Laboratory Modeling Techniques for Primary and Secondary Cataracts: Methodology and Analysis

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Abstract. This article is dedicated to the development of a laboratory methodology for modeling lens pathologies and cataract membranes of various etiologies. These models are essential for determining precise parameters of surgical or laser interventions for primary and secondary cataracts. The study explores two models based on chemical treatment leading to cataract formation. Two approaches to cataract modeling were investigated: soaking in formalin and exposure to reactive oxygen species (oxidative cataract). The optical properties of these models under laser treatment were also analyzed. It was found out that modeling intraocular lens opacification with hydrogen peroxide, a stable decline in optical response was observed during IR pulses—around 60% after 24 h, dropping to 30% after 72 h. This is attributed to increased scattering and capsule thickening. In contrast, formalin-induced capsule opacification initially caused a threefold increase in signal amplitude, likely due to increased tissue rigidity and greater displacement during heating. However, as tissue thickened, the optical response declined, returning close to baseline.

Keywords: laser; dynamics of laser radiation; primary cataract; secondary cataract; hydrogen peroxide; formalin.

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1 Introduction

Currently, research aimed at understanding the physical mechanisms and minimizing the side effects associated with laser ophthalmological surgeries is of significant relevance. The necessity for interdisciplinary approaches in this field is driven by the need to address several physical problems, such as investigating the heating processes of thin biological membranes under tension and the development of additional thermally induced laser stresses. These stresses indirectly affect the germinative (growth) zone of the lens capsule (LC), which retains proliferative activity and often initiates secondary postoperative opacification, leading to the development of so-called "secondary cataracts" (SC).

The proportion of lens pathologies within the structure of vision-disabling diseases remains high, accounting for 11–18%. Among these, the primary clinical manifestation of lens pathology leading to visual disability following cataract surgery is complicated pseudophakia (57%), which refers to the condition after replacing the native lens with an artificial one [1, 2]. The predominant postoperative complication in cases of pseudophakia is the development of so-called "membranous" opacities in the pupillary area. These opacities vary in nature and can form both in the early postoperative period and at later stages (ranging from 10 days to over 4 years) [3–5]. The term "membranous" refers to the postoperative reduction in the transparency of the LC. Posterior LC opacification is more common than anterior opacification. The incidence of posterior LC opacification ranges from 4% to 60% of cases [5–8], while anterior LC opacification occurs in 3–16% of cases [9, 10]. Furthermore, the development of capsular contraction syndrome often results not only in LC opacification but also in deformation of the elastic

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artificial lens, significantly altering the eye's optical system and refractive power. "Membranous" opacities can substantially reduce visual acuity and negatively impact quality of life, necessitating repeated interventions in some cases, either surgical or laser-based.

The frequency and severity of "membranous" opacities can be influenced by numerous factors, including age, the presence of concomitant ocular and systemic diseases, the state of the immune system and the blood-ocular barrier, the bioinertness of the artificial lens material, and its structural features [11]. The varying conditions of the LC affected by opacities dictate the strategy for laser intervention, requiring surgeons to determine the appropriate laser parameters during the procedure itself.

At present, there is no universal method for determining the optimal parameters of safe laser radiation. Consequently, each new laser intervention approach necessitates dedicated research, typically involving initial calculations, modeling of thermal field propagation and thermal stress fields, and experimental verification of the derived parameter ranges, taking into account the actual geometry and specific biological tissues. Due to the limited availability of biological tissues for experimental studies, it is often necessary to simulate both the tissues themselves and their pathological variations using various methods.

The development of cataracts can result from external adverse factors (chemical and physical), including various metabolic disorders, as well as genetic factors that affect the structure of the lens. Recently, genetic mutations disrupting the synthesis of crystallins, membrane proteins, cytoskeletal proteins, and transcription factors in the lens have been increasingly identified [12]. Among these, α-crystallin is the most important structural protein of the lens, comprising 40% of the total crystallin content in the human lens [12]. This protein regulates cell growth and genome stability, influencing both the cell membrane and the cytoskeleton. γ-crystallin is one of the most long-lived proteins, with complete turnover in humans occurring over several years [13]. Crystallins play a critical role in maintaining the lens's transparency and refractive properties. Therefore, disruption of the crystalline structure of the lens critically affects its functional properties, leading to opacification, or the formation of cataracts.

In addition to genetic disorders, oxidative stress accumulation can also contribute to lens opacification [14, 15]. Prolonged exposure to ultraviolet radiation induces photochemical production of reactive oxygen species, such as hydrogen peroxide, superoxide radicals, hydroxyl radicals, and others. These molecules gradually oxidize lens proteins, primarily crystallins, causing their aggregation and leading to loss of optical transparency. One well-documented mechanism of oxidant action is the oxidation of thiol (−SH) groups to sulfoxide (−S=O) groups, which subsequently form dimers via disulfide bridges [16]. Since hydrogen peroxide appears to be the most stable oxidant in the progression of oxidative cataracts [17, 18], modeling oxidative cataracts has been conducted using hydrogen peroxide solutions of varying concentrations.

Since the literature on oxidative cataracts primarily focuses on cataract modeling in the lenses of small animals (such as rats [19] and rabbits [20]), this study required selecting appropriate concentrations of solutions and exposure times for lens capsules of larger animals. This was done to modify the biological tissue to induce cataracts without causing capsule destruction. Limited studies on the application of oxidative techniques to pig eyes have demonstrated the success of this approach [21]. However, in our study, it was crucial to optimize tissue treatment to develop two distinct methods for laboratory cataract modeling with following laser exposure.

Thus, the task of simulating processes that lead to postoperative opacities of LC of various types, as well as modeling the properties of SC with subsequent laser treatment of modified biological tissue samples, is of significant relevance.

This article is dedicated to the development of a laboratory methodology for modeling lens pathologies and cataract membranes of various etiologies for primary and SC. The study explores two models based on chemical treatment leading to cataract formation. Two approaches to cataract modeling were investigated: soaking in formalin and exposure to reactive oxygen species (oxidative cataract). The optical properties of these models under laser treatment were also analyzed.

2 Materials and Methods

2.1 Laboratory Methodology for Oxidative Cataract Modeling

The pig lenses obtained from a slaughterhouse were used in the experiment. The extracted eyes were stored in a refrigerator at 4 °C in 0.9% NaCl physiological solution for no more than 24 h.

The oxidative solutions were based on a phosphate-buffered saline (PBS) with a pH of 7.4, prepared using the following composition: 6 g of $Na_2HPO_4 \cdot 12H_2O$, 0.19 g of K_2HPO_4, and 8 g of NaCl dissolved in distilled water. The pH was measured using Universal test paper, and the solution volume was adjusted to 1000 ml. The final pH corresponded to 7 on the indicator scale. Hydrogen peroxide (H_2O_2) solution was used to initiate oxidative processes in the lens tissue, with the peroxide concentration varying according to Table 1.

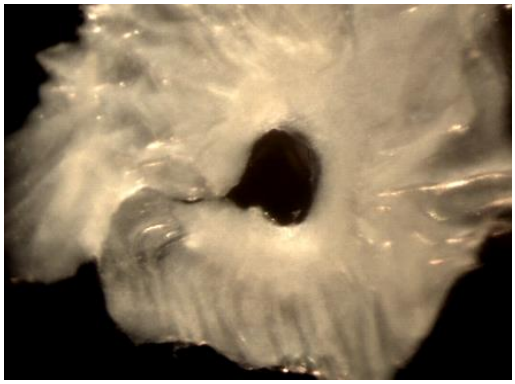
Immediately before the experiment, lenses were extracted from the eye samples, and their transparency was assessed. The lenses were then placed in PBS containing hydrogen peroxide. The reaction was conducted in a room without exposure to daylight. The changes were recorded on photo and video. The lenses were incubated for 72 h with monitoring at 24, 48, and 72-h intervals. Visual observations were recorded using a Celestron Amoeba Microscope (Model 44325). The experiment was carried out on a minimum of six samples at each point.

Table 1 Volumes of PBS and 3% hydrogen peroxide required to prepare solutions of the indicated concentrations.

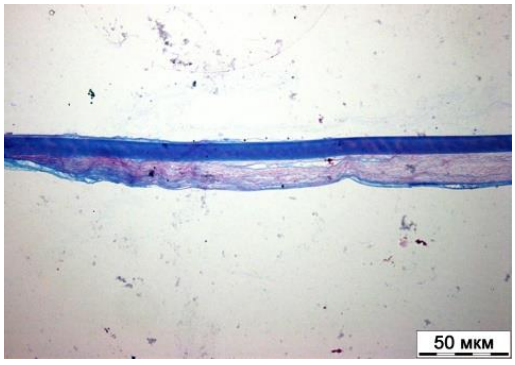
Concentration	PBS	H_2O_2 3%
4 mM	20 ml	0,1 ml
8 mM	20 ml	0.2 ml
13 mM	20 ml	0.3 ml

2.2 Laboratory Methodology for Modeling Cataractous Formations Using Formalin

The experimental model for LC opacification using formalin was based on the results from previous comprehensive studies of autopsy LC samples in pseudophakic eyes (with artificial lenses) exhibiting SC with varying optical densities [22]. The density of these dissected samples subsequently influenced the parameters for laser tissue ablation [23]. LC opacities were categorized as follows: SC with dense fibrous deposits on the internal surface of the posterior lens capsule (PLC) were classified as "hard" membranes (Fig. 1).



(a)



(b)

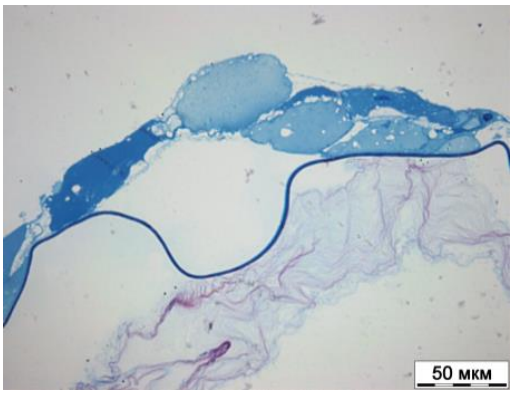
Fig. 1 (a) Macroscopic view of a sample of a "hard" posterior capsule (PC): an optically dense capsule with a shiny outer surface and a wavy relief, magnification ×1.2. (b) Morphological structure of a "hard" PC sample [24]: fibrous tissue on the inner surface of the PC. Wrinkling of the PC is observed. Semi-thin section stained with methylene blue and basic Fuchsine.

SC films with deposits of pseudoregenerative cellular components (Elschnig-Adamiuk pearls) on the inner surface of the posterior capsule were classified as "soft" films (Fig. 2).

This formalin-based method for modeling LC pathological changes was chosen to densify the tissue and create cross-links, as this approach is commonly employed by ophthalmologists to simulate cataracts and practice surgical skills for removing cataractous opacities [25]. It is also used for studying tissue properties during soaking in the solution [26].

The experiment was conducted over 60 min, with control intervals at 15, 30, and 60 min, with minimum three samples in every point. It was hypothesized that varying exposure times would result in LC opacities of differing densities (softer or harder), enabling the creation of conditions as close as possible to the actual scenarios described earlier. Visual observations were recorded using a Celestron Amoeba Microscope (Model 44325).

(a)



(b)

Fig. 2 (a) Macroscopic view of a "soft" PC sample: uneven optical density of the capsule (areas of transparency alternate with denser regions), magnification ×1.2. (b) Morphological structure of a "soft" PC sample: Elschnig-Adamiuk pearls on the inner surface of the PC. Semi-thin section stained with methylene blue and basic Fuchsine.

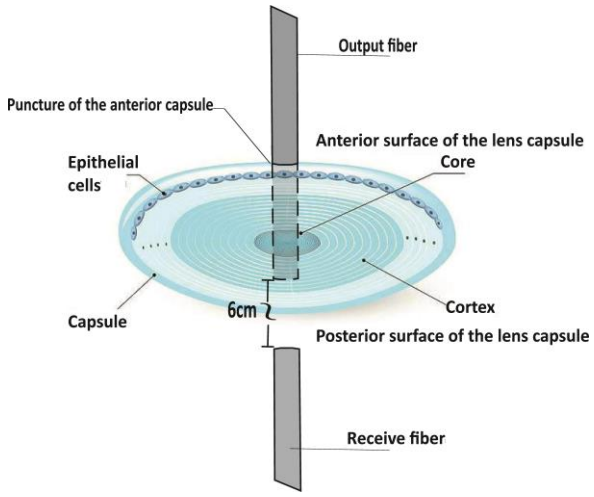


Fig. 3 Optical method for studying the dynamics of transmission of probing laser radiation [23], the diagram of the experiment is shown in cross section.

2.3 Optical Method for Investigating the Dynamics of Transmission of Probing Laser Radiation

Experiments on the study of the transmission dynamics of low-intensity probing laser radiation were carried out. The laser radiation penetrated a sample of a whole lens perpendicular to its surface using an optical fiber. Behind the surface of the sample, coaxially with the input optical fiber supplying the radiation, there was a "receiving" optical fiber connected to an optical multichannel analyzer in which the signal was digitized (Fig. 3). Peroxide soaking was conducted for 72 h with control intervals at 24, 48, and 72 h. Formalin soaking was conducted for 60 min with control intervals at 15, 30, and 60 min. The geometry and power of the visible radiation were consistent across all four irradiation series of each modeling process. The wavelength of the constant probing radiation was 0.53 μm (visible spectrum). Heating irradiation with a wavelength of 1.56 μm was performed simultaneously with a probing one in a pulsed-periodic mode, with a pulse duration of 100 ms and a pause of 1900 ms between pulses. The total irradiation time was 20 s (10 pulses), with power levels of 0.3 W and 0.5 W.

3 Results and Discussion

3.1 Visual Assessment of Cataract Modeling

Microscopic analysis conducted with the Amoeba Celestron Microscope 44325 revealed distinct patterns in cataract formation for oxidative cataracts (Table 2). The lens soaked in PBS solution showed negligible changes in light transmission within the visible spectrum after 24 h. However, at 48 and 72 h, structural opacities and damage began to appear.

Table 2 Visual assessment of lenses subjected to various modifications using hydrogen peroxide.

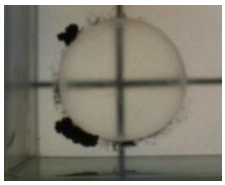
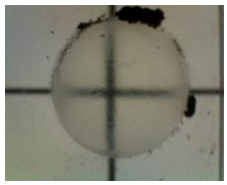
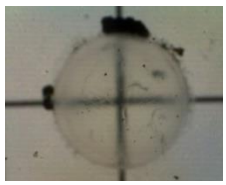
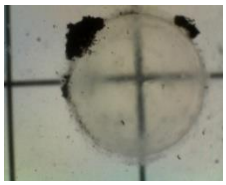
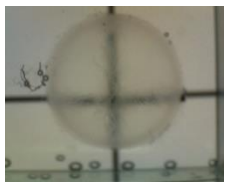
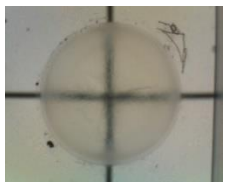
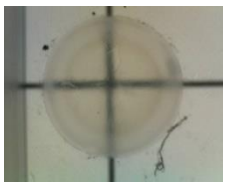
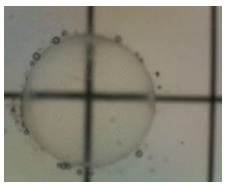
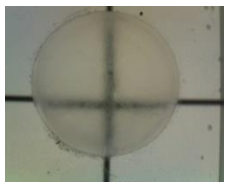
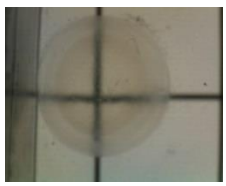
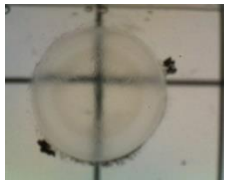
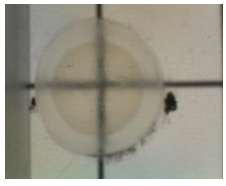
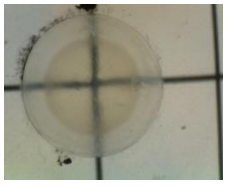
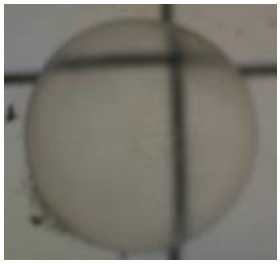
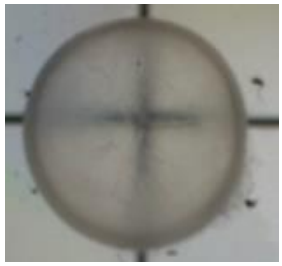
Time, h	0	24	48	72
PBS				
PBS + H_2O_2 3% 4 mM				
PBS + H_2O_2 3% 8 mM				
PBS + H_2O_2 3% 13 mM				

Table 3 Visual assessment of lenses subjected to various modifications using formalin [23].

0 min in formalin solution (Intact lens)	15 min in formalin solution	30 min in formalin solution	60 min in formalin solution
			

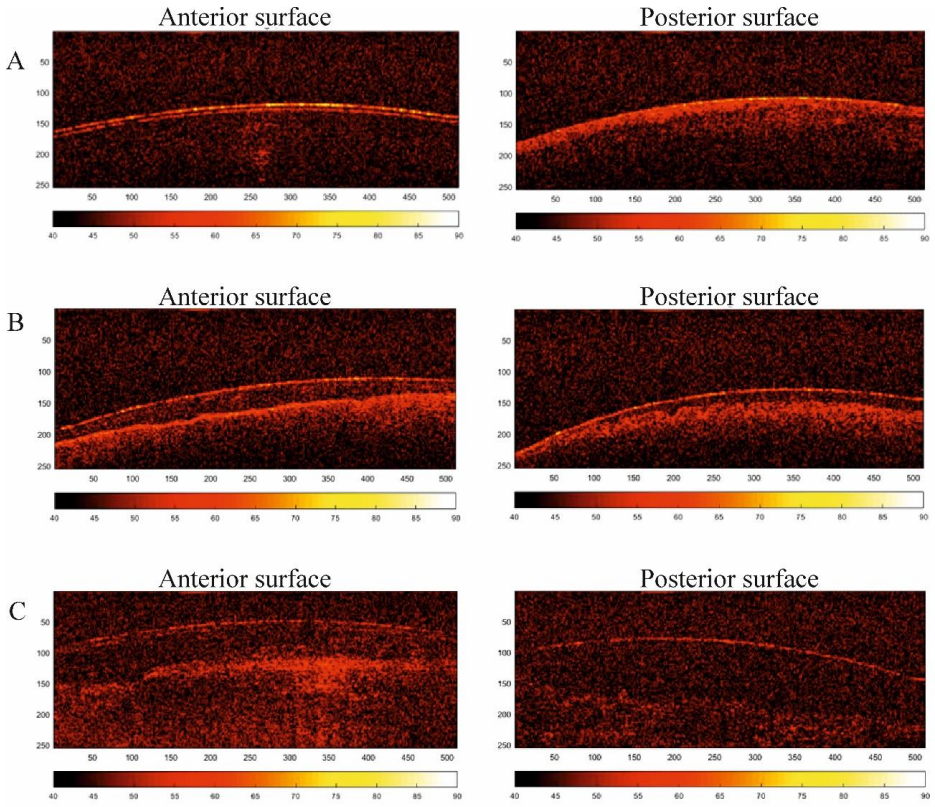


Fig. 4 OCT structural image of (A) the intact lens of the eye, no more than 4 h have passed since the material was extracted, (B) the eye lens 30 min after placing them in formalin, (C) the lens during modeling of oxidative cataract in PBS + 8 mM peroxide solution for 48 h.

The addition of hydrogen peroxide at varying concentrations had differential effects on structural changes and lens opacity. A 4 mM concentration led to noticeable separation of the capsule and body only after 72 h, whereas a 13 mM concentration caused clear structural separation as early as 24 h. However, at 13 mM and 24 h, frequent detachment of the lens capsule from its body was observed during sample handling, and prolonging exposure to 72 h was deemed unnecessary due to time constraints. Therefore, a concentration of 8 mM with an exposure time of 48 h was selected, achieving distinct structural separation in the lens without capsule detachment.

In formalin-based modeling, lens opacity became evident within 15 min of soaking (Table 3). Furthermore, the opacity appeared to affect only the superficial layers, as the peripheral band of the lens visibly thickened. This observation suggests the potential to model SC films, which are a common reason for laser capsulotomy procedures in cases of SC.

3.2 OCT Visualization of Lens Structure

The evaluation was performed on both sides of the lens (anterior and posterior surfaces) along the axial line (Fig. 4). For intact lens tissues, OCT imaging revealed a structural separation in the superficial layers of the anterior capsule, with a very thin gap between them, while no such separation was observed on the posterior surface.

Soaking the lens in formalin for 30 min or longer induced the following structural changes observed on OCT images:

- Anterior surface: in 60% of cases, there was a significant increase in the distance between the capsule (surface) and the lens body (cortical layers and nucleus). In the remaining cases, an increase in this distance was also observed, but it was less clear.
- Posterior surface: distinct separation of the surface was observed in only 30% of cases.

It is important to note that this pattern emerged as early as 15 min of formalin exposure and remained consistent regardless of the duration of soaking.

In the case of oxidative cataract modeling, OCT images revealed changes occurring within the lens body rather than on its surface, as seen with formalin treatment.

These OCT imaging results confirm the conclusions drawn from the visual assessment of lens changes during cataract modeling. Soaking in formalin primarily affects the superficial layers of the lens (its capsule), while oxidative cataract modeling leads to structural alterations within the lens body.

3.3 Results of the Study on the Dynamics of Transmission of Probing Laser Radiation

The main point of this study was to obtain information about optical response of lens to IR heating pulses. Data presented in Fig. 5 shows the average relative change of the constant visual signal due to periodical IR heating pulses for lens with varying exposure durations in various solutions. At least 3 samples were used to plot each bar in Fig. 5.

Using hydrogen peroxide modeling the opacification of the intraocular lens, a stable decrease in the relative amplitude of the change in the transmitted probing radiation during heating IR laser pulses is noted depending on the time of exposure to the solution. The intensity of optical response was around 60% for 24 h of solution influence in comparison to initial signal and decreases further down to 30% for 72 h. This is due to the general decrease in optical transparency due to the increase in scattering of the capsule and its thickening.

At the same time, modeling the opacification of the capsule using formalin, an initial significant increase up to 3 times in the amplitude of the change in the visible signal is noted. This behavior can be explained by a change in the mechanical properties of the capsule surface. Formalin increases the rigidity of the tissue, which leads to a greater spatial shift of the sample as a reaction to thermal laser exposure. Subsequently, an increase in the thickness of the tissue again leads to a decrease in the optical response and almost returns to its initial value.

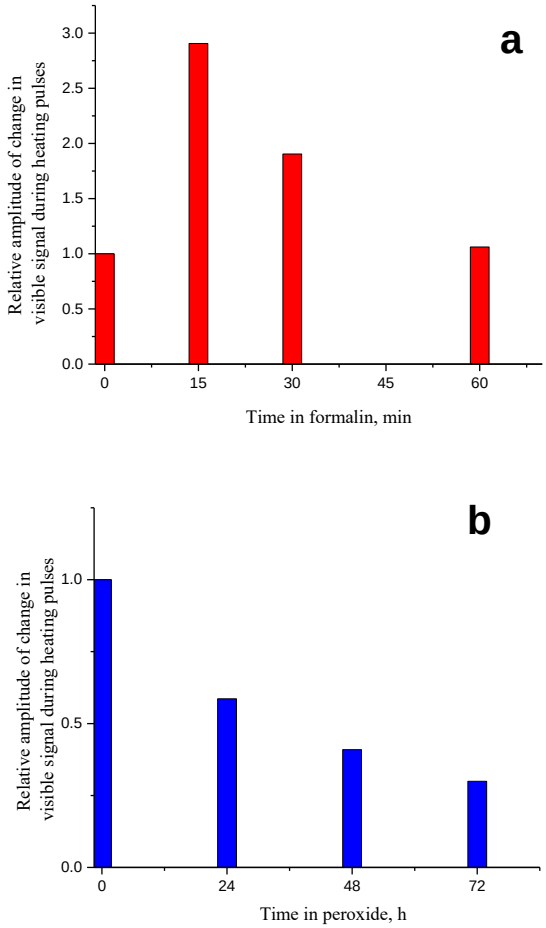


Fig. 5 Average relative amplitude of change in visible probing signal due to IR heating pulses for the samples with varying exposure durations in (a) formalin and (b) hydrogen peroxide.

4 Conclusions

A comparative study of two methods for creating models of pathologies in the “lens + capsule” system was conducted, corresponding to two of the most common forms of intraocular lens opacification that reduce vision and require surgical or laser treatment. These methods were tested on isolated eyes of large laboratory animals.

In the oxidative cataract model, changes occur within the lens body itself: the optical response of the lens mass alters, and its density changes. This effect on the density of the lens mass can be used to create cataract models for developing laser parameters aimed at fragmenting and removing lens material during lens replacement surgery. When modeling intraocular lens opacification with hydrogen peroxide, a stable decline in optical response was observed during IR irradiation − around 60% after 24 h, dropping to 30% after 72 h. This is attributed to increased scattering and capsule thickening, which demonstrates the model’s suitability for simulating the initial stages of cataract formation.

In contrast, formalin-induced capsule opacification initially caused a threefold increase in signal amplitude

change, likely due to increased tissue rigidity and greater displacement during heating. However, as tissue thickened, the optical response declined, returning close to baseline. In the formalin-induced cataract model, the changes are localized to the superficial layers of the lens (its capsule).

This approach allows for the modeling of two types of cataract films. These models can subsequently be used to refine photodestruction laser mode for treating SC films in eyes with artificial lenses.

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Disclosures

The authors declare that they have no conflict of interest.

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