



## Analyses of Natural Latex in Applied Membranes in the Human Eye for Treatment of Amblyopia

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### Abstract

Amblyopia is an ophthalmic disorder that consists in low visual acuity in an eye that developed inadequately during childhood, commonly known as lazy eye disorder. Nowadays, products used for treatment of amblyopia are conventional occluders fixed with adhesive tape directly on the child's eye region. These products generate great aesthetic damage and are easily removed by the child and thus bringing considerable delays to the treatment, rendering it unsuccessful in some cases. The aim of this study was to obtain and characterize natural rubber membranes and to assess their potential as an eye lens capable of altering the passage of light and replacing commonly used eye patches in the treatment of amblyopia. It was analyzed the properties of the polymers with the goal of obtaining data on the feasibility of using the latex membrane in the treatment of amblyopia with safety and comfort for the patient. The light crossing analysis of the M1 and M2 Brushstroke membranes presented partial and total occlusion, respectively. A prototype was built and its analysis was carried out based on biomaterial which already has several applications in tissues with satisfactory results.

**Keywords:** Amblyopia; Biomaterial; Latex; Lazy Eye; Membranes

### Introduction

The human eye is a complex optical system, formed by various transparent media, as well as a physiological system with several components. This system is responsible for most of external stimuli conveyed to the brain, in fact, approximately 85% of human learning happens through vision. The World Health Organization (WHO) estimates that 7.5 million school-age children have some kind of visual impairment. However, only 25% of them present symptoms; thus, the other 75% require specific tests to identify the problem [1].

A very common sight disorder is amblyopia, affecting between 1% to 4% of children under the age of 7 years all over the world [2-4]. The etiology of amblyopia (lazy eye) is the failure to consolidate unilateral or bilateral visual acuity (VA) resulting from lack of stimuli or presence of inappropriate stimuli during a critical period for vision development. It is the most common visual defect in children and its diagnosis and early treatment have satisfactory results [2,5], with a complete cure of the patient after a couple of years of therapy.

The treatment for amblyopia is carried out with the use of optical correction followed by occlusion of the eye with better visual acuity. This is done to allow the development of the "weaker" defective eye, forced to perceive all visual stimuli. Currently, occlusion is carried out with an adhesive eye pad placed on the healthy eye. This traditional form of treatment has been used for over 250 years. The external occlusion has some setbacks, such as i) discomfort due to the adhesive used in the eye patch in a very sensitive skin as the eye lid; ii) removal of the eye patch due social awkwardness; iii) necessity of occlusion for a period of at least 6 hours during the day. However, the method of partial occlusion combined with the latex based lenses can diminish the time of use to less than 6 hours [6] due to the latex characteristics discussed further on this paper; iv) prohibitive cost of the eye patches for some families, leading to a precocious interruption of the treatment [6]. This trend part-time patching of 6 hours has also been observed in Brazil among the ophthalmological community [7]. As an alternative to the conventional eye patch, some researchers have investigated the effectiveness of contact lenses that block vision in the nonamblyopic eye, presenting encouraging results in the treatment of amblyopia in children [8,9]. When compared with the external eye patch, one drawback remains when contact lenses are considered, namely, their high cost.

Looking for a more economic option of amblyopia directed contact lenses, latex, natural resin extracted from the *Hevea brasiliensis* rubber tree, presents itself as a promising candidate. Its properties have long been studied, latex is known to induce neovascularization and tissue regeneration in dogs [10], a high level of biocompatibility [11] and low allergy occurrence once properly prepared [12,13]. Natural latex also has the advantage of cost, resulting in cheap raw material that can be discarded after each use and consequently reducing the risk of pathogen transmission and ocular infections in the patient [14,15].

Latex has long been use in medicine in different pathologies and different organs, such as an esophageal prosthetic to aimed to treat obesity [16] or as a biomembrane used in a post-orbitary exenteration wound by basal cell carcinoma [17].

Given the positive use of latex in biological systems, this study presents a simple approach to eye occlusion based on an ophthalmic membrane derived from natural rubber latex. The natural rub-

ber membranes were produced with two different techniques, the brushstroke technique and the deposition technique and cured at two different temperatures (40°C and 70°C). The different membranes developed were characterized using by X-ray fluorescence (XRF) and diffraction (XRD), water absorption as function of time and light crossing analysis, in order to analyze the properties of the membranes and asses their potential to be used as a contact lens.

## Materials and Methods

### Preparation of the raw material

The latex used to make the membranes is a colloidal suspension of rubber particles and polymeric liquid, dispersed in water and sulfur, which is responsible for latex low viscosity and adhesive properties after vulcanization, a polymerization process common to natural rubber industry.

From the double centrifuged and pre-vulcanized natural latex, a final rubber compound was prepared by the addition of chemicals following the steps described in [18]. This procedure provides the latex membrane essential characteristics for a lens application, such as elasticity, softness, strength, impermeability [19] and hypoallergenicity. All lenses manufactured in this work were registered under the trademark LENCOC®.

### Preparation of the Mold

The development of the technique for obtaining the latex membrane was performed in the Biomaterial Engineering Laboratory – BioEngLab®, at the Faculdade do Gama - UnB. In the brushstroke (or Van Gogh) technique, the developed mold was consisted by plastic material coated with an acrylic layer, measuring 3 cm in width, 3 cm in length, and 1.5 cm in diameter as previously described [18,19]. This mold was designed based on the anatomy and characteristics of the eye, taking a spherical shape of a marble as a model for prototype purposes, based on its size, shape and proportion. The confection of the membrane was completely individualized. Its shape and proportions closely follow the characteristics of the patient's eyes, providing greater comfort.

### Preparation of the membranes

The Brushstroke technique was based on the immersion technique [10], which consists in immersing of the molds into the final latex compound, perpendicular to the plane of the latex in a

liquid form. This step represents the beginning of the vulcanization, which determines the final characteristics of the product. Next, the mold was slowly and gradually removed from the latex compound. The mold was then placed in the incubator and subjected to complete vulcanization at a temperature of 40°C to 70°C. The molds were removed from the incubator and the membranes were prepared using thick brushstrokes by means of a flexible rod with cotton-covered tips, about 1cm away from the mold, with forward and backward movements to completely cover the mold. This procedure was repeated three (sample M1) to four times (sample M2) in succession, followed by drying in an incubator at 40°C for 2 hours, resulting in membranes with thicknesses of approximately 0.2 mm. After this drying period, the brushstroke technique membranes were kept at rest for 24 hours at room temperature to complete the procedure. This procedure of obtaining membranes was named as the Van Gogh technique in honor of the Impressionist painter, Van Gogh.

The deposition membranes were obtained by simply pouring latex on a glass Petri dish following a protocol previously described [15,20,21]. The membrane would be more transparent if dried at room temperature, whilst vulcanization in the incubator usually renders the membrane yellowish and turbid. The preparation of these membranes required about 3 to 4 days for total drying and complete polymerization. The deposition membranes had 8.0 cm diameter and 0.30 mm thickness.

The latex membranes obtained by both techniques were developed using several different protocols to obtain models that maintains the main features-occlusion of light, appropriate thickness, and applicability. The latex membrane will henceforth be identified as brushstroke membranes obtained from three baths (M1) and four baths (M2), both vulcanized at 40°C; the deposition membranes vulcanized at 40°C will be referred to as M3 and those vulcanized at 70°C will be referred to as M4.

Latex has two stages of mass loss. The first extends to approximately 160°C gradually and steadily [16]. The literature shows that this stage is characterized by the loss of water, ammonia and by-products of non-rubber components such as proteins, amino acids, carbohydrates, lipids, nucleic acids [22]. The second step occurs between 240°C and 300°C and it is characterized as the main degradation phase [16]. Therefore, the range of values from 40°C to

70°C was chosen, since in this range important properties will be maintained for the lens and will prevent that they become dehydrated and, eventually, absorbing more water causing problems as will be discussed in the water absorption analysis.

### Characterization of the product

The methods used to characterize the materials were: Energy Dispersive X-ray Fluorescence (EDX), X-ray diffraction (XRD), water absorption analysis and light crossing analysis.

#### Energy dispersive X-ray fluorescence (EDX)

This technique was used to quantify the components of the membranes and to check the variation of the components vulcanized at 40°C and 70°C. The EDX were carried out at the Magnetic and Optical Analysis Laboratory (LAMOp) of the University of the State of Rio Grande do Norte, in a SHIMADZU EDX-7000 Energy Dispersive X-Ray Fluorescence Spectrometer equipment. For the measurements, 2x2 mm pieces of each samples were cut and placed at the spectrometer. The chemical specimen quantification was done by the spectrometer software.

#### X-ray diffraction (XRD)

X-ray diffraction measurements were performed at the Magnetic and Optical Analysis Laboratory (LAMOp) of the University of the State of Rio Grande do Norte, in a RIGAKU - MiniFlex II diffractometer model RV-200B using a Cu tube (1.5405 Å). The diffraction angle varied within the range of 5° < 2θ < 50° at a scan rate of 2°/min.

#### Water absorption analysis

The water absorption analysis was performed to assess the difference in water absorption rates for different preparation protocols. For this analysis, the membranes were cut into 5x5 mm samples which were in turn immersed in saline solution for several minutes. At predetermined periods, the samples were removed from the solution, rapidly dried with a paper towel to remove the excess water from the surface and then weighed at a precision scale with d = 0.1 mg. The chosen weighing times were 15, 30, 60, 105, 165, 255 and 375 minutes.

During the calculations of the mass variation, an instrumental error was added, considering the equipment error, of 0.0001 g, found by the equation 1:

$$\Delta = \frac{\Delta M_f}{M_f} + \frac{M_f}{M_i^2} * \Delta M_i \quad (1)$$

Where  $M_i$  stands for initial mass,  $M_f$  stands for final mass and  $\Delta M_i$  stands for initial mass variation.

A software (Qtiplot®) was used for calculating the time when the membrane stabilized and stopped gaining weight. Firstly, the normalization of the mass of the membranes and a non-linear function were determined considering the instrumental error (Figure 1). The function of time in relation to the variation of weight was calculated by the equation 2:

$$F(t) = A_0 - A_1 e^{-x/t} \quad (2)$$

Where  $A_0$  corresponds to the initial weight and  $A_1$  to the final weight.

Since the experiment is calculated manually, it was made an instrumental error verification using the equation 2 listed above. The graph in Figure 1 related to samples M1, M2 and M3 are quite similar due to their vulcanization at 40°C. However, sample M4, which had the lower water absorption as it vulcanization temperature was higher, followed a different pattern.

**Light crossing analysis**

A focimeter is an optical device that focuses light after transmission by a medium with a certain refraction index. It is ordinarily used to verify the diopter of correction lenses. In this work, a digital focimeter in an optometry clinic (Ótica Cristal) in Goiânia, GO, Brazil, was used. Through this analysis, it was possible to assess the distortion of light as it passes through the membranes. For the potential application in treatment of amblyopia it is important that light passes in such a way that it does not form an image in the good eye, so a good amblyopia lens presents no focimeter signal.

**Results and Discussion**

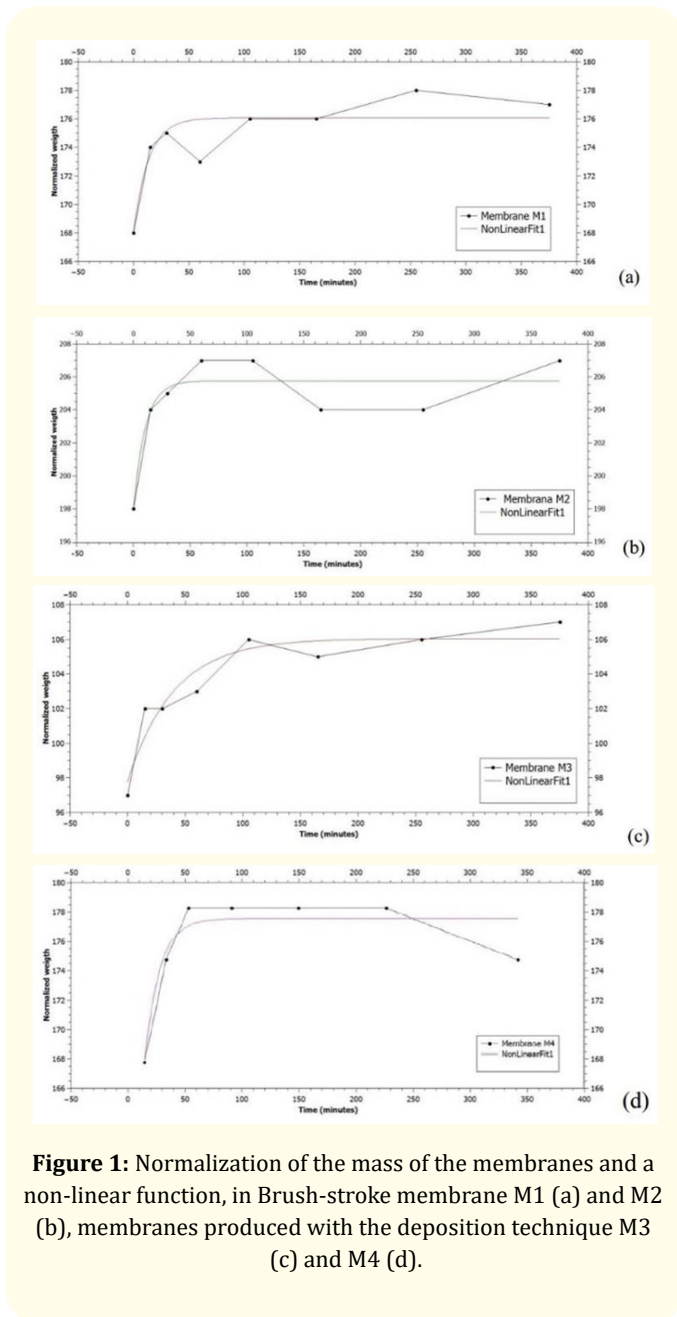
This section will present the results of characterization of latex using the techniques of Fluorescence Measurements, X-ray diffraction (XRD), water absorption analysis and light crossing analysis.

**Energy dispersive X-ray fluorescence (EDX)**

The most commonly found elements in natural rubber in addition to carbon and hydrogen, are phosphorus, sulfur and potassium, since sulfur and potassium are constituent elements of proteins and phospholipids that are present in latex, and potassium is a constituent element of plants. Other elements such as zinc and rubidium are the second most abundant group, and some transition metals are also present in very low amounts (namely copper, iron, manganese and nickel) [23].

Table 1 presents the chemical composition obtained from EDX data of the membranes. High amounts of sulfur and potassium were detected in all membranes and lower quantities of phosphorus, zinc, copper and iron. Other elements with values insignificant were cobalt and gadolinium and manganese, likely due to residual contamination or specific characteristic of the soil where the plants were grown.

Membrane M4 differs of M1, M2 e M3 because of its vulcanization temperature, which promotes a higher molecular mobility resulting in an increase of the amount of potassium and decrease of the amount of sulfur when compared to the other membranes.



**Figure 1:** Normalization of the mass of the membranes and a non-linear function, in Brush-stroke membrane M1 (a) and M2 (b), membranes produced with the deposition technique M3 (c) and M4 (d).

	S	K	P	Zn	Cu	Fe	Others
M1	79.70	16.80	2.20	1.00	0.17	0.08	0.05
M2	70.50	25.60	2.40	1.12	0.15	0.10	0.13
M3	69.70	21.18	1.45	6.36	0.49	0.22	0.60
M4	57.77	32.86	3.78	33.82	0.76	0.23	0.78

**Table 1:** Percentage of each chemical element that composes each membrane determined by quantitative EDX.

Using EDX, we had identified the components in the latex, in the preparation and centrifugation techniques according to the samples of natural rubber membranes, both belonging to isoprene, the main constituent of latex, as well as to the functional groups.

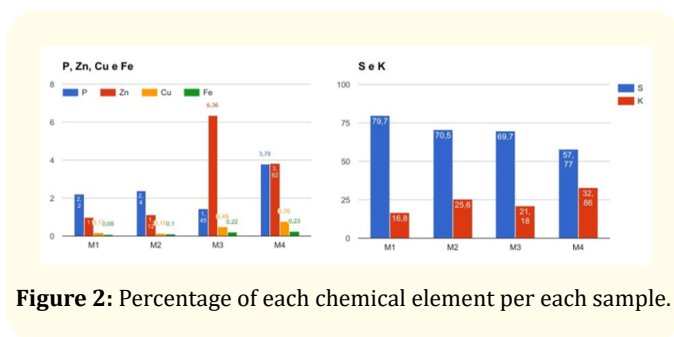
It is possible to see a better solubility of sulfur compared to potassium. Therefore, when the membrane is vulcanized at high temperatures, there is higher molecular mobility; therefore, the amount of sulfur decreases and the amount of potassium increases for M4 membrane. The sum of S and K remains roughly the same at all samples, accounting for more than 90% the total constituent content, aside from carbon. Zinc had an increase in M3 due to the slow vulcanization of the latex compound in the Petri dish.

Other metals, such as copper and iron, did not have significant representation. These minerals will not cause allergy in contact with the eye, since they are already part of the human body and can also be found in food which are mandatory for the function of biological systems.

### X-ray diffraction (XRD)

Figure 2 shows the X-ray diffraction profiles of M1, M2, M3 and M4 and the membranes display a predominantly amorphous character, as expected. The center of the amorphous peak at  $2\theta=19^\circ$  concurs with the expected position of diffraction from isoprene monomer as previously reported in the literature [24-26].

Although the membranes present an amorphous character, a notable difference between the diffractograms of the membranes can be observed depending on the preparation. The profiles of membranes M3 and M4 showed some crystallinity not noticeable in membranes M1 and M2. Notably, increased time involved in preparing the membranes has also increased the chemical crosslinking between isoprene segments, which explains the higher crystallinity shown by the latex membrane prepared using the deposition technique.



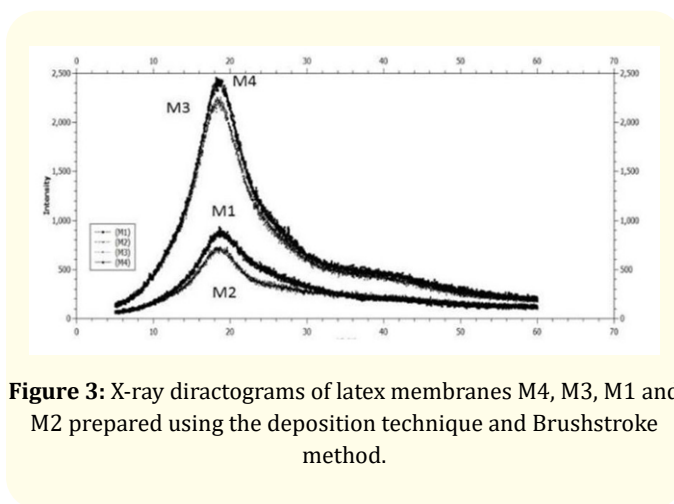
**Figure 2:** Percentage of each chemical element per each sample.

### Water absorption analysis

For a better analysis, the results are shown on Table 2 and Figure 3, which brings the values obtained from the variation of weight of the membranes M1, M2, M3 and M4, and the saturation time.

Sample	Time (minutes)	Weight
Membrane 1 (M1)	14	5.4%
Membrane 2 (M2)	10	4.4%
Membrane 3 (M3)	40	10.2%
Membrane 4 (M4)	10	4.2%

**Table 2:** Absorption times for all latex membranes, indicating the weight gain regarding to the primary weight.



**Figure 3:** X-ray diffractograms of latex membranes M4, M3, M1 and M2 prepared using the deposition technique and Brushstroke method.

In this analysis, we observed that M3 absorbed more water in a longer period and, consequently, gained more weight. The longer the period for saturation implies in a longer period for the system to reach equilibrium on the cornea surface. M2 and M4 gained less

weight in less time absorbing little water, which is desirable in contact lenses [27]. The variation in water absorption and saturation times of all membranes can be associated with the fabrication procedure. M3 extreme difference when compared to the other samples is associated with its thickness, possibly a critical thickness for water loss in ambient conditions, M3 was drier than the other samples and therefore absorbed more water and the process took longer time. Overall, the lenses manufactured showed good water absorption and saturation times, considering only 5% of the samples weight was increased due to water absorption. Considering a regular lens weight of about 20 mg, this results in 1 mg of water, over ten times less than a water drop (One water drop is equivalent to 0.05 mL, water's density is equal to 1 g/ml or 1000 mg/mL, thus 1 mg into 0.001 mL is 50 times smaller than a water drop). This absorption is complete in a very reasonable amount of time, ten minutes, showing it is a slow process and likely to remain stabilized throughout the day.

**Light crossing analysis**

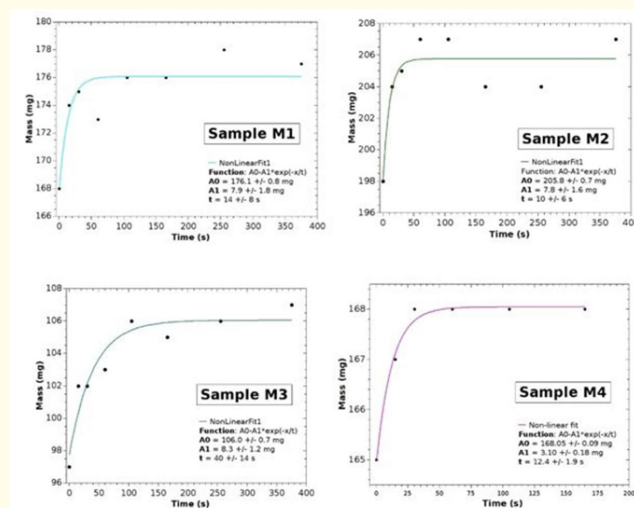
The focimeter used probes the spherical correction ("S"), the cylinder correction of the lens ("C"), which represent the degree of the lens. It also probes the lens' angle "A" and Δ, the variance of the light beam. For ordinary corrective lenses, all four values can be assessed with the focimeter. The control lens, an ordinary contact lens, the values found were S = 0.25, C = 0.25, A = 24 and θ = 0, representing the two values for diopters and two values for the axis. Once the latex membranes replaced the lenses, the numerical value of the instrument was nonsensical since such values cannot be assessed in latex - as is desirable for a lens dedicated to the treatment of amblyopia.

In the focimeter device, we observed the measurement of the light beam on the latex membrane as shown in Figure 4. In this test, five samples of each membrane were tested to verify their reproducibility. Table 3 presents these results.

Membrane M1 allows a slight focusing of light, indicating relatively small transparency. Due to the irregular surface caused by the brushstrokes, the equipment is unable to determine the four optical characteristics probed. Consequently, the brushstroke technique rendered the surface rough and thus resulted in diffuse scattering.

Sample	S (deg)	S (deg)	A (axis)	
M1	+0.00	+0.00	0	0.00
M2	Measurement error			
M3	+0.25	-0.50	75	0.75
M4	+0.50	-1.00	107	0.75

**Table 3:** Results of the membrane's measurement with focimeter.



**Figure 4:** Normalization of the mass of the membranes and a non-linear function taking into account the instrumental error.

As for M2 series, no measurements were possible with the focimeter, indicating an even more irregular surface and thicker samples, resulting in larger optical path and such diffuse scattering that no measurement was possible. This data prove that membrane is opaque, which is a characteristic of occlusion. Membranes M3 and M4 were successfully measured with the focimeter, and the surface was not so irregular. The focimeter performed the reading through the optical center, measuring the values, with no errors and total passage of light.

**Conclusion**

In this study, a complex system was elaborated to achieve the final product: a latex occlusive membrane for the treatment of amblyopia. Interfering factors were eliminated and several techniques were used to reinforce its applicability and viability.

In the technical test, it was used natural latex as a biomaterial for its qualitative properties, such as: impermeability, elasticity, smoothness, flexibility, and mechanical resistance.

To get to the final product, several vulcanization techniques were tested aiming a transparent membrane, with low thickness, low roughness and minimal water absorption. This process allowed an occlusion more biologically compatible with the eye. These results were significant, since the characteristics of the latex membrane enable an interaction with the eye without changing its properties.

During the characterization process, two distinct manufacturing techniques of membranes have been used at different curing temperatures, namely the brushstroke method or deposition technique. All samples have been tested using different physical and chemical analysis of the biomaterials. In Figure 5, we can observe the membranes developed with their morphological aspect.



**Figure 5:** Membranes prepared using the brushstroke and deposition techniques, in (a) M1 - brushstroke, (b) M2 - brushstroke, (c) M3 - deposition (40°C), (d) M4 - deposition (70°C).

EDX showed that the main components of the latex were preserved after vulcanization so that it maintains its ordinary characteristics as biomaterial, used in different biological applications, indicating the membranes huge potential as a lens for the treatment of amblyopia.

The performance of this material is directly related with the characterization of the biomaterial in light refraction, not fully eliminated the light and also maintaining the characteristics of the eye movement due the light incision. Since it is considered to be more comfortable and aesthetic than the external patch, and also allows complete motion of the eyeball and partial transmission of light, the latex lens is a strong candidate to replace the eyepatch in the treatment of amblyopia. Furthermore, its removal is not as easy, thus reducing the treatment period by insuring continuous use for the required time.

During the occlude manufacture, eye comfort aimed characteristics were considered, such as size, shape and homogeneity, since a sudden change in the eye may cause discomfort. In direct contact with the eye, the latex membrane consequently will be supporting structure that interacts with the organ mechanically and chemically, providing a biomaterial-tissue interface response. It is important, then, to study the behavior of eye in contact to the occlusive membrane, as subject of future research.

Thus, the brushstroke technique can be considered as viable for the manufacturing of latex membranes used for treating amblyopia. The passage of light was diffuse due to the rugosity and opacity, which will provide partial or total light occlusion in the eye of the patient, with low water absorption, light and malleable material. M1 membrane allowed the passage of diffuse light, causing partial occlusion, preserving stimuli to the eye due to the presence of light; while membrane M2 is opaque, thin and has low water absorption; so, it can be used to treat amblyopia, replacing the eyepatch with more comfort and providing a shorter treatment time.

The membranes were then produced and patented under registry BR 102012007483-4 on April 2012 with the name Latex-derived occluder contact lens, already published. Afterwards, the LENCOC® brand was registered under the number 906573572 for future commercialization.

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## Conflict of Interest

The authors declare there were no conflicts of interest.

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