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A NEW MODEL FOR IN VITRO TESTING OF VITREOUS SUBSTITUTE CANDIDATES

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ABSTRACT

Purpose: To describe a new model for *in vitro* assessment of novel vitreous substitute candidates.

Methods: The biological impact of three vitreous substitute candidates were explored in a retinal explant culture model; a polyalkylimide hydrogel (Bio-Alcamid®), a two component hydrogel of 20 wt.% poly(ethylene glycol) in phosphate buffered saline (PEG) and a cross-linked sodium hyaluronic acid hydrogel (Healaflo®). The gels were applied to explanted adult rat retinas and then kept in culture for 2, 5 and 10 days. Gel exposed explants were compared with explants incubated under standard tissue culture conditions. Cryosections of the specimens were stained with hematoxylin and eosin, immunohistochemical markers (GFAP, Vimentin, Neurofilament 160, PKC, Rhodopsin), and TUNEL.

Results: Explants kept under standard conditions as well as PEG exposed explants displayed disruption of retinal layers with moderate pyknosis of all neurons. They also displayed moderate labeling of apoptotic cells. Bio-Alcamid® exposed explants displayed severe thinning and disruption of retinal layers with massive cell death. Healaflo® treated explants displayed normal retinal lamination with significantly better preservation of retinal neurons compared with control specimens, and almost no signs of apoptosis. Retinas exposed to Healaflo® and retinas kept under standard condition showed variable labeling of GFAP with generally low expression and some areas of upregulation, PEG-exposed retinas showed increased GFAP labeling, and Bio-Alcamid® exposed retinas showed sparse labeling of GFAP.

Conclusions: Research into novel vitreous substitutes has important implications for both medical and surgical vitreoretinal disease. The *in vitro* model presented here provides a method of biocompatibility testing prior to more costly and cumbersome *in vivo* experiments. The explant culture system imposes reactions within the retina including disruption of layers, cell death and gliosis, and the progression of these reactions can be used for comparison of vitreous substitute candidates. Bio-Alcamid® had strong adverse effects on the retina, which is consistent with the results of prior *in vivo* trials. PEG gel elicits reactions similar to the control retinas whereas Healaflo® shows protection from culture induced trauma, indicating a favorable biocompatibility.

KEY WORDS

Vitreous Substitute; Immunohistochemistry; Retinal Culture; Vitreoretinal surgery; Hyaluronic Acid; Polyethylene oxide; Polyalkylimide;

INTRODUCTION

Vitreotomy is a common procedure for several eye disorders capable of severely impacting the vision of affected patients, and has an important role in the treatment of conditions such as rhegmatogenous retinal detachment, severe diabetic retinopathy, penetrating ocular trauma, macular holes, and epiretinal membranes. The removal of vitreous during vitreoretinal surgery mandates its replacement, either in the form of water or various tamponading agents. The compounds currently in widespread clinical use such as balanced salt solution, gases, silicon oils, and perfluorocarbon liquids all have considerable disadvantages, with complications such as cataract formation, uveitis, rise in intraocular pressure [1] and cytotoxicity [2, 3]. Further, current tamponading agents are either resorbed spontaneously after a few weeks or are not suitable for long-term use [4-9], and may require strict body positioning postoperatively.

The search for improved vitreous substitutes have been ongoing since the early days of the 20th century [10]. Early attempts were made to transplant animal and human vitreous [11], and investigations have been made into numerous semi-synthetic [12-14] and synthetic [15] molecules although few of them have reached a clinical setting, and none have fulfilled the requirements for long-term biocompatibility.

Traditionally, the interactions of vitreous substitutes with the tissues of the eye have been studied in various animal models *in vivo*. Such trials are, however, costly, time consuming, and might be considered ethically problematic. In some cases, *in vivo* experiments have been precluded by preclinical toxicological assays, mainly targeting apoptosis in cultures of cells from tissues outside the eye [16, 17], isolated retinal pigment epithelium (RPE) cells [18, 19] or dissociated cells from embryonal retinas [20]. The validity of these findings in relation to a clinical setting is however unclear since they represent a large transitional step regarding the impact on the adult neuroretinal sheet [21]. Therefore, a means to investigate the biological impact of vitreous substitutes more similar to the *in vivo* situation is desirable.

For this paper we wanted to explore a novel *in vitro* model for investigating the biological impact of vitreous substitutes on the neuroretina. To this end, we have used the well-established retinal explant model to study three polymer hydrogels of different chemical composition that theoretically may be considered as potential vitreous substitutes; 1) Cross-linked hyaluronic acid (Healaflo®), clinically used in glaucoma surgery [22, 23], 2) Poly(ethylene glycol) (PEG), widely used in different biochemical applications [24, 25], and 3) Polyalkylimide (Bio-Alcamid®), clinically used in reconstructive surgery [26-29].

MATERIALS AND METHODS

Three vitreous substitute candidates were investigated in the retinal explant culture model; a cross-linked sodium hyaluronic acid (22,5 mg/ml) hydrogel (Healaflo®), a two component hydrogel of 20 wt.% poly(ethylene glycol) in phosphate buffered saline (PEG) and a polyalkylimide hydrogel (Bio-Alcamid®). The gels were applied to explanted adult rat retinas and then kept in culture for 2, 5, and 10 days *in vitro* (DIV). Gel-exposed explants were compared with explants incubated under standard conditions (medium only). Cryosections of the specimens were stained with hematoxylin and eosin, immunohistochemical markers (GFAP, Vimentin, PKC, NF160, Rhodopsin), and TUNEL.

Animals

Retinas from adult Sprague-Dawley rats were used. All proceedings and animal treatment were in accordance with the guidelines and requirements of the government committee on animal experimentation at Lund University and with the ARVO (The Association for Research in Vision and Ophthalmology) statement on the use of animals in ophthalmic and vision research.

Gels

Healaflo® (Anteis S.A., Plan Les Ouates, Switzerland) is a commercially available translucent hydrogel, clinically used in glaucoma filtering surgery as a space-filler and to limit postoperative fibrosis [22, 23]. The hydrogel consists of over 97% water, sodium hyaluronic acid (22,5 mg/ml) of non-animal origin cross-linked with BDDE (1,4-Butanediol diglycidyl ether), and phosphate- and NaCl-salts to maintain physiological pH (7,0) and osmolarity (305 mOsm/kg). Estimated specific gravity is circa 1,03, and refractive index $n = 1.341$.

A custom made two component cross-linked hydrogel (PEG) consisting of 20 wt.% poly(ethylene glycol) in phosphate buffered saline (PBS) was prepared by mixing PEGDA in PBS into ETMP-1300 in PBS [30]. Poly(ethylene glycol) (PEG) is a synthetic water-soluble polymer that has been approved by the FDA for biomedical use in different applications including injectable hydrogels. It has been investigated for use in intravitreal drug delivery, repair of scleral incisions, and the sealing of retinal breaks in retinal detachment surgery [24, 25].

Bio-Alcamid® (Polymekon, Brindisi, Italy) is a commercially available clear hydrogel in clinical use as tissue filler for plastic and reconstructive surgery, mainly for lipoatrophic and posttraumatic conditions. The hydrogel consists of approximately 4% reticulated polyalkylimide and approximately 96% non-pyrogenic water (pH 6,9), it contains no free monomers, and is considered physically and chemically stable [29]. *In vivo*, a collagen capsule surrounding the implanted Bio-Alcamid® is formed.

Tissue handling and culture procedure (Fig. 1)

The rats were euthanized with CO₂, with subsequent decapitation, enucleation and immediate immersion of the eyes in ice-cold CO₂-independent medium (Gibco, Paisley, UK). The neuroretinas were dissected from the retinal pigment epithelium (RPE) and the vitreous with fine forceps, and either half or the entire neuroretinas were subsequently explanted on to culture plate inserts (Millicell Isopore-PCF 0.4 µm, 30 mm; Millipore, Billerica, ME) with the

photoreceptor layer against the membrane, and covered by 50-100 µl gel (Healaflo®[®], PEG, or Bio-Alcamid®[®]) depending on the size of the explant. The explants were cultured in 2 ml Dulbecco's modified Eagle's medium (DMEM)/F12 medium–L-glutamine (Gibco) supplemented with 10% fetal calf serum, with a drop of enriched medium applied directly onto the gels to ensure saturation. The cultures were also supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 ng/ml streptomycin (Sigma-Aldrich, St Louis, MO), and the retinas were kept at 37°C with 95% humidity and 5% CO₂. Four explants in each group (Standard conditions, Healaflo®[®], PEG, and Bio-Alcamid®[®]) were kept in culture for 2 days, and six explants in each group were kept for 5 or 10 days, with exchange of half the culture medium after 3, 5, 7 and 9 days. No exchange of gel was made during the change of medium.

Immunohistochemistry

In preparation for further histological studies, the explants were fixed for 1 hour in 4% formalin (pH 7.3) in 0.1 M Sørensen phosphate buffer (PB). The specimens were then washed with 0.1 M Sørensen PB, this was repeated with the same solution containing sucrose of increasing concentrations (5%–25%). Specimens were sectioned to 12 µm on a cryostat, and every tenth slide was stained with hematoxylin and eosin according to standard procedures.

For immunohistochemical staining, sections were washed in room temperature with 0.1 M of sodium phosphate-buffered saline pH 7.2 (PBS) with 0.1% Triton X-100 (PBS/Triton), and thereafter incubated overnight at +4°C with antibodies against the following antigens; Rhodopsin [rod photoreceptors] (Rho4D2, a kind gift from Prof. R.S. Molday, Vancouver, Canada; monoclonal, diluted 1:100), phospho-protein kinase C [PKC, rod bipolar cells] (K01107M; Cell Signaling, USA, diluted 1:200), Neurofilament 160 KDa [NF1 60, ganglion and horizontal cells] (clone NN18; Sigma, St. Louis, MO, USA, diluted 1:500), glial fibrillary acidic protein [GFAP, activated Müller cells] (clone G-A-5; Millipore, Sundbyberg, Sweden, diluted 1:200 with PBS/Triton with 1% bovine serum albumin), and vimentin [Müller cells] (Chemicon, USA, 1: 500). After incubation with the antibodies and rinse with PBS/Triton, the slides were incubated with fluorescein isothiocyanate (FITC)-conjugated antibodies (Sigma-Aldrich, St. Louis, MO, USA) for 45 min, rinsed, and mounted in anti-fading mounting media (Vectashield, Vector laboratories, Inc., Burlingame, CA, USA). Negative controls were obtained by performing the same procedure as above, but without any primary antibodies. The antibodies are summarized in table I. For identification of apoptotic cells, a commercial terminal transferase-mediated dUTP nick-end labeling (TUNEL) assay system with fluorescein-conjugated dUTP (Boehringer Mannheim, Mannheim, Germany) was used on the retinal sections according to the manufacturers instruction.

RESULTS

Retinal explant cultures

All gels (Healaflo®[®], PEG and Bio-Alcamid®[®]) could successfully be applied to the explanted retinal tissue. Healaflo®[®] and PEG formed even films over the retinal explants, whereas Bio-Alcamid®[®] retained a thick, uneven texture which did not cover the explants completely even after a prolonged time. The PEG gel was found to benefit from a 20 min incubation time prior to the administration of medium, allowing for some gelation and preventing dissolution. The gels remained translucent and could be visualized at every exchange of medium, and were confirmed to be macroscopically saturated with the colored medium by means of visual inspection. Two of the explant-cultures were subjected to infections and excluded from further analysis.

Cytoarchitecture and cell death (Fig. 2)

After two days *in vitro* (DIV), hematoxylin- and eosin-stained sections of explants kept under control conditions as well as PEG exposed explants displayed an abnormal retinal lamination with a wavy appearance of the outer nuclear layer (ONL). The ONL also displayed variable thickness, displacement towards the inner retina, and moderate pyknosis. Inner retinal layers displayed some variability in total thickness, and moderate pyknosis. Healaflo®[®] treated explants showed almost normal retinal lamination with significantly better preservation of retinal neurons compared with control specimens, whereas Bio-Alcamid®[®] exposed explants displayed a highly variable cytoarchitecture with severe thinning and disruption of all retinal layers in most parts, and less disrupted structure in minor areas. TUNEL labeling at 2 DIV demonstrated no or almost no apoptotic cells with explants kept under control conditions, with Healaflo®[®] and with PEG, and some apoptosis with explants cultured with Bio-Alcamid®[®]. After 5 and 10 DIV a progressive increase in pyknosis and laminar disruption was seen in all groups. Retinas kept under standard conditions, and especially with Healaflo®[®] exhibited less pyknosis and laminar disruption than those treated with PEG and Bio-Alcamid®[®]. TUNEL labeling of 5 DIV explants kept under control conditions and those subjected to PEG displayed moderate signs of apoptosis. Healaflo®[®] treated retinas showed almost no TUNEL labeling, whereas explants treated with Bio-Alcamid®[®] displayed massive cell death. At 10 DIV intense TUNEL labeling was observed in explants cultured under standard conditions, low labeling with Healaflo®[®]-treatment, and very low labeling in the PEG- and Bio-Alcamid®[®]-treated cultures.

Rod photoreceptors (Fig. 3)

Rhodopsin-labeled photoreceptor cells in standard cultures displayed high intensity labeling of the outer segments (OS) and in the outer plexiform layer (OPL), with mild intensity labeling present in the ONL. Similar patterns of labeling were seen at 2 and 5 DIV. At 10 DIV, stronger labeling was seen in the ONL. The Rhodopsin labeling pattern of Healaflo®[®] and PEG exposed explants was comparable to the standard control. Bio-Alcamid®[®] explants displayed intense labeling of the entire ONL already at 2 DIV.

Inner retinal cells (Fig. 4)

PKC labeling for rod bipolar cells at 2 DIV displayed a high variability, with most intense labeling towards the peripheral edge of the control explants. In 5 DIV specimens, only a few PKC labeled cell bodies were found, whereas 10 DIV specimens did not show any remaining rod bipolar cells. In Healaflo[®] treated specimens at 2 DIV, a few PKC labeled rod bipolar cells were found, but in older explants, no such cells were found. PEG and Bio-Alcamid[®] explants did not display any PKC labeled rod bipolar cells at any time-point.

Neurofilament 160 labeled ganglion cells were seen in all retinal cultures, with no clear differences between the different tested gels. No difference was observed between different incubation times.

Müller cells (Fig. 5)

GFAP labeling, indicative of Müller cell activation, showed very low intensity in most parts of the control retinas at 2 DIV, but intense labeling was present in astrocytes located in the innermost retina. A generally low labeling intensity was seen at 5 DIV, with some areas of moderate to high labeling of Müller cells (shown in figure 4). At 10 DIV some areas of moderate labeling was seen, with mostly fragmentary labeling with a tortuous appearance of the Müller cell fibers. Healaflo[®] subjected retinas displayed patterns similar to those of the control retinas at all time-points, although there was a tendency towards slightly lower labeling intensity at 5 DIV.

The retinas exposed to PEG and Bio-Alcamid[®] displayed high labeling intensity in the inner retina with labeled Müller cell fibers occasionally reaching the ONL at 2 DIV. After 5 DIV, moderate, variable expression both in the inner retina and in fibrils was exhibited on PEG exposure. Bio-Alcamid[®] exposed retinas exhibited low labeling intensity, almost exclusively in the inner retina. At 10 DIV, cultures with PEG showed moderate, variable expression, and those cultured with Bio-Alcamid[®] displayed only weak labeling present in the inner retina.

Vimentin labeling of Müller cell cytoskeletons was present in fibers through the inner parts of the retina, in some areas through to the ONL, with some labeling in the innermost retina. No significant differences were seen between the different groups but increased hypertrophy and disorganization of Müller cell fibers was seen over time with the labeling pattern appearing almost granular at late time points.

DISCUSSION

Summary

In this study, a new *in vitro* model for evaluating the effect of potential vitreous substitutes on adult neuroretinal sheets was explored. Three potential candidates were evaluated and compared to retinal explants cultured under standard conditions. Clear differences were seen between the groups, with similar effects observed in explants cultured under standard conditions and with Healaflo[®], and more degenerative findings in cultures with PEG and, particularly, Bio-Alcamid[®]. The relative degenerative morphological and immunohistochemical changes for the different gels compared to standard conditions are summarized as qualitative compound scores in table II.

The *in vitro* model

Research into novel vitreous substitutes has important implications for both medical and surgical vitreoretinal disease. An *in vitro* assay, analysed using immunohistochemistry and morphological stainings, can determine the biocompatibility and safety of potential vitreous substitutes. This may provide better predictions of the effects of novel substances on the retina than the traditional, more simplistic *in vitro* assays currently in use [16-19, 21, 31].

The *in vitro* model presented here provides a method of biocompatibility testing prior to more costly and cumbersome *in vivo* experiments [20]. In retinal explant cultures under standard conditions, there are several well-characterized reactions easily observable as early as 3 or 4 DIV [32-34]. These reactions include gliosis and neuroretinal degeneration, and can be visualized by GFAP upregulation, disruption of the cell layers, and the labeling of apoptotic cells. Using these reactions elicited by the explant culture system under standard conditions and comparing them to different vitreous substitute candidates, indicates the biocompatibility of the substances *in vivo*.

Our previous results and our hypothesis

The vitreous is often simplistically seen as a mere space filler inside the eye bulb. There is, however, evidence of a more intricate and purposeful composition [10] with important physiological implications on the micro-milieu of the retina including the upkeep of gradients of salts and nutrients, physical support, and more [35, 36]. An ideal vitreous substitute would replicate these influences on the neuroretina and surrounding tissues as well as providing a tamponading effect after vitrectomy [10].

In two recent papers our group investigated two different, promising new potential intravitreal substitutes in an *in vivo* rabbit model: Polyalkylimide (Bio-Alcamid[®]) [37] and a poly(ethylene glycol) (PEG) hydrogel [38].

Bio-Alcamid[®] is a translucent hydrogel with high biocompatibility [26, 27] used in plastic surgery, and in clinical use forms a surrounding collagen capsule giving it a degree of isolation from the surrounding tissue [28]. The synthetic polymer hydrogel PEG is used in different biomedical application and has been tested for intravitreal administration of drugs [24, 39], and is FDA approved for use intravitreally. The *in vivo* trials showed favorable biocompatibility but inadequate stability *in vivo* using PEG, where the substance was largely tolerated with minor changes in retinal cytoarchitecture and GFAP-upregulation, and minor electrophysiological changes [38]. On the other hand, Bio-Alcamid[®] displayed suitable physical properties but caused severe functional and morphological retinal damage with increased GFAP expression and cell death (TUNEL) [37].

The use of derivatives of sodium hyaluronic acid in vitreoretinal surgery predates their ubiquitous use in cataract- and anterior segment surgery [12, 14, 40-42], but their use in a clinical setting has been limited mainly due to concern about short term side effects and retention time [41, 43]. Healaflow® is a commercially available compound consisting of a cross-linked sodium hyaluronic acid hydrogel, and is FDA approved for use in glaucoma surgery [22, 23]. The composition of Healaflow® is akin to the natural vitreous: a reinforced hydrogel of hyaluronic acid with similar physical properties and thus considered a plausible candidate for vitreous substitution.

***In vivo* vs. *in vitro*: our earlier results and others**

It seems to us that a good correlation exists between the results of this *in vitro* explant culture system and earlier results for all the tested substances.

In this setting, retinal explants cultured with Healaflow® compare very well to specimens cultured under standard conditions, and even seems to lessen the trauma caused by the culture process. This is consistent with the excellent biocompatibility of hyaluronic acid seen in other studies [18-19]. Hyaluronic acid is one of the main constituents of the natural vitreous and consistently well tolerated in different biomedical applications. Healaflow® may exert a protective effect from culture-induced trauma on the retinal explants by providing a more physiologically similar microenvironment *in vitro*. Additionally, the positive effect on the retina could be due to biomechanical factors through physical interaction from the gel that might prevent retinal folds, and keep the explants under tension. This is a factor that previously has been showed to favorably affect retinas *in vitro* [44].

The PEG gel elicits reactions similar to the control retinas with comparable changes in the cytoarchitecture but with earlier, more intense TUNEL-labeling, consistent with previous *in vivo* findings [38]. In the retinal explant cultures with the longest duration (10 DIV) there was a decrease in the amount of apoptotic cells observed at earlier time points. This may be due to a loss of viable cells, as cell death occurred earlier than for Healaflow® and standard conditions, indicating a stronger adverse reaction to these gels than what is caused by the culture procedure.

Bio-Alcamid® caused severe retinal damage *in vivo* [37] and negatively affected the morphology of cells and cell layers, induced cell death and induced GFAP upregulation very early *in vitro*. Some of the variability in cytoarchitecture for retinas treated with Bio-Alcamid® might have been due to uneven coverage of the gel. The adverse effects may in part be influenced by uneven exposure to the medium, but cytotoxic effects from the gel itself is likely to play a part in this process. The explanted retinas were less affected in minor areas, which may not have been in direct contact with the gel, although this is difficult to discern due to the loss of gel in the preparation and sectioning procedures. This is in accordance with previous studies, which demonstrated pathological changes in the retina *in vivo*, primarily in parts more likely to have been in direct contact with the gel [37], suggesting at least in part a toxic or immunological response. Recently, clinical use of Bio-Alcamid® in reconstructive surgery has become increasingly controversial due to late complications such as inflammation, infection and excessive capsule formation [45-48].

Conclusion

The retinal explant assay described in this paper has the potential to be a useful tool for preliminary studies of vitreous substitute candidates prior to more costly and time-consuming *in vivo* testing. In addition, it may reduce the need for laboratory animals and limit the severity of the experiments from an ethical standpoint by excluding unfit substances from further testing, thereby providing refinement of the tests. *In vivo* tests will still be essential before

test on human subjects but this assay may minimize the translational step, which would prove valuable and beneficial in vetting out unsuitable biomaterials.

A need for better vitreous substitutes still remains, and more suitable substances would be highly valuable. Healaflow® and to a lesser extent PEG seem to be promising candidates for further development, and further *in vivo* testing of these and similar substances is clearly indicated.

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FIGURE LEGENDS

Fig. 1 Schematic overview of the retinal explant culture system with vitreous substitute candidate

Fig. 2 Cryosections of explants of rat retina at 2, 5 or 10 days *in vitro* (DIV) cultured with; standard conditions (**CTRL**), Healaflow® (**HF**), PEG-gel (**PEG**) and Bio-Alcamid® (**BA**). Hematoxylin and eosin staining (**top rows**), and TUNEL staining (**bottom rows**). Abbreviations: inner nuclear layer (**INL**), outer nuclear layer (**ONL**). Scale bar = 25 μm

Fig. 3 Cryosections of explants of rat retina at 2, 5 or 10 days *in vitro* (DIV) cultured with; standard conditions (**CTRL**), Healaflow® (**HF**), PEG-gel (**PEG**) and Bio-Alcamid® (**BA**). Immunohistochemical staining for Rhodopsin. Abbreviations: inner nuclear layer (**INL**), outer nuclear layer (**ONL**). Scale bar = 25 μm

Fig. 4 Cryosections of explants of rat retina at 2, 5 or 10 days *in vitro* (DIV) cultured with; standard conditions (**CTRL**), Healaflow® (**HF**), PEG-gel (**PEG**) and Bio-Alcamid® (**BA**). Immunohistochemical staining; (**a**): PKC α , and (**b**): Neurofilament 160. Abbreviations: inner nuclear layer (**INL**), outer nuclear layer (**ONL**). Scale bar = 12,5 μm

Fig. 5 Cryosections of explants of rat retina at 2, 5 or 10 days *in vitro* (DIV) cultured with; standard conditions (**CTRL**), Healaflow® (**HF**), PEG-gel (**PEG**) and Bio-Alcamid® (**BA**). Immunohistochemical staining; (**a**): GFAP, and (**b**): Vimentin. Abbreviations: inner nuclear layer (**INL**), outer nuclear layer (**ONL**). Scale bar = 12,5 μm

TABLES

Antigen	Antibody name	Target structure	Species	Dilution	Source
<i>GFAP</i>	Anti-glial fibrillary acidic protein	Astrocytes, activated Müller cells	Mouse monoclonal	1:200	Chemicon International, CA, USA
<i>Neurofilament 160 KDa (NF160)</i>	Anti-neurofilament 160 clone NN18	Ganglion and horizontal cells	Mouse monoclonal	1:500	Sigma, St. Louis, MO, USA
<i>PKC</i>	Phospho-PKC (pan)	Rod bipolar cells	Rabbit polyclonal	1:200	Cell Signaling, Beverly, MA, USA
<i>Rhodopsin</i>	Rho4D2	Rod photoreceptor	Mouse monoclonal	1:100	Kind gift of Prof. RS Molday, Vancouver, Canada
<i>Vimentin</i>	Mouse anti-vimentin	Müller cells	Mouse monoclonal	1:500	Chemicon International, CA, USA
Secondary antibody	Antibody name	Target	Species	Dilution	Source
<i>FITC</i>	Anti-mouse IgG FITC conjugate	Anti-mouse	Goat	1:200	Sigma, St Louis, MO, USA
<i>FITC</i>	Goat Anti-Rabbit IgM+IgG (H+L chain specific)	Anti-rabbit	Goat	1:200	Southern Biotechnology Associates, AL, USA

Table I. Specification of immunohistochemical markers

Gels	Cytoarchitecture and cell death	Rod photoreceptors	Inner retinal cells	Müller cell activation
<i>Healaflo®</i>	-	0	+	-
<i>PEG</i>	+	0	++	+
<i>Bio-Alcamid®</i>	++	+	++	++

Table II. Relative compound score for the degenerative retinal changes for the different gels compared to standard conditions ranging from – to ++.









