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Evaluation of long-term changes in physicochemical properties of hydrophobic intraocular lenses in a laboratory model

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ABSTRACT

BACKGROUND: In ophthalmology, the surface properties of implants such as intraocular lenses (IOLs) play a crucial role in the quality of vision because they may affect posterior capsule opacification (PCO), which is one of the most common long-term complications of cataract surgery, inevitably leading to revision surgery. The PCO effect increases when the epithelial cells of the lens remaining in the capsule's bag after IOLs implantation adhere, proliferate, grow, migrate and differentiate. For this reason, *in vitro* performance of IOLs, including protein adsorption on the implant surface (the initial step of cells adhesion), allows predicting its stability *in vivo*.

MATERIAL AND METHODS: We evaluated the physicochemical properties of six different hydrophobic commercial intraocular lenses (IOLs) in a two-step model experiment. During the first step, IOLs were immersed in the BSS solution. This stage simulated a 5-year implantation of the IOL within the eye. During the second step, IOLs were incubated in the albumin solution. These processes simulated protein adsorption onto the IOLs' surface.

RESULTS: On the surface of the examined IOLs glistening was observed in all the lenses after the *in vitro* condition. The model experiment showed that the transparency of the IOLs decreased by about 2–20% compared with the initial IOLs ($p < 0.05$). Surfaces of all IOLs became more hydrophilic after the glistening phenomena and after the time of protein (albumin) adsorption. Such surfaces are presumed to be more susceptible to adhesion of cells promoting the PCO effect.

CONCLUSIONS: The *in vitro* results (57 days/85°C/PBS) showed that the synergistic effect of two factors — glistening and protein adsorption — did not deteriorate the quality of visual acuity; however, it may facilitate cell adhesion that precedes the PCO phenomenon.

KEY WORDS: implant stability; intraocular lenses (IOLs); cataract; polymer biomaterials; biocompatibility

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INTRODUCTION

A cataract is a common disease among people over 60 (except for its congenital form that occurs at birth), which is treated surgically. The direct cause of the disease is opacification of the lens leading to its damage to the eye, and statistics claim that more

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than 20 million patients suffer from cataracts worldwide [1]. Opacification is a natural process associated with aging, although it quite often accompanies other diseases, e.g., diabetes. In extreme cases, cataracts may lead to blindness (approximately 50% of patients with cataracts), and the absence of symptoms (is painless) and gradual deterioration of the eyesight make the disease challenging to recognize. Nowadays, the best treatment method is phacoemulsification [1, 2], during which the damaged lens is emulsified and then mechanically removed. In the capsule formed after removing the natural lens, an intraocular lens is implanted using just small incisions.

Hydrophobic intraocular lenses (IOLs) are implants commonly used in cataract surgery. Foldable hydrophobic materials (such as acrylate, acrylate-methacrylate copolymers, and silicones) used to produce IOLs have become popular due to the minimally-invasive implantation technique and the efficiency of visual acuity after surgery. Additionally, the hydrophobic IOLs, thanks to their physicochemical properties, effectively adhere to the capsular bag, reducing the incidence of posterior capsule opacification (PCO) [3–5]. Unfortunately, long-term postoperative observations of IOLs show numerous problems occurring *in vivo*.

The first complication is glistening, supposedly caused by water retention in the IOL material, and such a phenomenon can be observed both in acrylic and silicone lenses [6–8]. Glistenings are microvacuoles (1–5 μm) observed in IOLs as tiny bright crystals or voids [9]. Reports have demonstrated that temperature fluctuations (e.g., during IOLs transportation, packaging, implantation surgery, and postoperatively) are involved in the formation of glistenings [10–11]. These phenomena do not induce changes in the material's properties, such as mass, molecular weight, or chemical composition alterations. Whether glistenings affect visual functions remains controversial, and some authors have shown that microvacuoles reduce visual acuity. Others indicated the lack of comprehensive optical methods to test the optical properties of the glistening-affected IOLs [9, 12].

Studies of explanted IOLs demonstrated that glistening phenomena appeared 6–16 months after the surgery, yet it was not the direct cause of explantation [7, 12]. The changes caused by glistening seem relatively safe for patients, unlike opacification of the optical surface, which requires another surgical procedure. PCO is supposedly caused by the deposition of biological substances onto the IOL surface, e.g., sodium phosphates or protein deposits diverge light and limit the patient's visual acuity [12–14]. The glistening may probably be the first step in the deposition process since it increases the surface roughness and thus facilitates adsorption.

Adsorption of proteins and salts and adhesion of endothelial cells onto the IOLs surface depends on the initial physicochemical properties of this surface. In the case of IOLs, data on such phenomena may help predict the prevalence of posterior capsule opacification (PCO). Thus, simulation of the above phenomena should be subjected to laboratory testing before application of the IOLs in clinical practice [7, 12–15].

The vitreous humor consists of proteins (human body serum, HSA) and α, β, γ globulins [16], which are mainly responsible for the physiological response to the eye implant. The interaction between IOLs and protein components of the intraocular liquid directly influences the ocular exudative reaction in the early postoperative period and the state of corneal endothelial cells in the distant postoperative period [16, 17].

The behavior of biomaterials and ready-to-use medical implants are subjected to a thorough investigation during their fabrication and *in vivo*. Appropriate tests (based on required standards) of the implants' *in vitro* performance constitute a decent simulation of the processes *in vivo*. In the case of stable, commercially available polymers for IOLs (silicone, acrylic ones), *in vitro* testing allows the monitoring of physicochemical and structural changes of the material and its ability to sustain visual acuity.

There are only a few papers in the literature documenting the results of *in vitro* studies of hydrophobic IOLs over a more extended period [18–20], while most long-term research focused on the analysis of the explanted IOLs [21–23]. The first works concerning long-term *in vitro* performance were completed in 2014 [18]. Thus it seems that there is a need to develop a relatively simple, inexpensive, and non-invasive procedure to assess the long-term durability of hydrophobic IOLs. This may also help organize knowledge from the literature about glistening — its presence and influence on the quality of vision (i.e., contrast sensitivity).

In the current study, we developed an *in vitro* test procedure to characterize the IOLs surface physicochemical properties and optical transmit-

tance. Six types of foldable acrylic hydrophobic IOLs and silicone lenses (as reference material) were investigated in a simulated 5-year degradation assay, followed by an albumin adsorption test. In the first step, the lenses were incubated under the conditions specified in the ISO 11979-5:2006 standard, i.e., 57 days in phosphate-buffered saline (PBS) at 85°C. The assayed *in vitro* degradation time at elevated temperature, calculated using equations available in the standard, corresponds to 5 years of *in vivo* testing. Microscopic observations revealed that all tested IOLs exhibited glistening phenomena. Wettability (contact angle measurement) decreased in the case of acrylic and methacrylic IOLs. After incubation, the polar component (γp) of surface free energy (SFE) was still higher than the dispersive one (γd). Structural changes of selected IOLs were also studied using attenuated total reflectance Fourier transform infrared spectroscopy (FTIR-ATR). The transparency of the aged IOLs measured in the wavelength spectrum of 400 to 800 nm showed that their visual acuity was similar to that of the initial IOLs. In the second step, aged IOLs were incubated in an albumin solution to simulate protein adsorption onto the IOLs surface. Changes in physicochemical properties were again monitored using wettability (Θ), surface free energy (SFE), and transparency (UV-Vis spectrometry) tests. The experiments indicated that the proposed conditions influenced the physicochemical properties of the IOLs surface, but neither glistening nor albumin adsorption affected their vision properties.

MATERIAL AND METHODS

Six commercial hydrophobic IOLs were used in the study: AcrySof® SN60AT (Alcon Laboratories, Fort Worth, TX); AcrySof®IQ SN60WF (Alcon Laboratories, Fort Worth, TX); EnVista (Bausch & Lomb, Rochester, NY) EyeCeeOne (Croma-Pharma GmbH, Leobendorf Austria), Tecnis® (Abbott Medical Optics, Santa Ana, CA) and Secura-sSA (Human Optics, Erlangen Germany). The data on IOLs provided by their manufacturers are presented in Table 1.

All experimental work on commercial IOLs was carried out at the Faculty of Materials Science and Ceramics, AGH University of Science and Technology in Krakow.

All IOLs were subjected to accelerated *in vitro* aging in a PBS solution (0.1 M NaCl, 0.086M KH2PO4, 0.041M Na2HPO4, pH 7.4; Alcon Laboratories, Fort Worth, TX). The incubation extracts had a ratio of 10:100 (IOL:PBS), and the incubation conditions were based on the requirements of the EN ISO 11979-5:2006 standard. According to the formula simulating the desired time of implantation *(F)* [24]:

$$F = 2.0^{\frac{Ta-To}{10}}$$

where: T_a — temperature accelerating the degradation time;

T_o — temperature inside the eye (35°C);

it was calculated that 57 days of incubation at 85°C corresponds to 5 years under the anticipated conditions of the implantation site (i.e. in the eyeball).

Changes in surface morphology of the IOLs before and after the degradation tests were observed using a Nikon ZT 400 optical microscope. Both sides of the IOLs were investigated, including the edges, and measurements were taken at a fixed microscope setting (50x magnification).

After simulated 5-year degradation, the IOLs were subsequently incubated in albumin solution: commercially available albumin of human serum (HSA, 66.5kDa, Sigma Aldrich, Germany) lyophilized powder dissolved in PBS (1.0 mg/mL); for 24 hours at 35°C.

Measurements of the contact angle were made by the direct method using the DSA 10 Kruss go-

Table 1. Product data of commercial intraocular lenses (IOLs) used in the experiment

IOL	Company	Material
SN60AT	Alcon Laboratories, Fort Worth, TX	Copolymer acrylate (2-HEMA-co-MMA)
SN60WF	Alcon Laboratories, Fort Worth, TX	Copolymer acrylate (2-HEMA-co-MMA)
EyeCeeOne	Croma-Pharma GmbH, Leobendorf, Austria	Hydrophobic acrylate
enVista	Bausch & Lomb, Rochester, NY	Hydrophobic acrylate
Tecnis®	Abbott Medical Optics, Santa Ana, CA	Hydrophobic copolymer acrylate
Secura-sSA	Human Optics, Erlangen, Germany	Silicon

niometer. The tests were performed in accordance with the EN ISO 11979-5:2006 standard [24]. In a typical experiment, the sessile drop of deionized water (0.15–0.25 µL in volume) was deposited at the center of the IOL surface. The measurements were performed at room temperature for initially hydrophobic IOLs, the samples after 57 days in PBS (simulated 5-year degradation), and after a further 24 hours in the albumin solution. Before the test, IOLs were removed from the immersion medium, air-dried (placed on wet support for 10–20 minutes at 25°C), and then cleaned with ethyl alcohol and air-dried again. The results of five measurements for each sample were averaged and presented as mean values with a significance level of $\alpha = 0.05$.

The surface free energy was calculated based on a mathematical analysis of direct measurements of the contact angle (as described above) for deionized water and diiodomethane (non-polar liquid, Avantor, Poland). The component method was applied (dispersive and polar component — Owens-Wendt method [26]) with the system of equations as follows:

$$\sqrt{\gamma_L^{\,d}} + 1,53\sqrt{\gamma_S^{\,d}} = 7,80 \cdot (1 + \cos\Theta_1)$$

$$\sqrt{\gamma_L^{\,d}} + 0,22\sqrt{\gamma_S^{\,d}} = 3,65 \cdot (1 + \cos\Theta_2)$$

where: Θ_1 — the contact angle when wetting the material with water;

Θ_2 — the contact angle when wetting the material with diiodomethane.

Significant structure changes during incubation of selected IOLs (EnVista) were characterized by the FTIR-ATR technique (ZnSe/Ge crystal, 600–4000 cm-1 range, penetration depth 2 µm). All lenses were tested after 15, 20, 30, and 57 days of incubation in PBS at 85°C, and the time steps selected for the IOLs degradation simulation correspond to 96, 128, 192 days, and 5 years, respectively.

The optical characteristics were performed using a Jasco V-630 spectrophotometer with a double-beam, a single monochromator, and a halogen/deuterium lamp as a light source operating in the 190–1100 nm range. The measurements were taken to evaluate the changes in visible light transmittance of the IOLs when subjected to the simulated conditions of the human eye. The measurements were made in the transmittance mode, in the 380–1100 nm range, using an integrating sphere (60 mm diameter) with a scanning speed of 400 nm/min. For each sample, measurements were taken 5 times on both sides of the IOLs, and the obtained spectra were averaged.

RESULTS

Designed experimental conditions allowed to investigate of the glistening of hydrophobic IOLs — a phenomenon observed in the optical microscope as the presence of bright vacuoles (Fig. 1). The extreme conditions of the accelerated degradation assay were supposed to mimic the extreme behavior of the IOLs material: from the possible decomposition of material to changes in its physicochemical properties. As shown in Figure 1, the optical part of all IOLs was altered due to the *in vitro* degradation assay. Salt crystals were visible near the edges of two IOLs (EnVista, EyeCeeOne). However, based on the performed tests, it was hard to evaluate the concentration of vacuoles on the IOLs surface. However, microscopic observations showed that their size ranged from 2 to 80 µma, and the changes resulting from the appearance of vacuoles in the outer part of the IOLs were permanent — glistening neither disappeared after IOLs air-drying nor after alcohol-cleaning. The influence of the incubation assay (57 days/85°C/PBS) on the IOLs morphology was determined by microscopic observations (Fig. 1), during which the phenomenon called glistening was observed as expected (formation of vacuoles) as a result of the elevated temperature effect.

Structural studies using the FTIR-ATR technique were performed for all IOLs at different stages of degradation (after 15, 20, 30, and 57 days in PBS at 85°C corresponding to 96, 128, 192 days, and 5 years in the eyeball at 35°C, respectively). However, structural changes, illustrating how the environment influences the implants, were only observed in the ATR spectrum of the enVista lens (Fig. 2). After 15 days of incubation, new bands appeared, indicating the change in the number of methyl and methylene groups. The variation of the bands in the 2860-2960 cm-1 region proved that the water molecules migrating into the lens material induce the reorganization of polymer chains. Hence, the groups endowed with hydrophobic properties were exposed to the environment (CH2, CH3).

Measurements of wettability made before and after the durability test showed that the hydrophobic surface of the acrylic IOLs changed into hy-

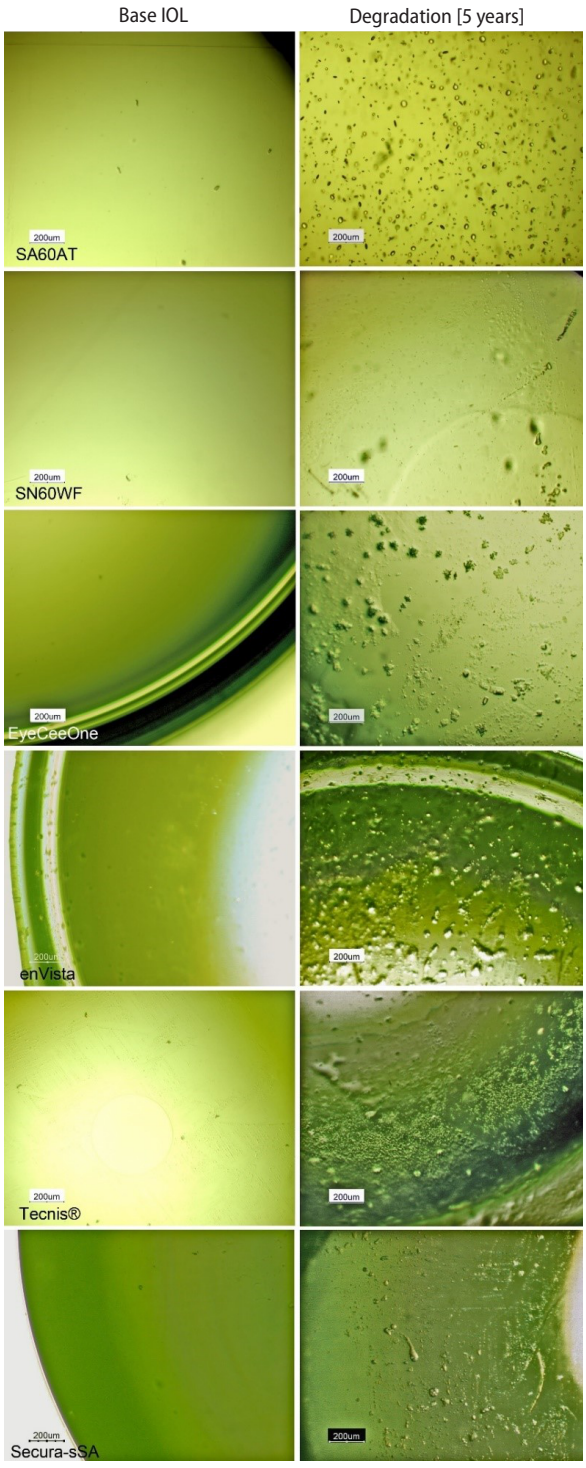

FIGURE 1. Representative microscopic images of initial intraocular lenses (IOLs) (base IOL) and IOLs after aging [simulated 5 years of *in vitro* degradation: 57 days/85°C/phosphate-buffered saline (PBS)] with glistening visible on the surface

drophilic (Fig. 3). In the case of two IOLs, i.e., AcrySof®SN60AT and EyeCeeOne, the contact angle decreased by approximately 50%. The surface nature of the AcrySof®IQ SN60WF IOL after the durability assay has changed completely (wettability 20.0°), while the enVista and Secura-sSA IOLs remained hydrophobic only. Moreover, their contact angle with water has increased. The measurements revealed inconsistency concerning the IOLs characteristics upon exposure to the albumin-containing immersion medium. Most IOLs became much more hydrophilic (AcrySof® SN60AT, AcrySof®IQ SN60WF, EyeCeeOne), while silicone IOLs Secura-sSA became more hydrophobic (compared to other IOLs).

A commonly used method of verification of wettability is the surface energy (SFE) test, which provides a better picture of the material's surface properties. Measurements performed for the initial and degraded IOLs have shown that in the case of pristine IOLs, the dispersive component comprises a more significant fraction of the SFE. In contrast, for IOLs exposed to accelerated degradation conditions, the polar component is larger (Fig. 4).

As a result of the glistening caused by the accelerated degradation, the SFE dispersive component of the IOLs has decreased, and the polar one has increased. This phenomenon is consistent with expectations: water molecules that penetrate inside the IOL have changed the character of its outer layer. The ratio of dispersive and polar components (Tab. 2) in the case of acrylic IOLs subjected to the durability test was similar — the polar component was about three times larger than the dispersive one. However, the behavior of the Secura-sSA silicone IOL was different, and after the durability assay, its wettability decreased, and the IOL became more hydrophobic. Moreover, the ratio of the dispersive to the polar part has increased.

In this study, a temperature of 35°C was used to simulate the conditions at the IOLs implantation site. In turn, the time of the experiment guaranteed stable (irreversible) binding of albumin to the lens surface. In conclusion, an albumin adsorption assay is a useful tool for assessing the tendency of glistening-susceptible lenses for further biodegradation and thus for opacification or PCO effect. Adsorbing proteins change the character of the IOLs surface, making it more hydrophilic. The analysis of energy components makes it possible to evaluate the relative proportion of the protein adsorbed onto the surface of the IOLs with glistening compared to the initial IOLs (Tab. 2). Higher concentration of albumin results in a possible change in protein conformation. It exposes polar groups to the en-

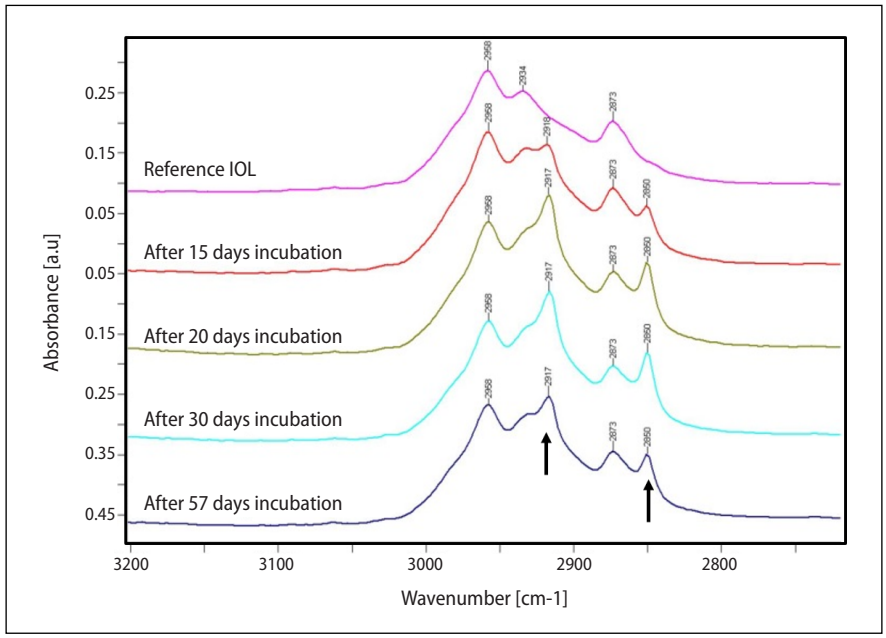

FIGURE 2. Attenuated total reflectance Fourier transform infrared spectroscopy (FTIR-ATR) spectra of the initial enVista intraocular lens and after 15, 20, 30, and 57 days in phosphate-buffered saline (PBS) at 85°C corresponding to 96, 128, 192 days

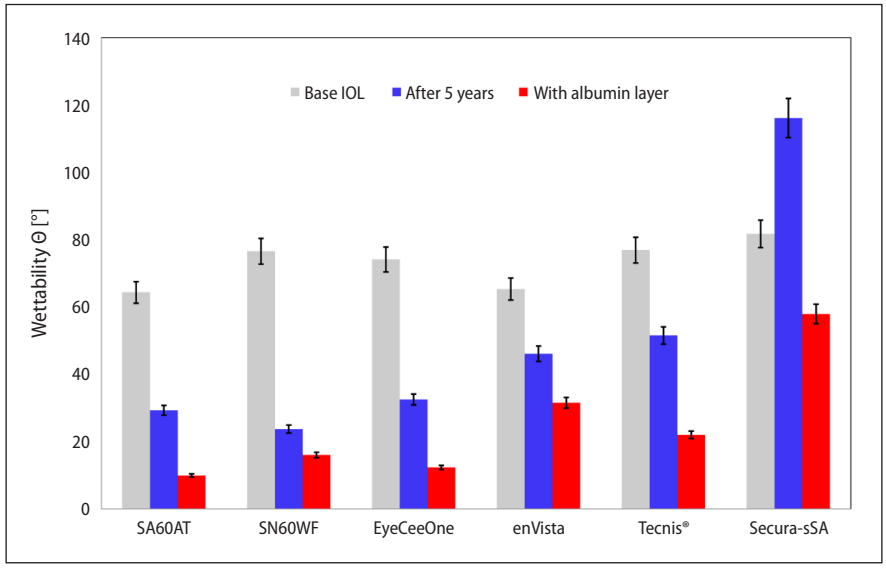

FIGURE 3. Wettability of the tested intraocular lenses (IOLs): initial, after 57 days in phosphate-buffered saline (PBS) at 85°C (simulation of 5 years in the eyeball), and with the albumin layer deposited after the durability assay

vironment, which was visible in the case of acrylic lenses (Fig. 2).

The optical characteristics of the lenses (Fig. 5) showed a slight decrease in transmittance after the degradation test (57 days/85°C/PBS as a 5-year simulation *in vivo*) and after immersion in the albumin solution. No significant changes in visible light transmission after *in vitro* degradation were observed in most of the studied lenses. Only in the case of the Acrysoft IQ SN60WF lens a more significant reduction in transparency (close to 8.0 %) was observed in all visible light ranges, due to which this lens can act as a grey filter. In addition, the silicone lenses exhibited a decrease in the transmission of blue light despite the absence of changes in physicochemical properties, e.g., wettability and surface energy. Such an optical effect may influence the acuity of color per-

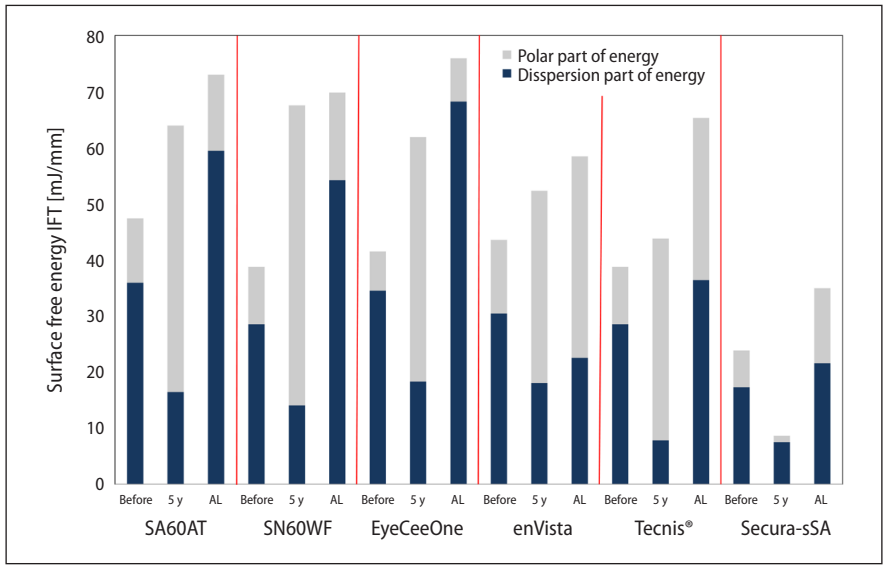

FIGURE 4. Changes of surface free energy with polar and dispersive part of the energy for all types of intraocular lenses (IOLs) tested: initial (denoted as "Before"), after 57 days in phosphate-buffered saline (PBS) at 85°C (simulation of 5 years in the eyeball, denoted as "5y"), and with albumin layer deposited after the durability assay (denoted as "AL")

Table 2. Values and ratios of the dispersive and polar parts of surface free energy of the tested intraocular lenses (IOLs): initial, after 57 days in phosphate-buffered saline (PBS) at 85°C (simulation of 5 years in the eyeball), and with the albumin layer deposited after the durability assay

IOL	Modification	γ_d [mJ/mm]	γ_p [mJ/mm]	γ_d/γ_p
Natural SN60AT	Initial	35.91	11.49	3.13
	After degradation	16.43	47.50	0.35
	With albumin layer	59.44	13.57	4.38
AcrySof®IQ	Initial	28.52	10.23	2.79
	After degradation	14.05	53.49	0.26
	With albumin layer	54.20	15.60	3.47
EyeCeeOne	Initial	34.50	6.98	4.94
	After degradation	18.30	43.58	0.42
	With albumin layer	68.21	7.73	8.82
enVista	Initial	30.45	13.10	2.32
	After degradation	18.02	34.29	0.53
	With albumin layer	22.53	35.93	0.63
Tecnis®	Initial	28.52	10.23	2.79
	After degradation	7.81	35.98	0.22
	With albumin layer	46.40	19.40	2.39
Secura-sSA	Initial	17.29	6.56	2.64
	After degradation	7.49	1.15	6.51
	With albumin layer	21.56	13.39	1.61

γd — dispersive part of surface free energy; γp — polar part of surface free energy

ception (contrast), but it does not adversely affect the quality of vision.

As a result of a 57-day accelerated degradation and subsequent 24-hour incubation in the albumin solution, the transmittance of all the studied IOLs within the visible spectrum ($p < 0.0001$; Tab. 3, Fig. 5) was reduced. In the red-orange (589–780 nm) and green-yellow (530–589 nm) spectra,

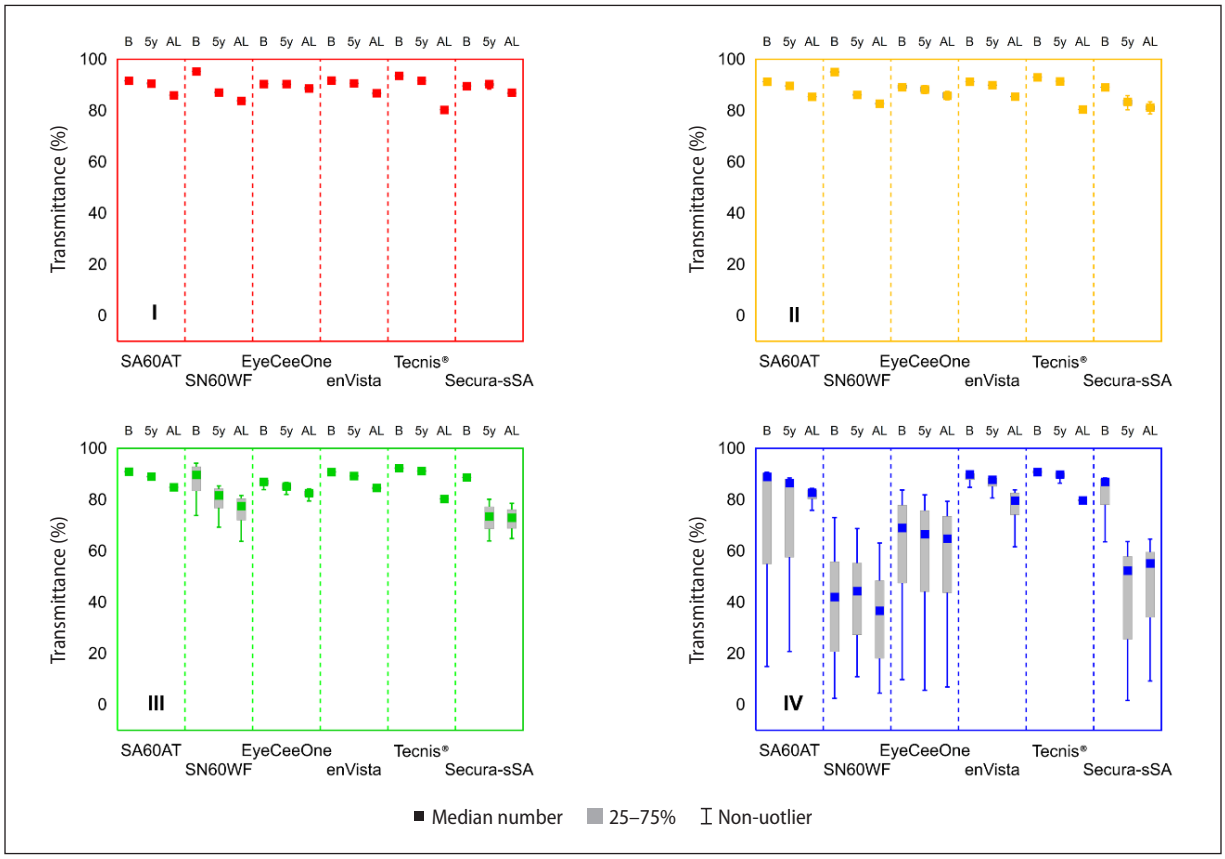

FIGURE 5. The transmittance of the tested intraocular lenses (IOLs): initial (denoted as "B"), after 57 days in phosphate-buffered saline (PBS) at 85°C (simulated 5 years in the eyeball, denoted as "5y"), and with the albumin layer deposited after the durability assay (denoted as "AL"). I — red-orange (589–780 nm), II — green-yellow (530–589 nm), blue-green (470–530 nm), violet-blue (380–470 nm) part of the visible light spectrum

the transmittance was reduced to a maximum of about 80.0%. The translucency reduction rate (between 'initial' and 'with AL' samples) fluctuated from 2.0 to 12.9%, depending on the IOLs.

The highest levels of translucency reduction were observed for the AcrySof®IQ SN60WF and Tecnis® IOLs, which may result from the fact that their initial transmittance values were the highest. In the blue-green spectrum (470–530 nm), the transmittance of the AcrySof®IQ SN60WF and Secura-sSA IOLs dropped below 80.0%. Similarly, a significant decrease in transmittance was observed in this part of the visible spectrum in the case of Tecnis® IOL.

The process of degradation and incubation in albumin had the most significant impact on the transmittance in the violet-blue spectrum (380–470 nm), in which, e.g., the transmittance of the Secura-sSA IOL was reduced by 42.3%. More minor changes (below 10.0 %) were observed only in the case of AcrySof® SN60AT and EyeCeeOne IOLs. It should also be noted that in this part of the visible spectrum, there were large fluctuations in the initial transmittance values of individual IOLs, and the transmittance values of the initial AcrySof®IQ SN60WF and EyeCeeOne IOLs were significantly lower than 80.0%. The transmittance of the AcrySof®IQ SN60WF IOL was the lowest in the violet-blue spectrum (380-470 nm) of visible light due to the presence of a filter for this part of the spectrum. In addition, significant statistical differences were noticed in the initial translucencies of the IOLs included in the study. The observed differences were maintained even after degradation and incubation in albumin ($p < 0.0001$; Tab. 3, Fig. 5).

DISCUSSION

The objective of the study was to mimic the behavior of the hydrophobic foldable IOLs during long-term implantation (glistening as the first step, followed by protein adsorption that precedes epithelial cells adhesion *in vivo*) and to develop *in*

Table 3. Transmittance (%) of the intraocular lenses (IOLs) tested: initial, after 57 days in phosphate-buffered saline (PBS) at 85°C (simulated 5 years in the eyeball), and with the albumin layer deposited after the durability assay

	SN60AT	SN60WF	EyeCeeOne	enVista	Tecnis®	Secura-sSA	†p
Red-orange (589–780 nm)							
Initial	91.7 ± 0.2	95.3 ± 0.1	90.4 ± 0.1	91.8 ± 0.2	93.7 ± 0.3	89.6 ± 0.1	< 0.0001
After degradation	90.6 ± 0.4	87.1 ± 0.3	90.3 ± 0.3	90.6 ± 0.2	91.7 ± 0.1	90.0 ± 1.0	< 0.0001
With AL	86.0 ± 0.2	83.9 ± 0.3	88.6 ± 0.6	86.7 ± 0.4	80.3 ± 0.2	86.7 ± 0.8	< 0.0001
% reduction	6.2	11.9	2.0	5.6	14.3	3.2	
*p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
Green-yellow (530–589 nm)							
Initial	91.3 ± 0.1	95.0 ± 0.3	89.2 ± 0.6	91.3 ± 0.1	93.0 ± 0.2	89.2 ± 0.1	< 0.0001
After degradation	89.7 ± 0.2	86.2 ± 0.3	88.3 ± 1.9	90.0 ± 0.2	91.5 ± 0.1	83.3 ± 1.4	< 0.0001
With AL	85.4 ± 0.1	82.7 ± 0.4	85.9 ± 1.1	85.5 ± 0.2	80.5 ± 0.1	81.2 ± 1.1	< 0.0001
% reduction	6.4	12.9	3.7	6.4	13.4	9.0	
*p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
Blue-green (470–530 nm)							
Initial	91.0 ± 0.1	87.7 ± 6.0	86.7 ± 1.2	90.9 ± 0.2	92.3 ± 0.2	88.7 ± 0.1	< 0.0001
After degradation	89.0 ± 0.2	80.2 ± 4.8	85.0 ± 1.3	89.3 ± 0.2	91.2 ± 0.2	72.9 ± 5.0	< 0.0001
With AL	84.9 ± 0.2	75.8 ± 5.3	82.4 ± 1.3	84.6 ± 0.4	80.3 ± 0.1	72.4 ± 4.2	< 0.0001
% reduction	6.7	13.6	5.0	6.9	13.0	18.4	
*P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	
Violet-blue (380–470 nm)							
Initial	87.1 ± 28.6	38.4 ± 22.0	59.8 ± 23.6	88.0 ± 4.2	88.8 ± 5.2	80.0 ± 14.5	< 0.0001
After degradation	83.1 ± 25.0	41.1 ± 17.9	57.0 ± 24.4	85.6 ± 4.9	88.1 ± 4.4	41.7 ± 21.1	< 0.0001
With AL	81.0 ± 4.5	33.7 ± 18.4	55.8 ± 23.0	77.3 ± 6.7	77.9 ± 4.6	45.9 ± 18.4	< 0.0001
% reduction	7.0	12.2	6.7	12.2	12.3	42.3	
*p	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

AL — albumin layer; % reduction — difference between transmittance of the initial lenses and the ones with the albumin layer deposited after the durability assay, expressed in %; *Friedman ANOVA; †Kruskal-Wallis ANOVA

vitro test procedure that will predict IOLs stability *in vivo*. To date, most of the methods published in the literature concerning the simulation of glistening were based only on short-term IOLs incubation with heating-cooling cycles. According to other sources, incubation times from 24 to 48 hours, water as an immersion medium, and temperatures 37.0, 40.0, or 50.0°C [20–23] were tested. Such conditions also allowed to obtain a glistening effect *in vitro*, testing whether the biomaterial itself and the manufactured IOLs were prone to glistening. The literature data confirmed that all materials **used to produce [UNCLEAR]** IOLs were prone to glistening [5–8, 20–23]. The longer incubation time made it possible to strengthen this phenomenon — upon prolonged incubation time, the vacuoles became larger and more numerous compared to the shorter immersion in water [18, 25]. According to published studies, the higher the incubation temperature, the bigger water retention in the implant was (for the acrylic IOLs 0.5%, for the silicone IOLs 0.1%) [25]. The conditions of the presented method ensured the formation of glistening, the presence of which was indispensable to perform a series of simple physicochemical tests verifying the durability of IOLs *in vitro*.

The results of the wettability measurements performed on the selected group of the foldable hydrophobic IOLs indicated that after incubation all materials based on acryl resins became strongly hydrophilic (Fig. 3). This may be explained by the nature of acryl resins that facilitate water migration at high temperatures. Water retention in the material takes place due to the presence of voids in the polymer network (non-homogeneous polymer structure is the result of polymerization): water gets trapped in the voids during the cooling phase, which results in a microscopic morphology known as glistening

[11, 23, 25]. The silicone lens remained hydrophobic — lower wettability of this material resulted from its homogeneous structure. The observed decrease in wettability of the IOLs was associated with an increase in the interfacial tension of the implants. Tests showed that the hydrophobic IOLs had a high content of polar components (except the silicone IOL). The goniometry method allows for determining the changes in the surface structure [18, 23]. The sessile drop technique with polar and non-polar liquids seems to be an efficient tool for evaluating the quality of IOLs.

The proposed *in vitro* stability test procedure for investigating the IOLs durability also included short-term incubation (24h) in albumin solution, which is an essential step in assessing biocompatibility and biofunctionality of contact lenses and IOLs in the eye environment. It has been proven that hemolytic tests with hydrophobic IOLs and albumin-coated IOLs do not induce the complement system [17]. Thus, interaction with albumin may be treated as a model of protein behavior on the lens surface. Some authors claimed that the chemical composition of the polymer used for implant manufacturing affects the amount of adsorbed albumin (the more hydrophobic MMA with low HEMA content, the less efficient protein adsorption) [26]. Others did not find a correlation between the amount of adsorbed albumin on the lens and its physicochemical character (hydrophobic/hydrophilic) [17]. However, the majority of works proved that the surface most prone to strong interaction with albumin is a hydrogel lens in a highly hydrated environment [26–28]. An important role is played by the experiment conditions, namely time and temperature — the number of albumin increases when the temperature drops (optimal is 8–12°C). The same authors suggested that the interaction between albumin and the implant surface is the effect of weakening hydrogen bonds with increasing temperature [29].

The second stage of the procedure aimed to show how the surface of the lenses with glistening adsorbs the proteins. Protein adsorption plays an important role in the biocompatibility and functional properties of intraocular lenses made from both hydrophobic and hydrophilic biomaterials [30]. In the case of foldable hydrophobic lenses, this step was used to determine the material's tendency to attract cells (e.g., epithelium) and the influence of the protein layer's adsorption on the lens's transparency. Finally, a sufficient amount of proteins irreversibly adsorbed onto the surface may trigger another cataract-like effect after a long implantation period [31]. The results confirmed that when the lenses with glistening are immersed in the albumin solution, even for a short time, the protein layer deposits on the surface of the lenses. This phenomenon was confirmed by a significant decrease in the contact angle value, observed both in acrylic and silicone lenses (Fig. 3), in which the protein layer increased the wettability of the surface, and all the lenses became strongly hydrophilic.

On the other hand, surface energy measurements showed an increase in the dispersive component fraction due to the protein adsorption (Fig. 4). Such results may be explained by the specific behavior of proteins, whose hydrophilic groups of macromolecules, after adsorption, attach to the hydrophilic surface of the lens. At the same time, the hydrophobic fragments of the protein chains are moved away and exposed to the outside — that is why the fraction of the dispersive component has increased. The coexistence of these two factors, i.e., the high level of glistening and protein adsorption, slightly affected the light transmittance of the examined IOLs, which as a result of extreme incubation test conditions, decreased only by about 10.0%. However, this should not negatively impact the overall quality of vision because the decrease was consistent throughout the visible range [32, 33]. The presence of the albumin layer on the surface of hydrophobic IOLs reduced visible light transmission compared to non-degraded IOLs, mainly for Tecnis® and AcrySof® IQ SN60WF (Fig. 5). This may probably be explained by the fact that the surfaces of these IOLs were covered with a thicker albumin layer in comparison with the rest of the examined group. This assumption was confirmed by the value of wettability and only a slight difference between the polar and dispersive components. The transmittance values of the initial AcrySof® SN60AT and EyeCee-One IOLs were very similar to those observed after the degradation test. The decrease in transmittance of the Secura-sSA and enVista (380–530 nm) samples might be associated with the fogging process occurring over time in natural crystal lenses.

CONCLUSIONS

This experiment strongly suggests that the degradation process of the IOLs promotes glistening, a common phenomenon in all polymer materials (acrylates, silicones) exposed to the aqueous envi-

ronment. Nevertheless, this phenomenon does not impair visual function, and glistening inclusions do not deteriorate visual quality. Glistening leads to alterations in the wettability of the IOLs surface, as it changes its character from hydrophobic to hydrophilic. The results showed that *in vitro* had an effect on all materials that, regardless of their original character, were covered with a protein layer. The synergistic effect of two factors — glistening and protein adsorption — did not deteriorate the quality of visual acuity; however, it may facilitate cell adhesion that precedes the PCO phenomenon. The test procedure developed to evaluate the physicochemical changes of IOLs *in vitro* (microscopy, goniometry, UV-VIS spectrometry) constitutes a successful model to verify the effect of long-term IOLs implantation *in vivo*.

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