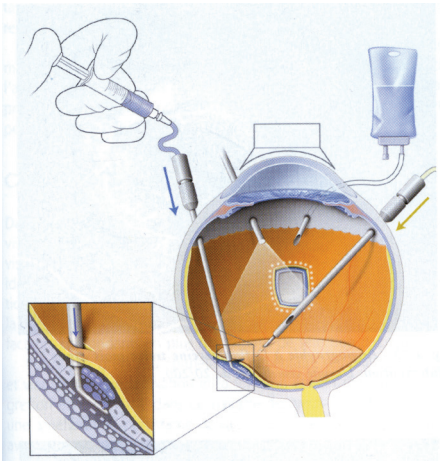
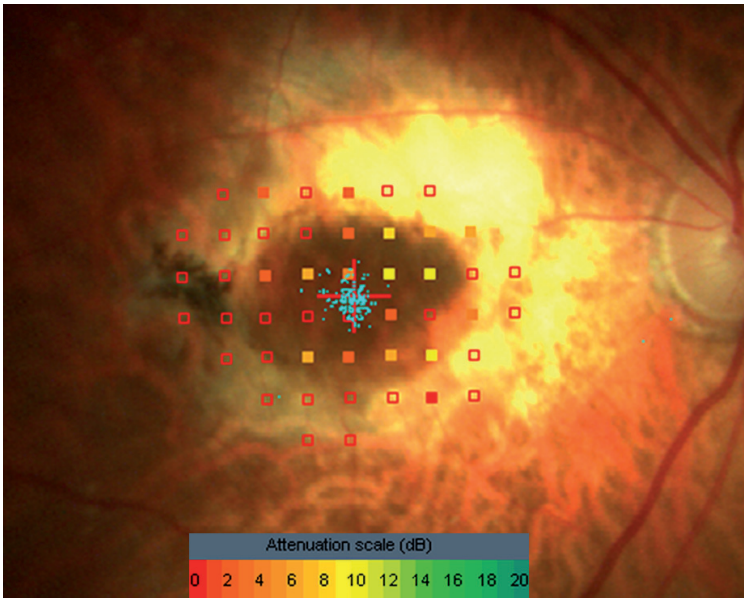


Chapter 2.2, Figure 4



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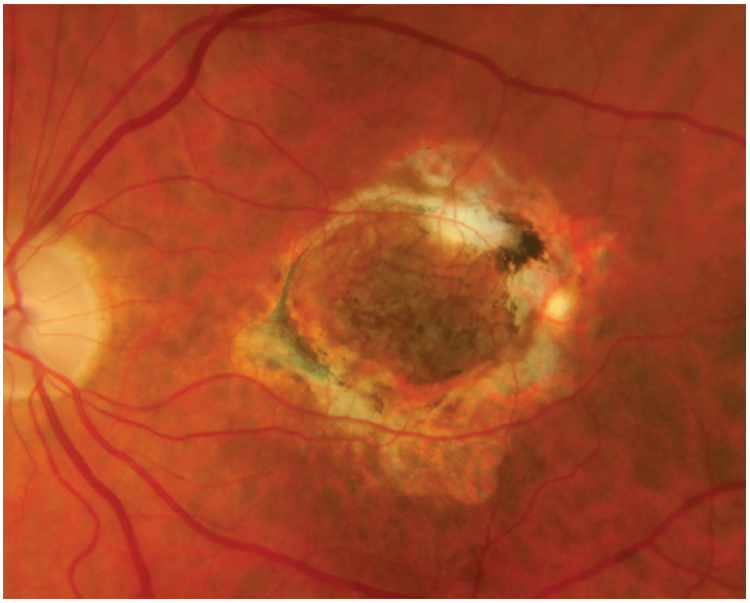

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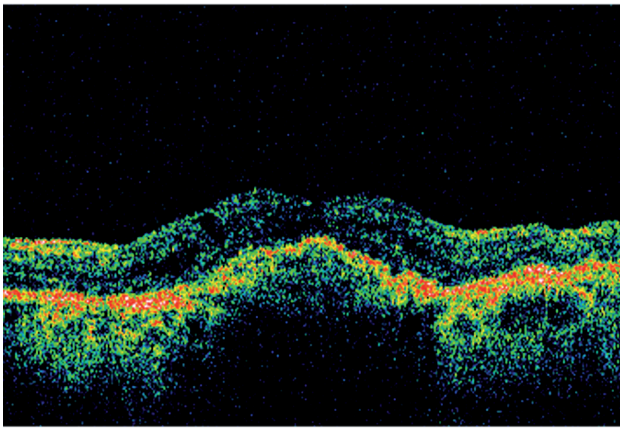
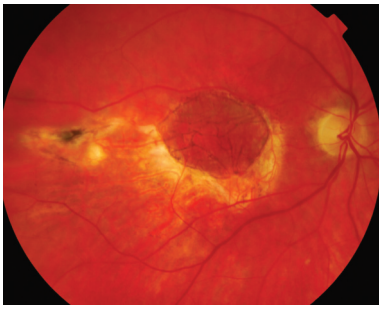
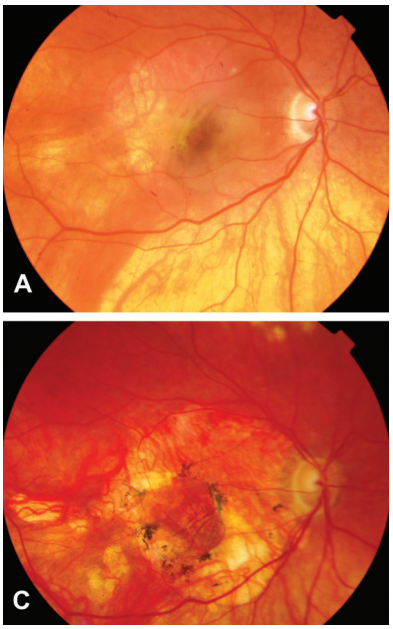


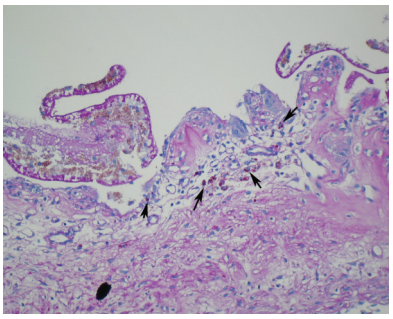
Chapter 2.2, Figure 10



Chapter 2.3, Figure 2



Chapter 2.4, Figure 1



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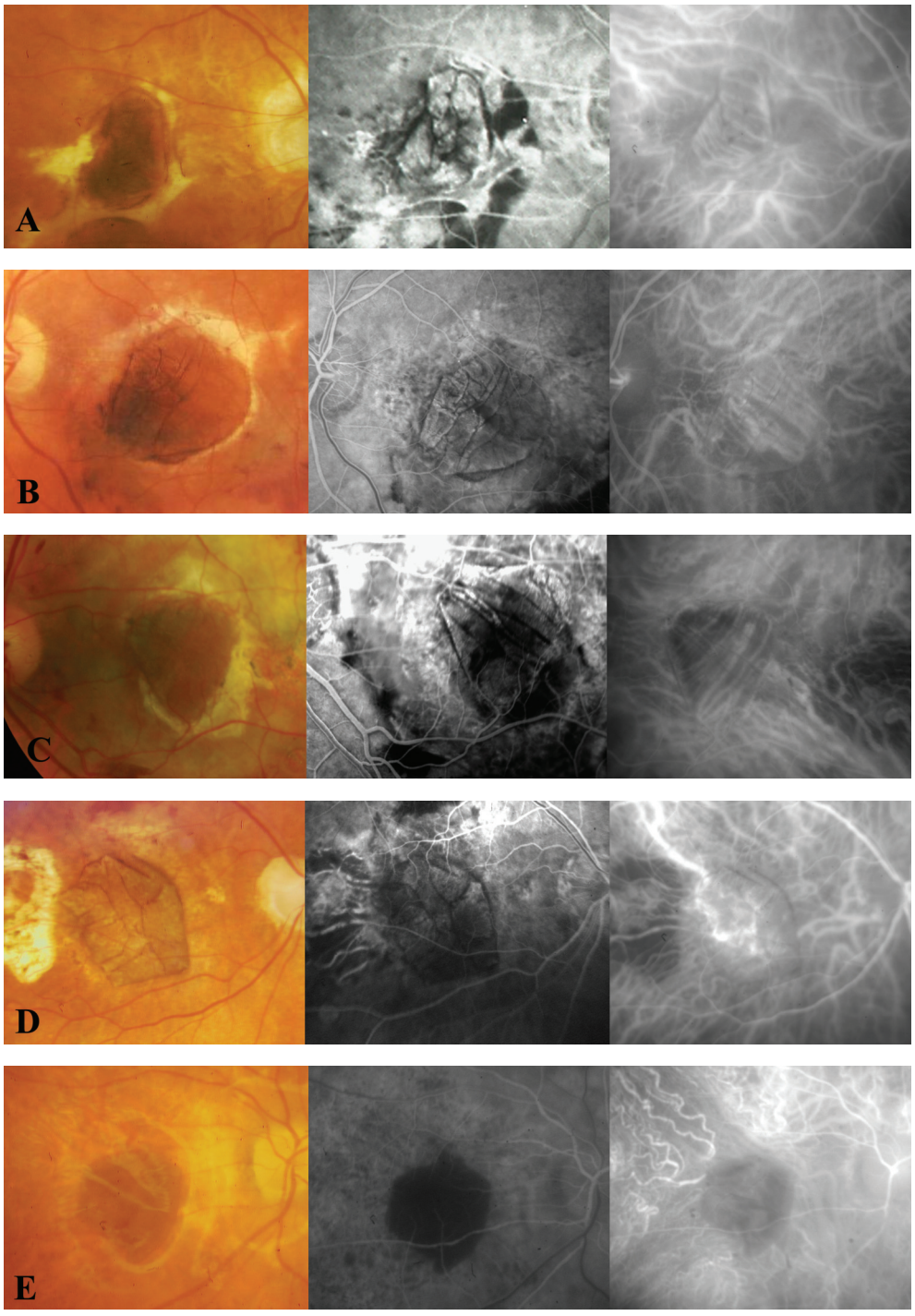
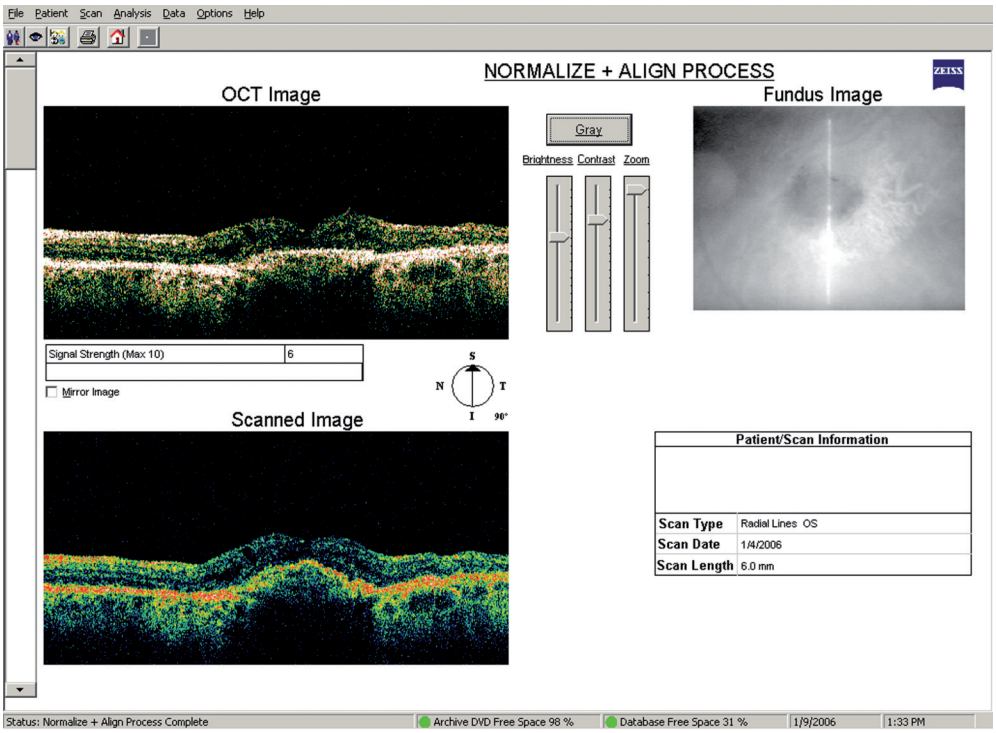
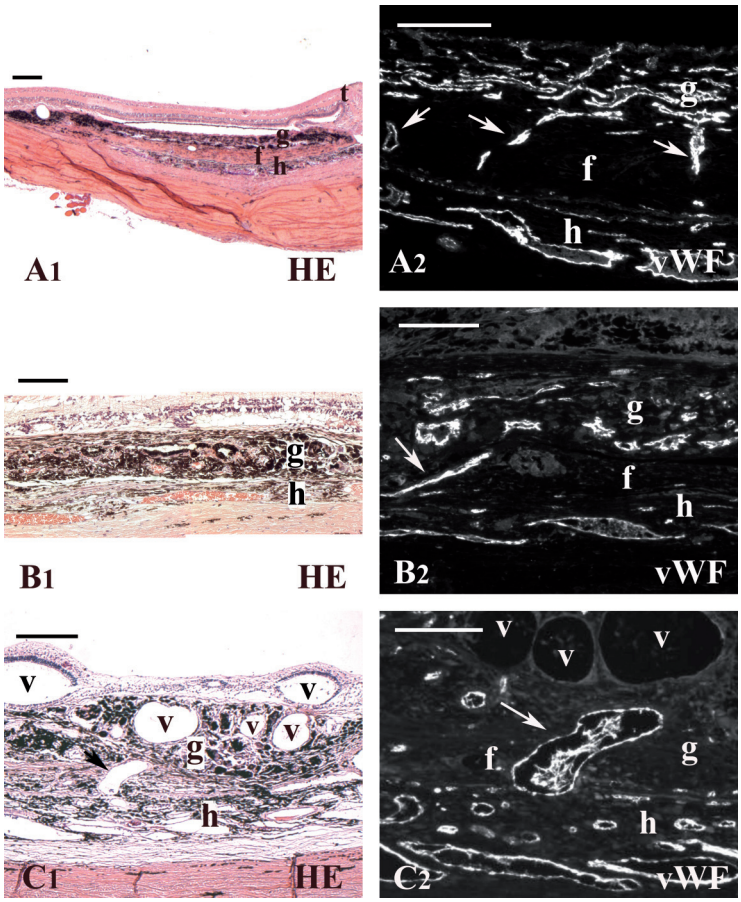


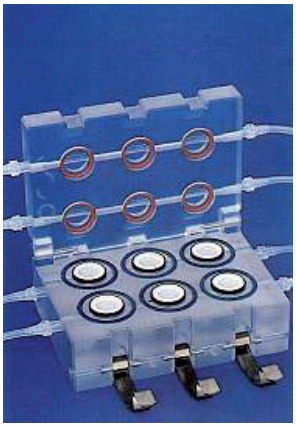
Chapter 2.5, Figure 1



Chapter 2.7, Figure 1



Chapter 3.1, Figure 1



Chapter 4.1, Figure 1

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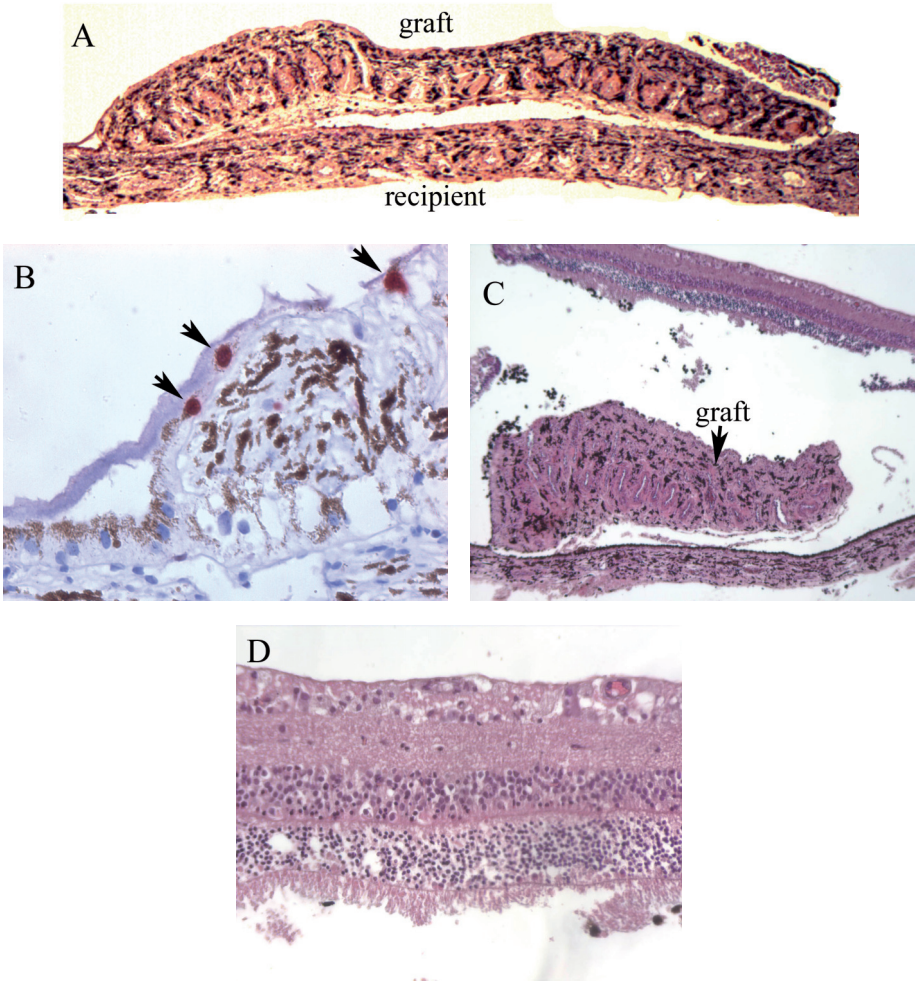

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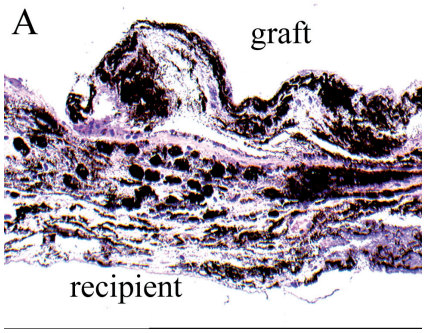

Chapter 4.1, Figure 4