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Alginate- and Hyaluronic Acid–Based Hydrogels as Vitreous Substitutes: An In Vitro Evaluation

André Schulz[1,2], Annekatrin Rickmann[1,2], Silke Wahl[1,2], Anja Germann[3], Boris Viktor Stanzel[1–3], Kai Januschowski[1,2,4], and Peter Szurman[1,2,4]

[1] Eye Clinic Sulzbach, Knappschaft Hospital Saar, Sulzbach, Germany
[2] Klaus Heimann Eye Research Institute, Sulzbach, Germany
[3] Fraunhofer Institute for Biomedical Engineering, Sulzbach, Germany
[4] Centre for Ophthalmology, University Eye Hospital Tuebingen, Tuebingen, Germany

Correspondence: André Schulz, An der Klinik 10, 66280 Sulzbach, Germany.
e-mail: andre.schulz@kksaar.de

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Purpose: To study alginate- and hyaluronic acid–based hydrogels in vitro as vitreous substitutes.

Methods: Biopolymeric hydrogels based on high-molecular alginate (0.5% and 1.0%) and hyaluronic acid (1.0% and Healaflow) were compared with extracted human vitreous bodies and silicone oil (SIL-5000) regarding their optical properties (refractive index, transmission) and viscoelastic characteristics (storage modulus G′, loss modulus G″). The cytotoxic (metabolic activity, apoptosis) and antiproliferative profiles were determined using cultured human fibroblasts, ARPE-19, and photoreceptor cells. The hydrogel systems were applied to human fetal retinal pigment epithelial cells cultured for two months until maximum transepithelial electrical resistance (TEER) to investigate the effect of the gel matrices on tight junctions using TEER measurements and immunostainings against the tight junction protein ZO-1.

Results: Tested alginate- and hyaluronic acid–based hydrogels resembled the natural refractive index of human vitreous bodies (1.3356–1.3360) in contrast to SIL-5000 (1.4034) and showed high optical transparency (>90%) within the visible light region. The biopolymeric hydrogels exhibited viscoelastic properties similar to juvenile vitreous bodies with G′>G″ adjustable via different gelation times, contrary to SIL-5000 (G′<G″). The metabolic activity, apoptosis and tight junctions of all tested ocular cells were unaffected by the alginate- and hyaluronic acid–based vitreous substitutes.

Conclusions: The present in vitro study demonstrates good optical, viscoelastic, and biocompatible properties of alginate- and hyaluronic acid–based hydrogels required for their use as vitreous substitutes.

Translational Relevance: Biopolymer-based hydrogels represent a promising vitreous replacement strategy to treat vitreoretinal diseases.

Introduction

The therapy of complicated retinal detachments includes the removal of the human vitreous body (vitrectomy) and the stabilization of the retina with an endotamponade (perfluorocarbons, air, gases, silicone oils; reviewed by Kleinberg et al.).[1] However, even modern tamponade materials have not significantly improved the primary reattachment rate in the last 30 years.[2–6] In particular, they do not provide a satisfactory response to multiple breaks at different sites, the challenges of proliferative vitreoretinopathy (PVR) or persistent hypotension.

The reason for this limitation is the hydrophobic character of all tamponade materials in clinical use. These act in the hydrophilic (aqueous) milieu of the vitreous cavity via the two physical functions "buoyancy vector" and "surface/interfacial tension." However, this has disadvantages, because

their tamponade vector only acts in one direction. More importantly, it is physically impossible to completely fill the hydrophilic vitreous cavity with a hydrophobic material.[7] A small amount of liquid at the opposite pole of buoyancy vector always remains, wherein growth factors accumulate and promote proinflammatory processes such as proliferative vitreoretinopathy.[8] Additionally, the hydrophobic character leads to complications such as emulsification, cataract formation, and the need for revision surgery.[6,9,10]

A solution to the above-mentioned may be the development of a hydrogel-based vitreous body substitute that mimics the physiological properties of a natural, young, and healthy vitreous body. The tamponade effect of a hydrogel (analog to the vitreous body) is not exerted by the surface tension and buoyancy vector, but via viscosity and swelling pressure, the latter via the high water-binding property. The pressure force of the swelling pressure is distributed homogeneously over all retinal areas, top and bottom, so that a uniform tamponade force is obtained in all directions.

Recent tamponade strategies therefore aim at hydrophilic, hydrogel-based systems (reviewed by Su et al.[11]). In addition to synthetic polymers, biopolymers such as hyaluronic acid,[12–17] collagen,[17,18] gellan,[16,19] chitosan,[20,21] and alginate[21] were investigated for the preparation of hydrophilic retinal tamponades. However, previous concepts were mainly limited by intransparency, deviating refractive indices, fast degradation, or insufficient biocompatibility, especially because of lack of substance purity or toxic cross-linking agents. For alginate gels, retinal toxicity[21] and lack of transparency[21–23] were limiting factors, because previously used alginates were low molecular weight and of technical purity with potentially increased impurities such as endotoxins, polyphenols, or proteins affecting the biocompatibility.[24,25] In contrast, vitreous body replacement strategies based on photo-cross-linked methacrylated hyaluronic acid or Healaflow, a hyaluronic acid–based hydrogel,[26] showed good biocompatibilities in rabbits,[12,13] whereas the evaluation of transparency and viscoelasticity, as well as in vitro biocompatibility studies on different ocular cells, however, is still pending.

In the present study, therefore, hydrogels of high purity based on high molecular weight alginate and hyaluronic acid were synthesized and examined in vitro with regard to the optical, viscoelastic and biocompatible properties after injection through a cannula required for their use as vitreous substitutes to treat vitreoretinal diseases.

Materials and Methods

Preparation of Alginate- and Hyaluronic Acid–Based Hydrogels

Different hydrogels were produced as potential vitreous substitutes (Fig. 1). For alginate hydrogels (ALG), only sterile alginates of high purity (endotoxin $\leq$ 100 EU/g, proteins $\leq$ 40 mg/L) and high molecular weight (Ø $\sim$ 1 MDa) were used. Five milliliters of alginate solution (0.5% and 1.0% w/v; Alginatec, Riedenheim, Germany) were transferred into a dialysis membrane (8 kDa, Ø 11.5 mm; VWR, Darmstadt, Germany), cross-linked for one to four hours at room temperature (RT) in an aqueous 11.6 mM calcium sulfate dihydrate solution (290–300 mOsmol, pH = 7.4; Alfa Aesar, Kandel, Germany) and thoroughly washed with balanced salt solution (BSS; Thermo Fisher Scientific, Waltham, MA, USA).

Hyaluronic acid was methylacrylated using glycidyl methacrylate (Sigma-Aldrich, St. Louis, MO, USA) and triethylamine (Merck, Kenilworth, NJ, USA) as described previously,[12] excluding the use of tetrabutylammonium bromide. The gelation of 5 mL glycidyl methacrylated hyaluronic acid (GMHA) was performed after addition of 0.25 mL N-vinylpyrrolidinone and 100 mg Irgacure 2959 (I2959; Sigma-Aldrich) using ultraviolet light (UV) exposure (365 nm, 100 mW/cm^2) for five or 20 minutes at RT. The resulting hydrogels were thoroughly washed with BSS to remove remnants of non-cross-linked substrates. In addition to the prepared UV-cross-linked hyaluronic acid hydrogels (UVHA), Healaflow (Aptissen, Geneva, Switzerland) was studied as a hyaluronic acid–based gel system, which is commercially available and applied in ophthalmic surgery.

Vitreous Body Dissection

All procedures including the acquisition and dissection of the donor eyes has been carried out in accordance with the tenets of the Declaration of Helsinki for the use of human tissue, as well as the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the local ethical committee (ec-number: 97/17). Human cadaver eyes donated for research purposes were obtained from The German Society for Tissue Transplantation (DGFG, Hannover, Germany) with a written consent from the donor's next of kin. Under a sterile bench, the globes were treated for five minutes with 10% povidone iodine (Mundipharma, Limburg an der Lahn, Germany) and washed twice with phosphate buffered saline solution

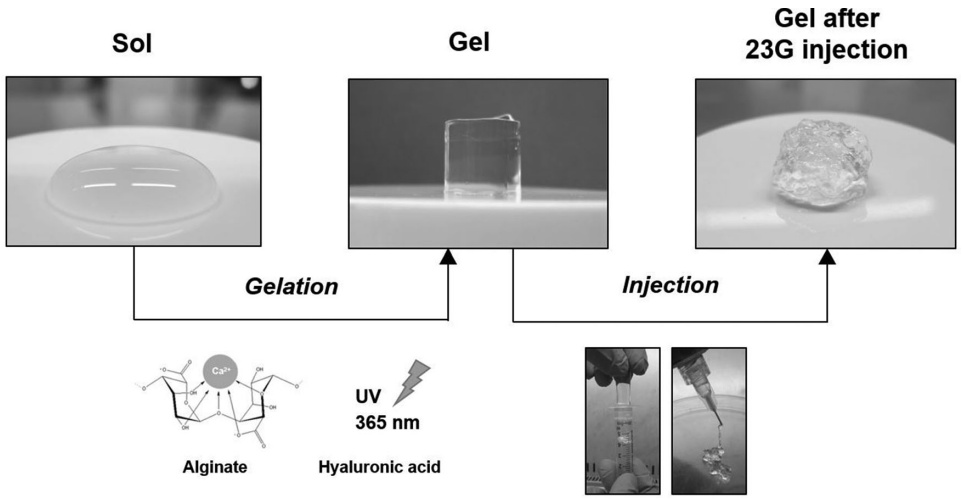

Figure 1. Processing of alginate and hyaluronic acid towards vitreous substitutes. High molecular weight alginates are gelled with sparingly soluble calcium sulfate under formation of ionic interactions. In contrast, methacrylic hyaluronic acids are covalently cross-linked with N-vinylpyrrolidinone by UV exposure (365 nm). Passing the gels through a 23G cannula breaks up the block structure to form a continuous gel chain. In the representative images, the composition of the biopolymers before (Sol) and after gelation (Gel) as well as after injection (Gel after 23G injection) is shown using alginate as an example.

(PBS; Sigma-Aldrich). After an initial incision into the sclera 3.5 mm posterior to the limbus, a circular 360° incision was performed using a surgical scissors (Geuder AG, Heidelberg, Germany) to remove the anterior segment of the eye. Then the vitreous was gently luxated with a blunt forceps (Geuder AG), transferred to a six-well plate (Greiner Bio-One, Kremsmünster, Austria) and put into closed interim storage at 4°C.

Refractive Index and Transparency Analysis

The refractive indices of alginate- and hyaluronic acid–based matrices were examined at RT ($N = 3$) using the refractometer HRB18-T (A. Krüss Optronic GmbH, Hamburg, Germany) in comparison to the human vitreous body and silicone oil (SIL-5000; Dutch Ophthalmic Research Center, Zuidland, The Netherlands). The optical transparency was determined by transmission analysis using the photometer DU 730 (Beckman Coulter, Fullerton, CA, USA) in the wavelength range 200 to 800 nm at 37°C ($N = 3$).

Dynamic Mechanical Analysis

The dynamic moduli of alginate- and hyaluronic acid–based matrices were determined in comparison to SIL-500 using shear tests ($N = 5$). In a frequency range of 0.1 to 100 s^{-1} the dynamic-mechanical analyses were performed using the rotational rheometer Physica MCR 101 (Anton Paar, Graz, Austria) with a parallel plate set-up and Peltier element at 37°C. Furthermore, the influence of the gelling time on the viscoelastic properties was investigated. Using the dynamic moduli (storage modulus G′ and loss modulus G″), the viscoelasticity of a system is analyzed. The concept of viscoelasticity is based on the behavior of materials to respond to deformations partly elastic/reversible (introduced energy is available after the deformation; storage modulus G′) and partly viscous/irreversible (energy is lost, loss modulus G″). Liquids/sols show a dominant viscous behavior ($G' < G''$) and gels a dominant elastic behavior ($G' > G''$).

Cell Culture

Human fibroblasts and human retinal pigment epithelial (RPE) cell line ARPE-19 (American Type Culture Collection, Manassas, VA, USA) were cultivated with Dulbecco's modified Eagle's medium (DMEM) supplemented with 4 mM L-glutamine, 10% fetal bovine serum (FBS), 100 U/mL penicillin G, 100 µg/mL streptomycin sulphate, and 1 ng/mL basic fibroblast growth factor (Gibco, Karlsruhe, Germany). The murine photoreceptor cell line 661W was provided by Dr. Muayyad Al-Ubaidi (Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA) and cultured in DMEM supplemented with 4 mM L-glutamine, 10% FBS (Gibco), 32 mg/L putrescine, 40 µg/L progesterone, 30 µg/L hydrocortisone, and 20 µL/L β-mercaptoethanol (Sigma-Aldrich). Cells were grown at 37°C with 5% CO_2 and passaged using

0.05% trypsin/0.02% ethylenediamine tetra-acetic acid (EDTA; T/E; Gibco) twice a week or at a confluency of ca. 80%. Human fetal RPE cells were provided by the MRC-Wellcome Trust Human Developmental Biology Resource (HDBR, Newcastle, UK) and cultured on Millicell cell culture inserts (50,000 cells/well, growth area: 0.33 cm^2, pore size: 1 µm; Merck) coated with Synthemax (2.5 µg/well; Corning, Corning, NY, USA) in modified Minimum Essential Medium (see Supplementary Table S1). Medium was changed every two to three days.

Metabolic Activity Assay

Using the CellTiter-Glo assay (Promega, Madison, WI, USA), the viability of the cells was determined by the content of adenosine triphosphate present after 24 hours' exposure to sol, cross-linker, and gel. After applying the luciferase-based reagent to the cell culture (2 minutes' mixing, 10 minutes' incubation at RT), the emitted luminescent signal, which correlated with the amount of ATP of metabolic active cells, was detected using the Infinite F200 microplate reader (Tecan, Maennedorf, Switzerland). Human fibroblasts, ARPE-19, photoreceptor cells: $n = 3$, $N = 8$; human fetal RPE cells: $n = 2$, $N = 8$.

Cell Proliferation Assay

By quantifying 5-bromo-2-deoxyuridine (BrdU), which was incorporated in place of thymidine into the DNA of proliferating cells, the cellular proliferative activity was analyzed after 24 hours' exposure to alginate or hyaluronic acid hydrogels using the BrdU Cell Proliferation Assay (Sigma-Aldrich). After incubating with BrdU for two hours, the cells were fixed at RT and stained with the manufacturer's antibodies. The reaction was then stopped by the addition of 1 M sulphuric acid (Sigma-Aldrich) and quantified by measuring the absorbance at 450 nm using the Infinite F200 microplate reader (Tecan) ($n = 3$, $N = 8$).

Flow Cytometry Analysis

Flow cytometry analyses were performed to study the apoptosis of human fibroblasts, ARPE-19 and photoreceptor cells, which were exposed to staurosporine (1 µg/mL; Sigma-Aldrich), alginate- or hyaluronic acid gels for 24 hours. Cells were detached using T/E treatment (3 minutes, 37°C, 5% CO_2) and washed with PBS. For surface staining, cells were incubated with saturating amounts of Fluorescein isothiocyanate (FITC)-conjugated Annexin V and propidium iodide (Sigma-Aldrich) for 10 minutes at RT. Flow cytometry was performed using BD FACSCalibur (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using Flowjo software (LLC) ($n = 3$, $N = 3$).

Transepithelial Electrical Resistance (TEER) Analysis

TEER of human fetal RPE cells was measured every seven days until its maximum using Millicell-ERS voltohmmeter (Merck) ($n = 2$, $N = 48$). After 24 hours' exposure to alginate- and hyaluronic acid–based hydrogels at 37°C, the TEER of human fetal RPE cells was re-examined ($n = 2$, $N = 8$). To disrupt tight junctions, the cells were treated with T/E for 15 minutes at 37°C ($n = 2$, $N = 8$).

Immunofluorescence Staining

Human fetal RPE cells were fixed for 20 minutes at RT in the fixation buffer Cytofix (BD Bioscience, San Jose, CA, USA), permeabilized in 0.2% TritonX (Sigma-Aldrich) for 20 minutes at RT and blocked with 1% bovine serum albumin (Sigma-Aldrich) in 0.2% TritonX for 30 minutes at RT. After staining the samples against the tight junction protein ZO-1 for 24 hours at 4°C and secondary antibody Cy5 (Thermo Fisher Scientific) for one hour at RT, NucBlue (Molecular Probes, Eugene, OR, USA) were added for nuclei staining 20 minutes before imaging with the Leica TCS SP8 confocal laser microscope (Leica Microsystems, Mannheim, Germany).

Statistical Evaluation

Graphical illustration of data and statistical analyses were performed using OriginPro and IBM SPSS Statistics. Data are presented as mean ± standard deviation. Differences between groups were considered significant by $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***) and evaluated using univariate analyses of variance with simple contrasts or using Dirichlet regression (flow cytometry analyses).

Results

Optical and Viscoelastic Properties

To evaluate the optical properties of the biopolymer gels, the refractive index and transparency were determined in comparison to the natural vitreous body and the clinically used endotamponade SIL-5000 (Fig. 2). The gellike, human vitreous body had a refractive

A

	Refractive index		
	Sol	*Gel*	*Gel after 23G-injection*
Human vitreous body	1.3356	1.3360	-
0.5% Alginate	1.3357	1.3360	1.3357
1.0% Alginate	1.3359	1.3363	1.3359
1.0% Hyaluronic acid	1.3353	1.3356	1.3353
Healaflow	-	1.3388	1.3385
Silicone oil 5000	1.4034	-	-

B

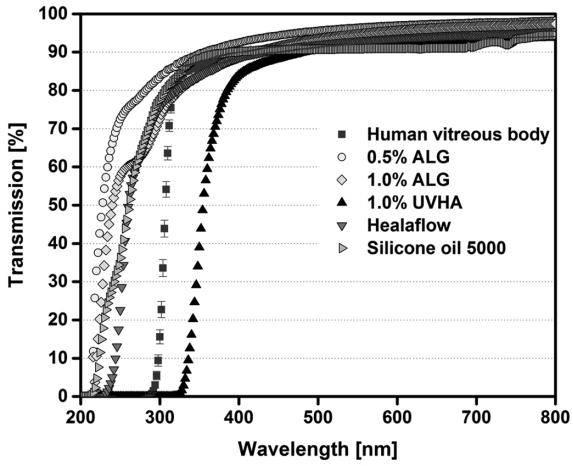

Figure 2. Optical properties of alginate and hyaluronic acid gels in comparison to human vitreous body and silicone oil. Biopolymer-based hydrogels resemble the natural refractive index of human vitreous bodies contrary to silicone oil (A; $N = 3$) and exhibit high optical transparency (> 90%, B; $N = 3$) within the visible light region (400–700 nm).

index of 1.3360, which reduced to 1.3356 in the liquefied state. Tested alginate- and hyaluronic acid–based hydrogels resembled the natural refractive index of human vitreous bodies in contrast to SIL-5000 (1.4034). Here, the refractive index increased slightly both by increasing the polymer concentration and by gelling. In contrast, the refractive indices were slightly reduced after passing through a 23G cannula.

The optical transparency of the materials after 23G injection was examined in comparison to the human vitreous body and was found to be high (>90%) within the visible light region. With decreasing wavelengths the light transmittance was reduced, UV-A: UVHA, UV-B: human vitreous, UV-C: Healaflow, 1.0% ALG, 0.5% ALG. Here, changes in light transmission were observed at the substance-specific absorption wavelengths of alginate (210 nm), hyaluronic acid (260–300 nm), and the photoinitiation using I2959 (365 nm).

Using dynamic mechanical analyses, the viscoelastic properties (storage modulus G′ and loss modulus G″) of alginate and hyaluronic acid–based matrices were studied and compared with SIL-5000 (Fig. 3). The dynamic moduli of the tested solutions shows the behavior of liquid, viscous samples with $G' < G''$ at low frequencies. With increasing polymer concentration the dynamic moduli of the sol increased. Both 0.5% and 1.0% alginate solutions were more viscous than 1.0% GMHA. ALG, UVHA, and Healaflow showed with $G' > G''$ a dominant elastic behavior of gels dependent on the polymer concentration and gelling time. However, there was no difference in the viscoelastic properties of ALG after three or four hours' gelation. The dynamic moduli of hyaluronic acid gels were higher than of alginate gels, according to 0.5% ALG < 1.0% ALG < Healaflow < 1.0% UVHA. After passing through 23G cannulas, the dominant elastic behavior was still present, but in inverse order: 1.0% UVHA < Healaflow < 0.5% ALG < 1.0% ALG. Here, longer gelling times of UVHA resulted in lower dynamic moduli after 23G-injection (20 minutes < 5 minutes). In contrast to the biopolymeric vitreous substitutes, SIL-5000 possessed dominant viscous properties ($G' < G''$).

In Vitro Biocompatibilty of Alginate and Hyaluronic Acid Hydrogels

The cytotoxicity of alginate- and hyaluronic acid–based hydrogels and their individual components (sol, cross-linker) was studied on human fibroblasts, ARPE-19 and photoreceptor cells (Fig. 4A). After 24 hours of exposure to biopolymeric gels, the different cell types showed high metabolic activity > 85%. Likewise, the metabolic activity of the cells was almost unaffected after contact with non-cross-linked 0.5% alginate. In contrast, exposure to highly viscous sols (1.0% alginate, 1.0% GMHA) and individual cross-linkers resulted in reduced cell viabilities.

The comparative studies on the influence of the substances on cell proliferation showed an antiproliferative effect of the gels on fibroblasts, whereas the proliferation of ARPE-19 and photoreceptor was unaffected (Fig. 4B). Approximately 50% of fibroblasts showed proliferative activity after exposure of the gels.

In addition, flow cytometry using FITC-conjugated Annexin V and propidium iodide was used to

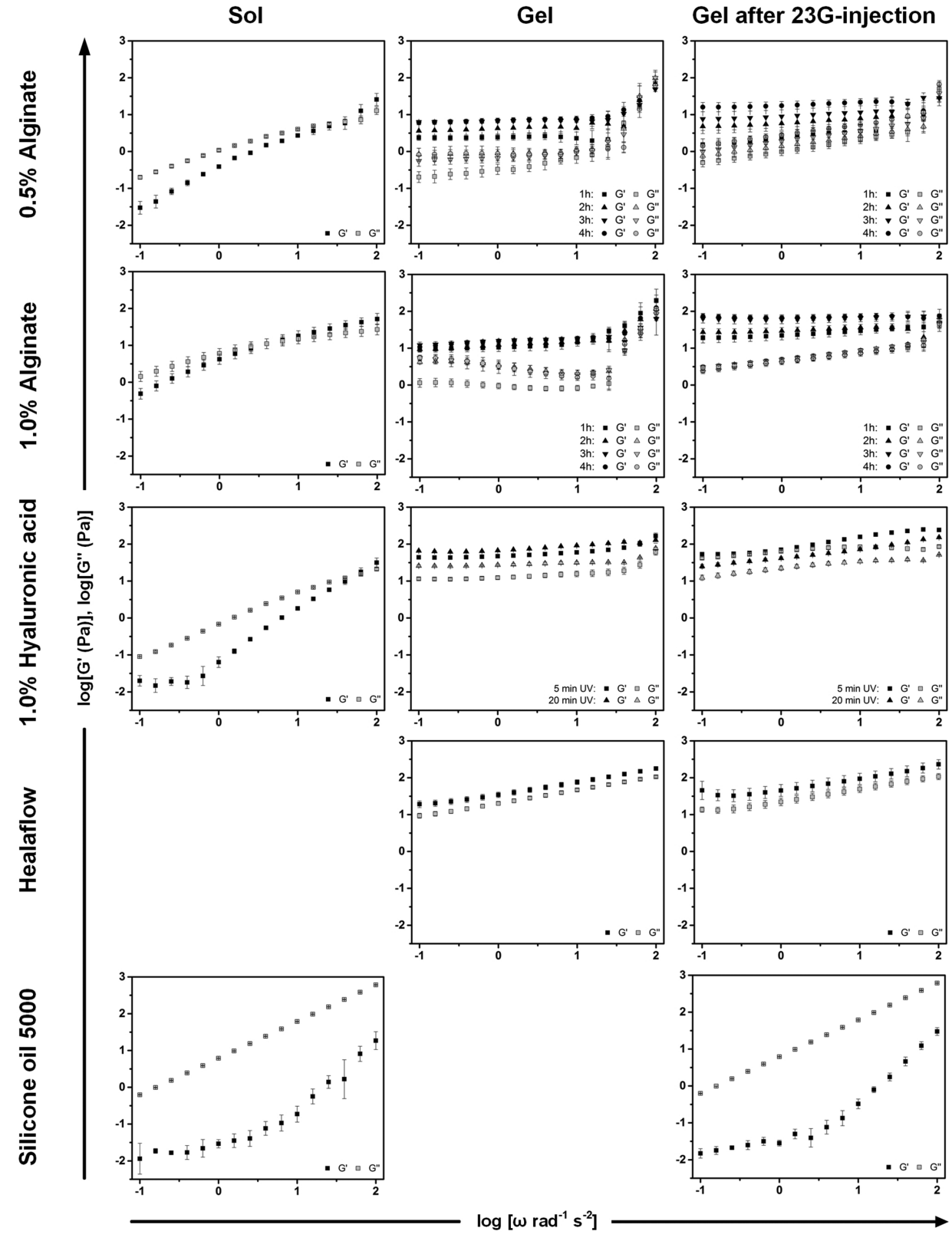

Figure 3. Viscoelastic properties (storage modulus G′ and loss modulus G″) of alginates and hyaluronic acids during different process steps compared to Healaflow and silicone oil. Biopolymeric hydrogels exhibit viscoelastic properties similar to juvenile vitreous bodies with G′ > G″ adjustable via different gelation times, contrary to silicone oil (G′ < G″), $N = 5$.

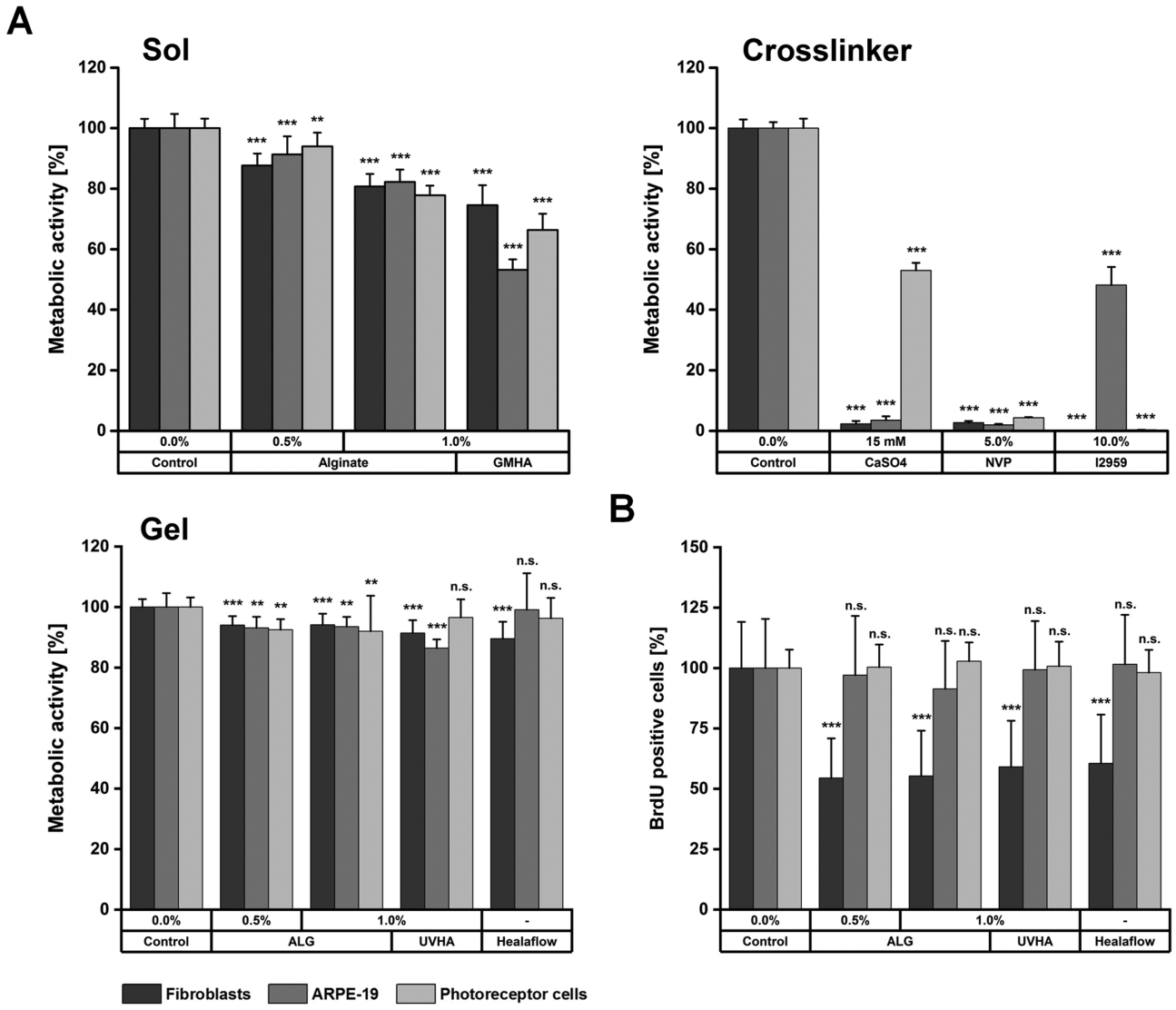

Figure 4. Comparative studies on the cytotoxicity of alginate and hyaluronic acid–based gels and their individual components (A; $n = 3$, $N = 8$) and on the antiproliferative profile of biopolymeric gels (B; $n = 3$, $N = 8$) with respect to fibroblasts, ARPE-19 and photoreceptor cells. The gels had no cytotoxic effect indicating that excess, partly cytotoxic substrates (sol, cross-linkers) were successfully removed. Although the biopolymeric gels showed an antiproliferative effect on fibroblasts, the proliferation of ARPE-19 and photoreceptor cells was unaffected by gel contact. Differences between groups were significant with $P < 0.01$ (**) and $P < 0.001$ (***). NVP, N- vinylpyrrolidinone; I2959, Irgacure 2959.

investigate whether direct contact of the cells with the alginate and hyaluronic acid–based gels induces apoptosis (Fig. 5). Staurosporine treatment (positive control) resulted in apoptosis of human fibroblasts, ARPE-19, and photoreceptor cells. In contrast, no increased numbers of apoptotic or necrotic cells were observed after exposure to alginate-based gels compared to the untreated negative control. However, a significant, slightly increased number of late apoptotic cells was found after contact with UVHA with human fibroblasts and of late apoptotic and necrotic cells after contact with Healaflow with human fibroblasts and ARPE-19.

The in vitro biocompatibility of gels was also evaluated using human fetal RPE cells (Fig. 6). The TEER value reached its maximum at 1375 ± 76 Ohm • cm^2 56 days after cell seeding (Fig. 6A). The exposure to different biopolymer gels resulted in no alteration of TEER. In contrast, tight junctions were effectively reduced by the administration of T/E (Fig. 6B). The preservation of cell-cell interactions was also confirmed by immunofluorescence staining against the tight junction protein ZO-1 (Fig. 6C). The pigmentation and hexagonal shape of human fetal RPE cells were maintained after exposure to alginate and hyaluronic acid–based hydrogels. Treatment with T/E, on the other hand,

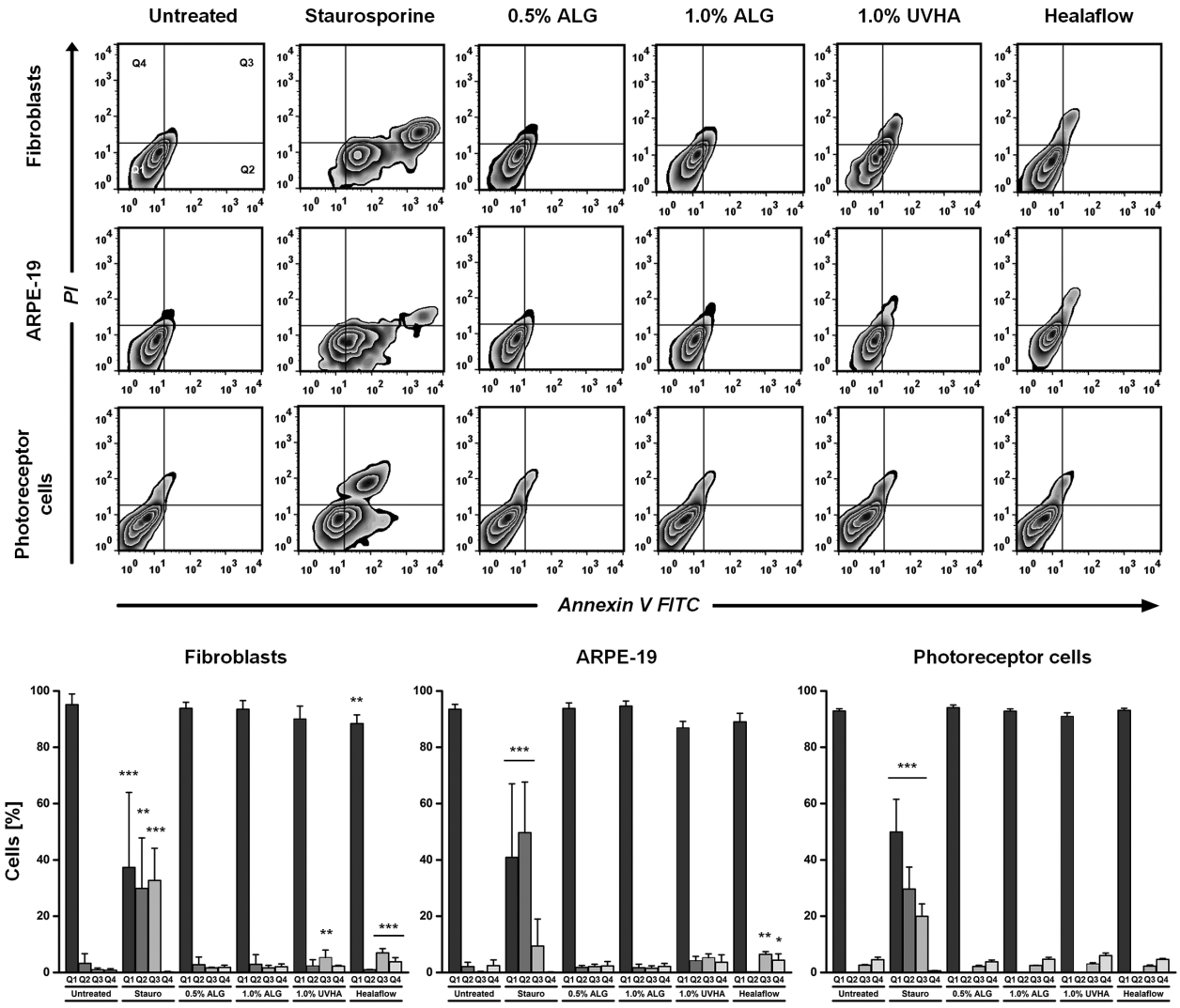

Figure 5. Comparative studies on the influence of alginate and hyaluronic acid–based vitreous substitutes on the apoptosis of fibroblasts, ARPE-19 and photoreceptor cells compared to untreated (negative control) and staurosporine-treated cells (positive control, Stauro). Flow cytometry analyses using FITC-conjugated Annexin V and propidium iodide (PI) revealed no apoptotoses induced by the hydrogel systems. Differences between groups were significant with $P < 0.05$ (*), $P < 0.01$ (**) and $p < 0.001$ (***), $n = 3$, $N = 3$. Q1, viable; Q2, early apopototic; Q3, late apoptotic; Q4, necrotic cells.

resulted in the disintegration of the cell network toward rounded and detached cells with diffuse ZO-1 distribution. Additionally, the metabolic activity of human fetal RPE cells was not affected by contact with the gels (Fig. 6D).

Discussion

The present in vitro study demonstrates good optical, viscoelastic, and biocompatible properties of alginate- and hyaluronic acid–based hydrogels required for their use as vitreous substitutes. Both the refractive index and the optical transparency of the gels reflect the optical properties of the healthy, human vitreous body. The transmission of visible light (400–700 nm) to the retina is essential for the patient's vision, as well as for diagnostic and therapeutic purposes such as the visualization of fundus details or laser photocoagulation. Like the human vitreous body, both the hydrogels and the silicone oil showed high transparency within the visible light spectrum. In the range of ultraviolet light, the human vitreous body absorbed radiation with wavelengths < 300 nm (UV-B and UV-C), which, like the cornea, protects the retina from toxic UV radiation.[27] While ALG, Healaflow, and silicone oil absorbed light in the UV-C spectrum, UVHA

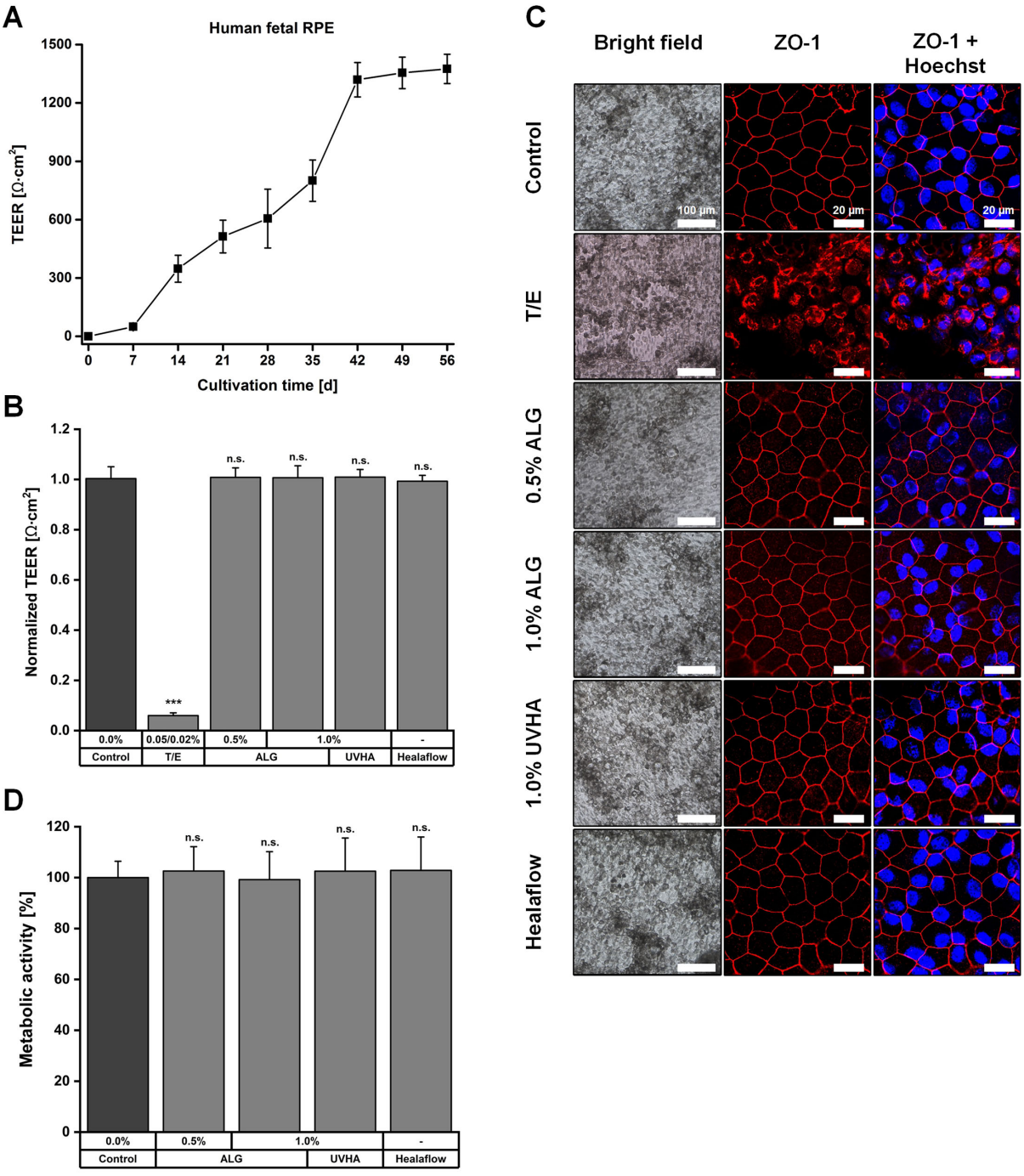

Figure 6. Exposure of human fetal RPEs cultivated for two months until maximum TEER (A; $n = 2$, $N = 48$) to alginate and hyaluronic acid–based vitreous substitutes compared to untreated (negative control) and trypsin-EDTA–treated cells (positive control, T/E). TEER measurements (B; $n = 2$, $N = 8$) and immunostainings against the tight junction protein ZO-1 (C; representative images) demonstrate the preservation of the RPE's tight junctions after gel contact. No loss of vitality of human fetal RPE cells was observed confirming the gels' biocompatibility (D; $n = 2$, $N = 8$). Differences between groups were significant with $P < 0.001$ (***).

also absorbed light from the UV-B and UV-A range, which could possibly indicate incomplete photo-cross-linking of UVHA. Because of the high water content ≥99%, ALG and UVHA, like the human vitreous body, exhibit a refractive index similar to water (1.336). Any deviations from the natural refractive index will lead to a reduction in visual acuity. Thus SIL-5000 with an increased refractive index of about 1.4 induces

a hyperopic shift of about 4 to 6 diopters,[28] whereas the hydrophilic gels would not cause any alterations. The analyses further confirmed that the density of a material influences the refractive index.[29] The gelling process formed dense polymer networks, which slightly increased the refractive properties. By contrast, the polymeric gel network was partially broken by passing through a 23G cannula, resulting in a slight reduction of the refractive indices. In addition, the refractive properties of the gels were adjustable via the polymer concentration, because the density is dependent on the concentration. Therefore Healaflow with an increased content of hyaluronic acid (22.5 g/L) and a lower water content (97%) has a slightly higher refractive index (1.338) but still resembles the natural vitreous body.

Tested alginate and hyaluronic acid based hydrogels possessed viscoelastic properties similar to those of human, juvenile, and healthy vitreous bodies.[30] Apart from the tamponading effect, the viscoelasticity of the vitreous body is critical because it protects the eye from physical impacts ranging from internal low frequency mechanical stress and vibration to external mechanical trauma.[1] Therefore appropriate vitreous substitutes should be viscoelastic and correspond to the viscoelasticity of young, healthy vitreous bodies, because the vitreal viscoelasticity decreases with age as a result of liquefaction.[30] This requirement is met by the biopolymeric gels investigated, so that because of their viscoelastic properties the gels possess both tamponading and protective properties. Contrary to liquid tamponades, the use of viscoelastic gels could also reduce the risk of the tamponade dislocating behind the retina and causing retinal (re-)detachment. The viscoelastic properties of the biopolymer gels were adjustable via the polymer concentration, the gelling time and the type of cross-linking. ALG were formed physically by formation of charge-stabilized complexes after incubation in a Ca^{2+} cross-linking bath. In contrast, hyaluronic acid was conjugated with light-sensitive units and cross-linked by UV exposure (UVHA) or by cross-linking with 1.4-butanediol diglycidyl ether (Healaflow) under formation of covalent bonds. Because of the different types of cross-linking, different viscoelastic properties occurred after passing through a cannula. The induced mechanical stress partially breaks the gel network, so that after injection the gel matrices appear as a ball of coiled pearl strings (see Fig. 1). Whereas charge-stabilized ALG gained a higher degree of freedom by fracturing and showed increased viscoelasticities, the fractures in covalently cross-linked UVHA resulted in reduced viscoelastic properties. Nevertheless, both alginate- and hyaluronic acid–based gels revealed viscoelastic properties that are beneficial for retinal tamponading. In contrast, SIL-5000 as a clinically applied endotamponade lacked viscoelasticity. SIL-5000 showed a dominant viscous behavior and is therefore inferior to gels for the stabilization of the retina.

In addition, alginate- and hyaluronic acid–based gels demonstrated high biocompatibility in vitro. Using human fibroblasts, RPE (ARPE-19, fetal RPE) and photoreceptor cells, relevant ocular cells of the posterior segment of the eye were considered. Because the metabolic activity, apoptosis, and tight junctions of the applied cells were unaffected by the biopolymeric gels, non-cytoxicity is demonstrated. Thus the in vitro biocompatibility on several ocular cell types was added to the previous in vivo studies in rabbits,[12,13] providing the proof of compatibility of both UVHA and Healaflow for clinical use. In contrast, the compatibility of alginate-based vitreous bodies has to be confirmed in further in vivo studies. With regard to PVR, the antiproliferative effect of the gels against fibroblasts might be interesting, because fibroblasts occur in advanced stages of PVR and can cause tractional redetachments.[31]

For application during vitreoretinal surgery the tamponading agent should be injectable through a needle. The present study examined the properties of the material systems before and after passing a 23G cannula. Although partial network fractures occurred, suitable optical and viscoelastic properties of the gels are still present after injection. Passing the gels through a 23G cannula was accomplished by hand. Under surgical conditions, the injection of the tamponade gains further standardization by applying constant pressure using appropriate vitrectomy equipment. Furthermore, the biocompatibility studies presented revealed that the dosage of the gels did not generate cytotoxic products. The administration of the tested biopolymeric gels via injection is thus feasible and supports the trend toward minimally invasive methods of treatment.

The presented comparative studies demonstrate that the hydrophilic biopolymers based on alginate and hyaluronic acid resemble the physical properties of natural vitreous bodies and are superior to silicone oil in terms of refractive and viscoelastic properties as a vitreous substitute. In addition to existing hyaluronic acid–based approaches, the present study provides transparent, biocompatible alginate gels for vitreoretinal surgery. It is important to note that the biocompatibility of alginate-based vitreous substitutes is highly dependent on the sterility, purity,[24,25] and molecular weight[32] of the alginates used. Here, it becomes

apparent that the use of sterile, high-purity, and high-molecular weight alginates may counteract previous complications such as intransparency[21–23] and retinal toxicity.[21] Further studies on alginate-based vitreous substitutes should address the maintenance of intraocular pressure and biostability in vivo as important parameters for future clinical success. Here, of particular interest are studies that examine the extent to which the tamponading is favored by slight swelling caused by the administration of slightly hyperosmolar gels. In addition, hyaluronic acid–based vitreous replacement strategies, such as UVHA and Healaflow, will be subject to the degradation of the system, because hyaluronic acid will be degraded enzymatically in the human eye. In vivo studies of UVHA in rabbits demonstrated stability of at least six weeks.[12] In contrast, because alginates are inherently nondegradable in mammals,[33,34] alginate-based hydrogels may be a valuable alternative for long-term vitreous replacement.

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