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Published in:
Graefe's Archive for Clinical and Experimental Ophthalmology

DOI:
10.1007/s00417-011-1652-6

2011

Link to publication

Citation for published version (APA):
Crafoord, S., Andréasson, S., & Ghosh, F. (2011). Experimental vitreous tamponade using polyalkylimide hydrogel. *Graefe's Archive for Clinical and Experimental Ophthalmology*, *249*(8), 1167-1174. https://doi.org/10.1007/s00417-011-1652-6

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EXPERIMENTAL VITREOUS TAMPONADE USING POLYALKYL-IMIDE HYDROGEL

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ABSTRACT

Purpose: To evaluate polyalkyl-imide as a possible vitreous tamponading agent.

Methods: A 20 gauge pars plana vitrectomy and posterior vitreous detachment were performed in the right eye of six pigmented rabbits. Approximately 1 ml of viscoelastic gel, polyalkyl-imide (Bio-Alcamid®) was thereafter injected into the vitreous space. Full-field ERG and intraocular pressure (IOP, Tonopen) was measured pre-and postoperatively at regular intervals up to 28 days. At day 6 or 28, the rabbits were sacrificed and the eyes were examined macroscopically, photographed, and prepared for histological examination with routine microscopy.

Results: The viscoelastic hydrogel was successfully injected and remained translucent with preserved gel properties throughout the postoperative period. The postoperative IOP was unchanged compared to preoperative values. Five of six eyes displayed retinal edema or pigmentary changes centrally while the periphery appeared intact. ERG recordings showed a radical decrease in rod and cone derived B-wave amplitudes. Histological examination confirmed varying degrees of edema combined with neuronal cell death within the retinal layers in the central part of the fundus while the peripheral part appeared intact.

Conclusion: Polyalkyl-imide displays favourable physical properties when used as a vitreous tamponade. However, the hydrogel causes functional and morphological retinal damage when in direct contact with the inner retina. Possible pathological mechanisms include osmotic imbalance and direct toxic effects, and modification of biochemical properties is warranted before clinical use will be possible.

KEY WORDS:

Vitreous; Vitrectomy; Photoreceptor death

INTRODUCTION

Several eye disorders such as rhegmatogenous retinal detachment, severe diabetic retinopathy, and penetrating trauma affect the retina to such an extent that surgical removal of the vitreous and replacement with a tamponading agent is often necessary. The pars plana vitrectomy paradigm in such cases includes vitreous removal and replacement with saline solution or tamponades in the form of air, perfluorocarbon liquids, intraocular expanding gas or silicone oil. However, current clinical tamponading agents are all associated with complications. Gas and silicone oil disturb the normal optical properties of the eye, induce cataract and IOP elevation, and demand awkward posturing regimes. In addition, the tamponading effect of gas is limited in time while oil and perfluorocarbon liquids require a second procedure for removal (Parel et al.2001; Kirchof et al 2002). Finding novel long-term tamponading agents with less adverse effects is thus a justified challenge.

The ideal vitreous tamponading agent requires surface properties restricting inflow of water through existing breaks and interfacial tension preventing the tamponade itself to enter these breaks. However, several other factors also need to be considered such as optical clarity, refractive index, long-term molecular stability, biological atoxicity, and molecular transport.

Chemically different compounds such as hyaluronic acid (Healon^{R}), perfluorinated organic liquids and various synthetic polymers have been tested in the laboratory (Denlinger et al 1980, 1980b, Koster et al 1986, 1986b, Gerke et al 1984, Wilson et al 1995; Swindle et al 2007, Maruoka et al 2006, Soman et al 2003, Hong et al 1998). So far, their potential use has been limited due to degradation problems, opacification, retinotoxicity and IOP elevation.

A fairly recent idea is to explore substances used successfully in other surgical areas (Yang et al 2008). One potential compound is the acrylamide-derivative Bio-Alcamid® (Polyalkylimide), a clear hydrogel used as subcutaneous filler in reconstructive plastic surgery (Lahiri et al 2007). Bio-Alcamid® has been found to be highly bio-compatible, chemically and physical-

ly stable. Interestingly, from a vitreous tamponading point of view, this compund has also been demonstrated to form a thin collagen capsule isolating it from the host tissue. These attributes together with the possibility of uncomplicated removal makes Bio-Alcamid® a theoretically attractive option for intravitreal use.

In the present paper, we present an experimental protocol involving vitrectomy, retinal morphology and function to test the feasability of Bio-Alcamid® as a vitreous tamponade.

MATERIAL AND METHODS

The study was approved by The Regional Ethics Committee for Animal Experiments in Lund and it also conformed to the ARVO Resolution on the Use of Animals in Vision and Ophthalmic Research.

Six pigmented rabbits, aged 4 months were used in the experiment. The right eye was operated upon and filled with Bio-Alcamid® while the left eye served as control. Additional two rabbits were vitrectomized in their right eyes and filled with BSS®. They served as controls to the surgical procedure.

Examination including ophthalmoscopy and IOP measurement (Tonopen) was performed at postoperative day 1, 6 and 28. The rabbits were sacrificed at day 6 (n=1) or 28 (n=5), at which time the eyes were gross examined, photographed and prepared for histological examination with routine microscopy.

Gel properties

Bio-Alcamid® (Polymekon, Brindisi, Italy) is a gel polymer comprised exclusively of networks of alkyl-imide groups (approximately 4%) and non-pyrogenic water (approximately 96%). Alkyl-imide belongs to the family of acryl-derivatives and its polymeric structure does not contain free monomers. The gel is available on the market and used clinically in plastic surgery as non-degradable filler in aesthetic lipoathrophic conditions and after posttraumatic or therapeutic atrophy of subcutaneous tissue. (Claoue et al 2004, Lahiri et al 2007, Protopapa et al 2003). When placed in the subcutaneous space, the gel forms a thin collagen capsule, and it is extractable even after several years without signs of degrading.

Surgery

All procedures were performed by clinically well experienced vitreoretinal surgeons. General anesthesia was provided with a combination of ketamine (35 mg/kg) and xylazine (5

mg/kg) intramuscularly. The right eye was instilled with cyclopentolate (1%) and phenylephrine (10%) 30 minutes before surgery. Topical tetracaine (0.5%) was applied just before surgery. The conjunctiva was incised limbally 270° from 9 to 6 o'clock with a vertical incision at 12 o'clock, creating two flaps. A 20G infusion cannula was sutured to the sclera in the 4 o'clock position 1 mm posterior to the limbus and a balanced salt solution (BSS, Endosol, Allergan Medical Optics) was started. Two 20G sclerotomies were made in the 10 and 2 o'clock positions. A BIOM 90-D lens (Oculus) was used to visualize the fundus, and an Accurus surgical system machine (Alcon, Fort Worth, TX) was used for surgery. A standard endo-illuminating light probe (Alcon) was introduced through the 10 o'clock sclerotomy (illumination level 80%), and a vitreous cutter (Innovit, Alcon) was inserted through the 2 o'clock sclerotomy. Posterior vitreous detachment (PVD) was created by positioning the vitrectomy probe at the margin of the disk and applying suction (100 mmHg) while pulling on the probe. PVD was confirmed visually as the posterior vitreous cortex separated from the posterior pole. All vitreous in the central fundus (approximately 50% of the total volume) was removed while peripheral parts were left because of the risk of instrument touch to the comparatively large lens. In two cases pin-point hemorrhages appeared at the central retina after PVD induction, and in one case a retinal touch with the vitrector occurred causing a minimal retinal hemorrhage. The hemorrhage stopped quickly and the touch did not lead to any retinal break or detachment in the postoperative period. After vitreous removal, a fluid-air exchange was performed after which Bio-Alcamid® was injected under visual control through a 19 gauge needle. The amount of gel injected varied from 0,4 to 1,2 ml (Table 1). The sclerotomies and conjunctiva were sutured and 25 mg gentamicin and 2 mg betamethasone were injected subconjunctivally. No postoperative treatment was given. The eyes were examined externally daily and with an external ophthalmoscope at days 1 and 10. Two eyes, used as controls, un-

derwent the above surgery but were injected with balanced salt solution (BSS®) instead of Bio-Alcamid®. These 2 animals were followed for 41 days.

Full-Field ERG

A standardized full-field electroretinography (ERG) was recorded 7 days before surgery and 28 days postoperatively on the right eye using a Nicolet Viking analysis system (Nicolet Biomedical Instruments, Madison Wisconsin) as previously described (Gjörloff et al. 2004). During examination the rabbits were sedated with Hypnorm®, (fentanyl 0.2 mg/ml and fluanisone 10 mg/ml) 0.1 ml/kg, intramuscularly and the pupils were dilated with Cyclogyl® (cyclopentolate hydrochloride 1%) to a pupil diameter of 8–9 mm.

After 30 minutes of dark adaptation a Burian-Allen bipolar ERG contact lens electrode was applied on the topically anesthetized cornea together with a subcutaneous ground electrode on the neck. The lens was lubricated with methylcellulose (2%). Responses were obtained with a wide band filter (-3 dB at 1 Hz and 500 Hz), stimulating with single full-field flashes (30 µs) with dim blue light (Wratten filters # 47, 47A and 47B) and of white light (0.8 cd.s/m2) without a background and of white light (3.8 cd.s/m2) with a background light of 10 fL. Cone responses were also obtained with 30 Hz flickering white light (0.8 cd s/m^2) averaged from 20 sweeps without a background light. The luminances of the three different light stimuli refer to the light reflected from the Ganzfeld sphere.

Tissue preparation

At day 6 or 28, the rabbits were sacrificed and the eyes were dissected, gross examined and fixed for 1 h in 4% formalin, pH 7.3 in a 0.1 M Sørensen's phosphate buffer (PB). After fixation, the specimens were washed with 0.1 M Sørensen's PB, and then washed again using the same solution containing sucrose of rising concentrations (5-25%). The specimens were sec-

tioned at 12 μm on a cryostat, and each 10th slide was stained with hematoxylin and eosin according to standard procedures.

For glial fibrillary acidic protein (GFAP) immunolabeling, sections were washed in 0.1 M of sodium phosphate-buffered saline pH 7.2 (PBS) with 0.1% Triton X-100 (PBS/Triton) and incubated with the primary antibody (anti-GFAP, clone G-A-5; Millipore, Sundbyberg, Sweden, diluted 1:200 with PBS/Triton with 1% bovine serum albumin) overnight at +4°C. After incubation, the slides were rinsed in PBS/Triton, incubated with fluorescein isothiocyanate (FITC)-conjugated antibodies (Sigma-Aldrich, Saint Louise, USA) for 45 min, rinsed, and mounted in vectashield (a custom-made anti-fading mounting media, Vector laboratories, Inc., Ca, USA). Unoperated eyes served as controls. For negative controls, the same labeling procedure without the primary antibody was performed on both the normal left and the operated right eye of the animals.

RESULTS

Macroscopic findings

No adverse conjunctival swelling or inflammation of any eye injected with poly alkyl-imide hydrogel was observed postoperatively. Postoperative IOP (9-15 mmHg) was unchanged compared with preop values (8-13 mmHg). On postoperative day 1, moderate lens opacities were noted in 4 eyes making fundus examination difficult. In one eye, the retina appeared normal, and in the remaining one, a limited retinal detachment was seen. In postoperative day 6, all eyes displayed posterior cataract, with fundus visualization possible in 4 eyes (Fig. 1a). At this time, one eye (#5) displayed profound choroidal swelling with spot hemorrhages on the retina and this animal was therefore terminated (Fig. 1b). At dissection, this eye was found to be well filled with the hydrogel which remained clear, but displayed a substantial degree of fragmentation (Fig. 2a). The remaining eyes were kept until postoperative day 28 at which time some extent of lens opacification was still present in 4 out of 5 cases, but fundus visualization was possible in all eyes. One eye displayed total retinal detachment, three eyes had pigmentations in the posterior fundus, and the remaining one appeared normal. No signs of proliferative vitreoretinopathy (PVR) were seen. The translucent gel filled the vitreous space in 4 eyes and was well apposed to the retinal surface (Fig. 2b and c). In 3 eyes, some degree of fragmentation of the gel was noted. In the eye with retinal detachment, the gel could not be identified.

The BSS® injected eyes displayed no cataract, clear vitreous and intact retina.

Histology

In the eye terminated on postoperative day 6, light microscopy using hematoxylin and eosin staining revealed neuroretinal destruction of all retinal layers and RPE within the central

part of the fundus while the peripheral part appeared intact (Fig. 3a-c). The choroidal space in this eye was enlarged without any normal vessels. No signs of inflammation were seen.

In eyes terminated at day 28, varying degrees of neuroretinal degeneration was seen. In one eye, the retina appeared normal, 3 eyes displayed neuroretinal degeneration centrally with profound changes in the inner retinal layers, and in the eye with retinal detachment, total neuroretinal destruction was seen. In most eyes, a sharp demarcation was seen between degenerated and more normal retina indicating a variation of locations of the neuroretina cellular destruction (Fig. 3d). Two eyes displayed invasion of leucocytes in the subretinal space (Fig. 3e). No other signs of inflammation were seen. At the areas of retinal degeneration, disruption of RPE cells was evident, in areas of more normal retina, also the RPE appeared normal. No enlargement of the choroidal space was seen in these specimens.

The 2 BSS injected eyes displayed normal retinal morphology.

GFAP immunolabeling

All Bio-Alcamid injected eyes displayed upregulation of GFAP as a sign of Müller cell activation.No particular difference in labeling intensity was found between central and peripheral retina, but in degenerated parts, Müller cells were not arranged in the normal vertical manner (Fig. 4). In BSS injected eyes GFAP labeling was comparable with normal unoperated controls with discrete labeling of Müller cells seen only in the periphery.

ERG

Full-field ERG measurements were obtained pre- and post-operatively in five eyes (one rabbit was sacrificed after 6 days and no ERG performed). These recordings demonstrated a reduction of both cone and rod b-wave amplitudes 28 days postoperatively compared with preoperative values in 4 cases (Fig. 5). In the eye with detachment (#1), no residual response

could be measured, while in the remaining four, varying degrees of functions was still present.

DISCUSSION

Attempts to develop new vitreous tamponading agents have been ongoing for several decades. Various substances and approaches have been explored, but to date clinically available alternatives to conventional tamponades are still very limited (Denlinger et al., 1980; Swindle et al 2009; Katagiri et al 2005; Gao et al 2008). To identify and develop a novel agent is a challenging task, albeit with a large therapeutical potential. The various conditions in which a synthetic long-term tamponade could be used may demand different properties, but all require compound-to-retina contact preventing water inflow through existing breaks.

In the present study we have evaluated Bio-Alcamid® as a vitreal tamponade using a previously described protocol involving vitrectomy with postoperative evaluation of retinal morphology as well as function (Wallentén et al., 2008). We found that the viscoelastic polyalkylimide hydrogel developed for reconstructive surgery can be injected in the vitreous space in the normal rabbit eye. The gel remained clear with retained viscosity for at least 28 days, factors that are important when considering its use as a tamponading agent. In contrast to previous reports in reconstructive surgery, a surrounding capsule was not found; instead the gel displayed uninterrupted apposition with the retinal surface without signs of PVR.

When the natural vitreous is removed, a new metabolic and physiologic environment is created within the eye. Specifically, oxygen concentration, osmotic balance and molecular transport in the vitreous space are altered in a manner that may affect all interior tissues of the eye (Stefansson 2009). In this setting, the rabbit retina may be especially vulnerable to biochemical changes due to its merangiotic nature (partly vascularized, i.e., the retinal layers are dependent on nutritive supply from the choroid and also the vitreous (De Schaepdrijver et al., 1989). It has been demonstrated that the oxygen concentration changes from 15 to 80 % in the vitreous space after vitrectomy (Stefansson 2009). This phenomenon has also been shown in rabbits by Barbazetto et al 2004. The normal PO_2 gradient from the lens to the retina disap-

pears after vitrectomy and the oxygen concentration near the lens increases 2-3 times. In humans, the increased oxygen concentration close to the crystalline lens has been implicated in cataract formation post-vitrectomy (Holekamp et al 2005). We observed pathological changes in Bio-Alcamid® filled eyes early in the postoperative period, not only in the retina, but also in the lens. We detected no such changes in BSS injected eyes, indicating that the polyalkyl-imide gel and not the surgical procedure is responsible for pathological changes. Due to the relatively large size of the lens, PVD and vitrectomy is only possible in the central part of the rabbit eye, which in turn prevents a complete fill of the gel. Degenerative retinal changes were seen almost exclusively in central parts of the retina and not in the periphery where the natural vitreous remained; suggesting that direct gel-to-retina contact is responsible for the adverse influence on the retina. The amount of removed vitreous, and as a consequence, the space that could be filled with gel varied between 0,4 – 1,0 ml which was probably attributed to variations in lens size and volume of the rabbit eye (Sarnat 1978). Interestingly, the eye which was filled with the largest volume of hydrogel (#1, 1,0 ml) displayed the most profound pathological changes while the eye with only 0,4 ml (#3) of gel had only minimal changes. BioAlcamide® contains polyalkyl-imide which belongs to the family of acryl-derivatives. In monomeric form, some members of the acryl-derivatives are well known to produce neurotoxicity characterized by neuronal swelling (LoPachin, 1994). The polyalkyl-imide macromolecular gel is formed by cross-linking of amide-imide alkyl-type molecules. This gel has been found to be atoxic when applied on the skin or on cultured fibroblast, but to induce swelling and hyperemia after subcutaneous injection in the rat (Ramires et al., 2005). In the present work, we noted abnormal retinal edema early in the postoperative period, and we can therefore not rule out the possibility of a direct toxic effect of the compound.

To sum up, intravitreal polyalkyl-imide gel induces severe retinal pathological reactions within the rabbit eye but displays physical properties which fulfill several of the requirements

of an ideal vitreous tamponade. Future work will now be focused on eliminating toxic influence without altering the gel structure.

ACKNOWLEDGEMENTS

The authors thank Karin Arnér for excellent technical assistance.

This study was supported by The Faculty of Medicine, University of Lund, The Swedish Research Council, The Torsten and Ragnar Söderberg Foundation, and research funds of Departments of Ophthalmology Örebro. BioAlcamid® was kindly provided by Richard Leo, P.O.Medica AB, Sparsör, Sweden.

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TABLES

Case #	Amount of injected gel (ml)	Follow-up time (days)	IOP Preop (mm Hg)	IOP Postop (mmHg)	Cataract	Retinal Status Macro/Microscopically
1	1.0	28	8	9	No	Retina thick and detached. Total degeneration centrally GFAP upregulated
2	0.5	28	9	10	Moderate	Minimal retinal folds and pigment clumps. Thin retina with no photoreceptors centrally. Inflammatory cells subretinally. GFAP upregulated
3	0.4	28	10	10	Mild posterior	Minimal pigment clumps otherwise ok macroscopically. Normal morphology (htx). GFAP upregulated
4	0.5	28	12	12	Mild posterior	Macroscopically ok. Degenerated centrally. Inner retina with cell-loss. GFAP upregulated
5	0.6	6	13	13	Mild posterior	Retinal edema centrally Total degeneration centrally. GFAP upregulated
6	0.6	28	13	15	Moderate	Minimal pigment clumps otherwise ok macroscopically. Degeneration centrally. Inner retinal cell-loss. Inflammatory cells subretinally. GFAP upregulated
Cntrl 1	0.6 (BSS)	41	10	10	No	Macroscopically ok. Normal morphology (htx). No GFAP upregulation
Cntrl 2	0.6 (BSS)	41	10	10	No	Macroscopically ok. Normal morphology (htx). No GFAP upregulation

Table1. Pre-, per- and post-operative data

Case #	Blue light		White light		30 Hz flicker dark		Single flash light	
	Preop	Postop	Preop	Postop	Preop	Postop	Preop	Postop
1	85,9	0	105	0	20,7	0	83,3	0
2	76,8	5,2	138	18,2	15,7	1,5	109	6,5
3	49,5	61,2	85,9	78,1	12,3	4,2	54,7	46,9
4	43	27,3	85,9	49,5	9,3	6,5	54,7	41,7
5	56	-	100	-	13,9	-	79,4	-
6	70,3	27,3	74,2	41,7	8,1	5,3	52,1	19,5
Median	63,2	27,3	93,0	41,7	13,1	4,2	67,1	419,5
Cntrl 1	76,8	53,4	164	99	20,9	12,6	109	53,4
Cntrl 2	39,1	89,8	19,5	17,6	10,1	3,96	45,6	53,4

Table 2. ERG data. Values are given of the b-wave amplitudes (μV) for rod ERG (blue light), combined ERG (white light), dark adapted cone ERG (30-Hz flicker) and single white flash light adapted.

FIGURELEGENDS

Figure 1. Ophthalmoscopic examination 6 days postoperatively. **a**: Posterior lens opacification is seen (Case #4). **b**: Clear media, with massive neuroretinal swelling is seen in Case #5.

Figure 2. a: Dissection. Case #5, 6 days postoperatively. The gel has remained clear, but has fragmented severely. **b** and **c**: Case #2 displays clear gel with no apparent fragmentation 28 days postoperatively. The gel is well apposed to the retinal surface.

Figure 3. Hematoxylin and Eosin staining, cryosections. **a** - **c**: Case #5 displays choroidal edema and total neuroretinal destruction centrally (**a** and **b**) 6 days postoperatively. The peripheral neuroretina appears normal (**c**). **d**: In Case #4, the border between severe neuroretinal degeneration, and less affected tissue is seen (arrow). **e**: In Case #2, invasion of inflammatory cells can be seen in the subretinal space. The RPE is disrupted, and degeneration of photoreceptors as well as inner retinal cells is evident. Scale bar = 200 µm (**a**), 50 µm (**b** - **d**), 25 µm (**e**).

Figure 4. GFAP immunolabeling, cryostat sections. Case #2 displays upregulation of GFAP in Müller cells. In the peripheral part (**a**), Müller cells display the normal arrangement of vertically arranged cells while labeled cells in the central part with retinal degeneration (**b**) has a more disorganized appearance. Scale bar = 50 µm.

Figure 5. Full-field ERG demonstrating reduction of a- and b-wave amplitudes in all stimulation protocols, indicating diminshed cone and rod function 28 days after injection of Bio-Alcamid®.

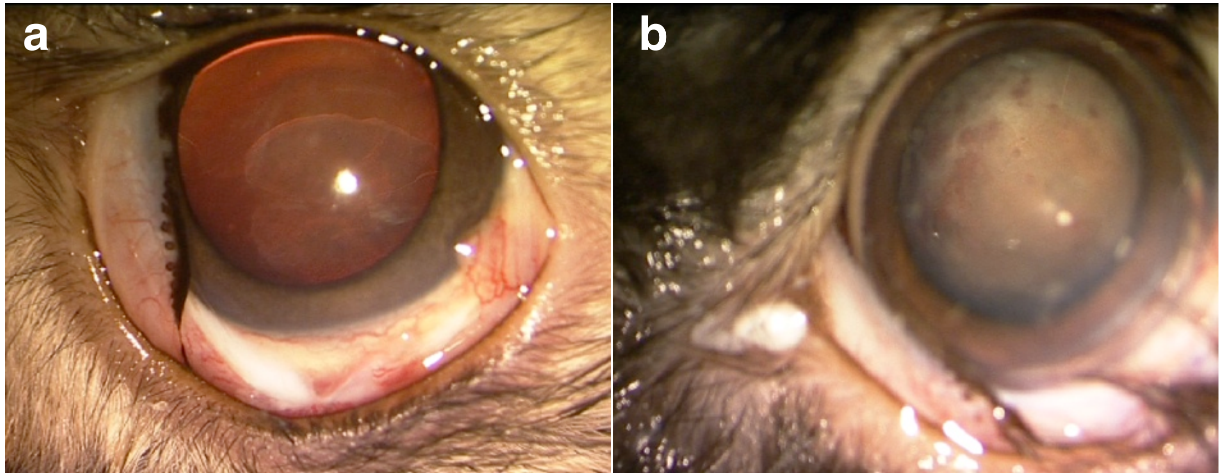

Figure 1. Crafoord et al

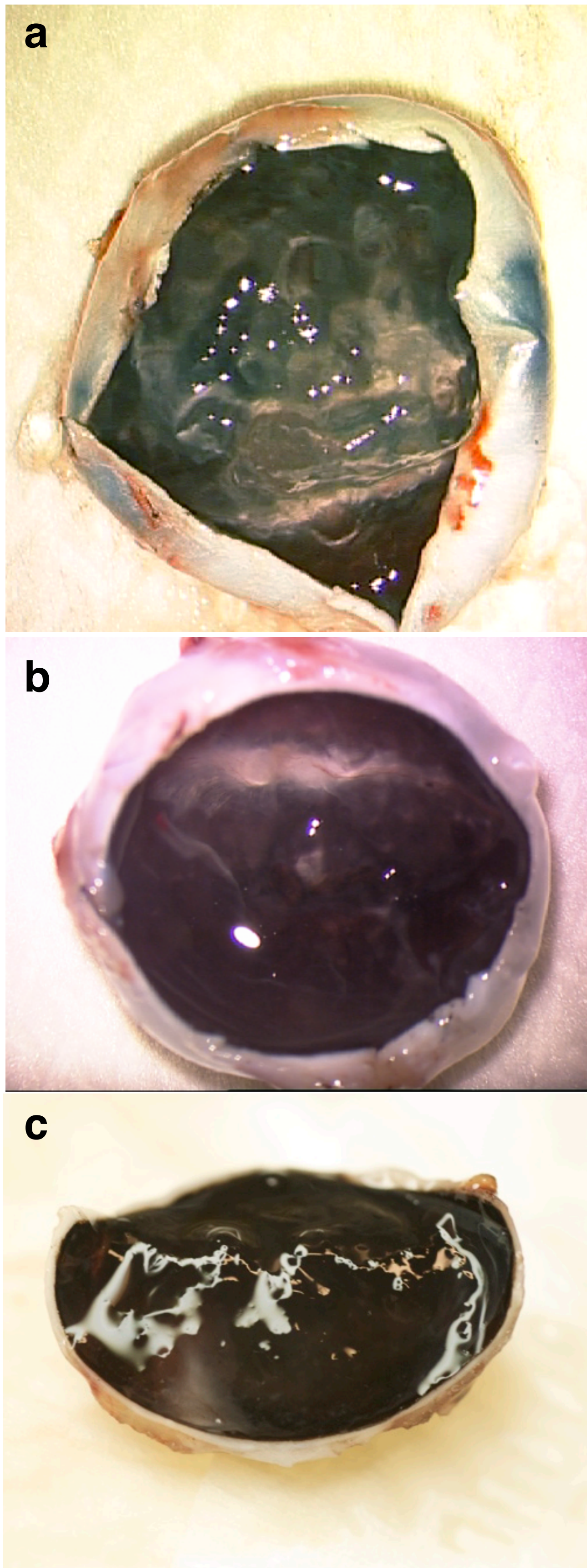

Figure 2. Crafoord et al

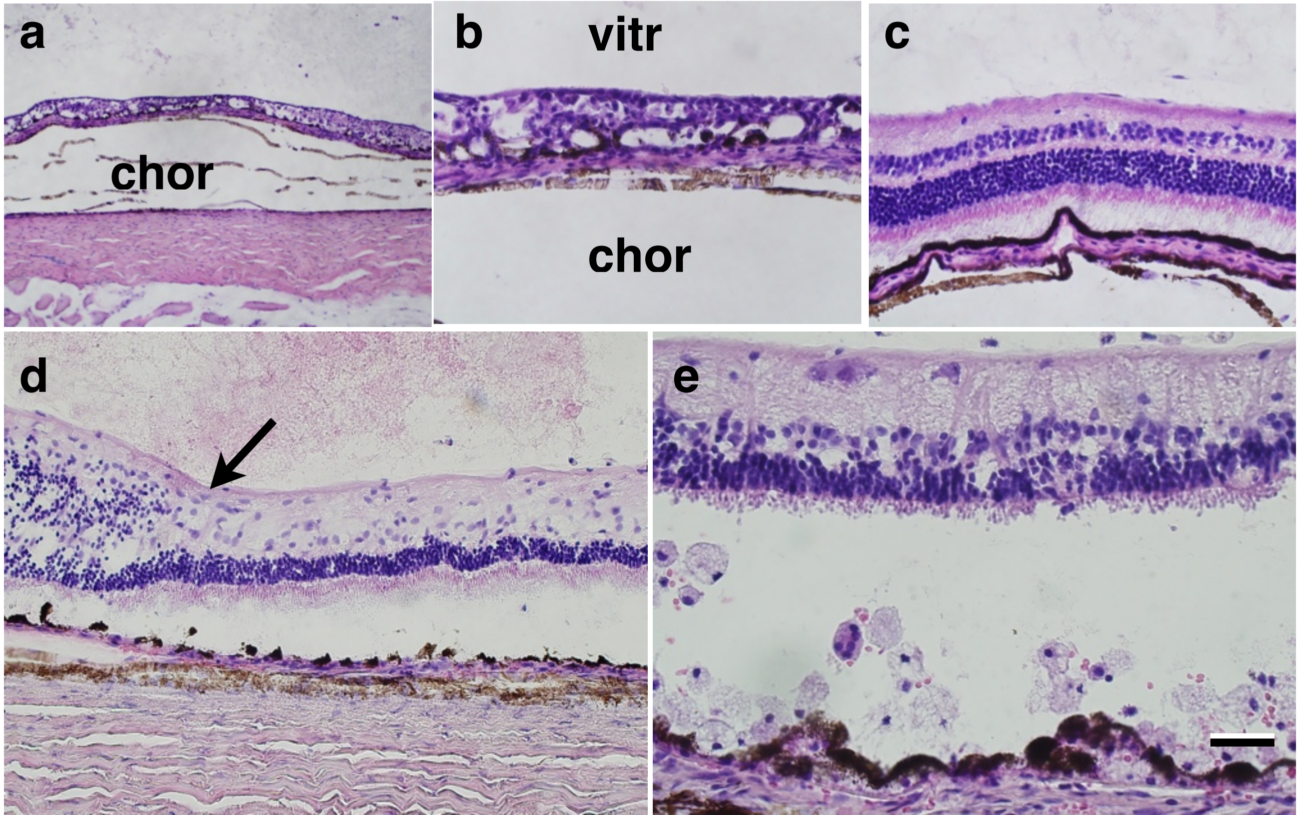

Figure 3. Crafoord et al

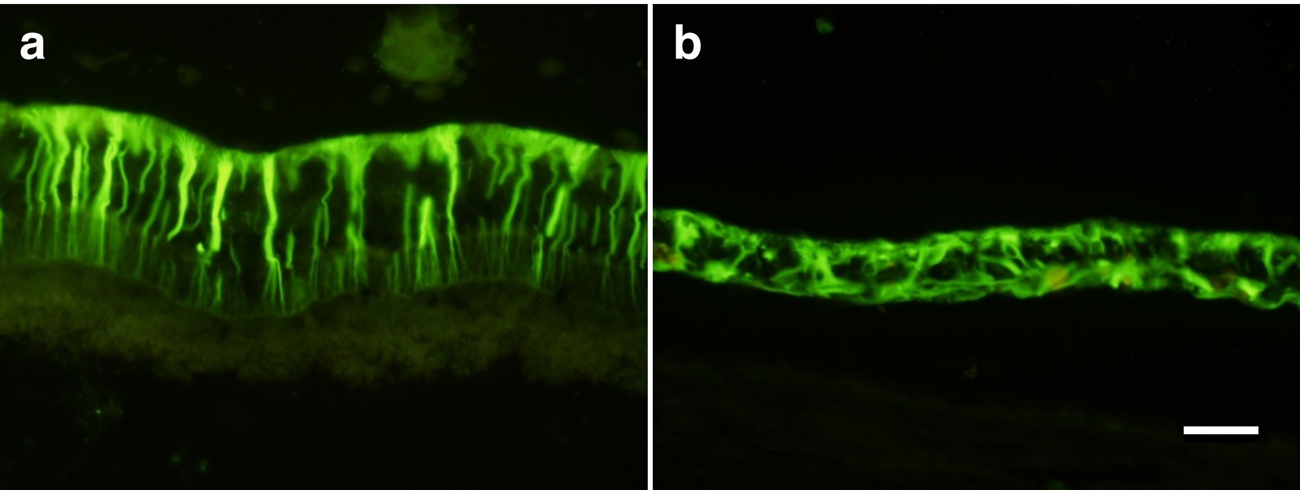

Figure 4. Crafoord et al

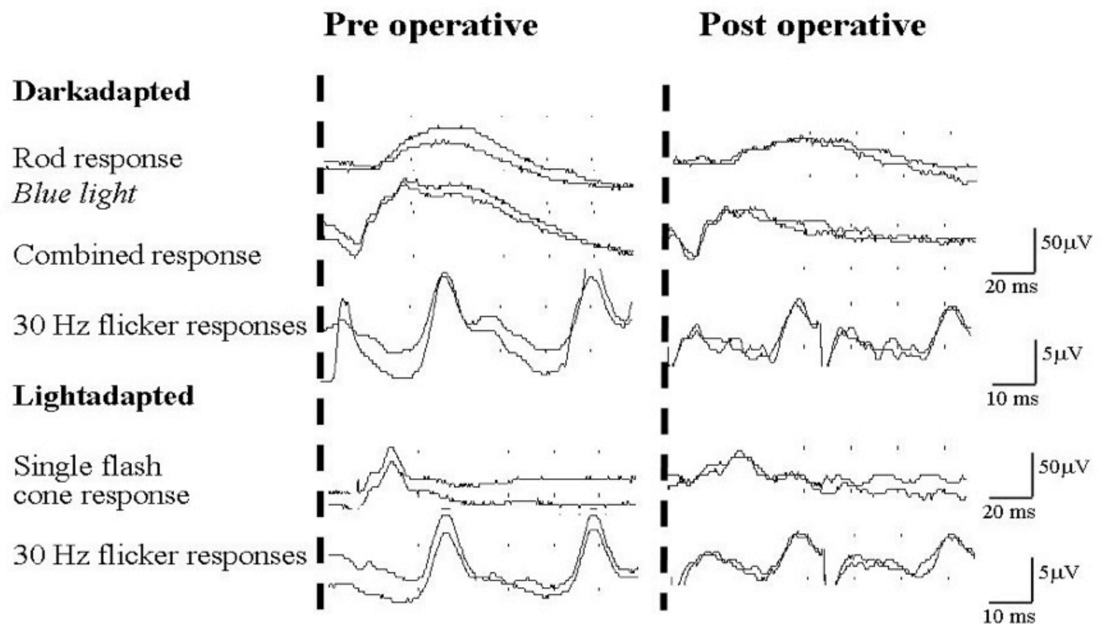

Figure 5. Crafoord et al