Colloids and Surfaces B: Biointerfaces 107 (2013) 124–129

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

An intraocular dye solution based on lutein and zeaxanthin in a surrogate internal limiting membrane model: A Langmuir monolayer study

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ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form 30 January 2013
Accepted 31 January 2013
Available online 10 February 2013

Keywords:
Lutein
Zeaxanthin
Internal limiting membrane
Chromovitrectomy
Retina
Langmuir films

ABSTRACT

Investigating the role of biomolecules and bioactive molecules associated with membranes is fundamental to comprehend at the molecular point-of-view biochemical and clinical processes that occur at biointerfaces. In this paper we exploit the interaction of an intraocular dye solution based on lutein and zeaxanthin in surrogate internal limiting membrane (ILM) models, consisting of dipalmitoyphosphatidylcholine (DPPC) Langmuir monolayers, pure or mixed with collagen, proteoglycan and laminin. The interactions between the film components occurring at the air–water interface were investigated with surface pressure–area isotherms and polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS). A natural dye solution based on lutein and zeaxanthin, employed to label ILM in ophthalmic surgery, was incorporated in the ILM model, and the data suggested non-rupture of the structure of the membrane, with predominance of interactions based on intermolecular forces.

1. Introduction

In ophthalmology, vital dyes have been used for a long time as a diagnostic tool for surgeons to better visualize the semitransparent intraocular membranes and tissues, such as the internal limiting membrane (ILM) [1,2].

Vitreoretinal surgery involves complex and delicate vitreoretinal techniques for the management of diseases, such as macular hole, epiretinal membrane (ERM), or diabetic macular edema [3]. A special surgical technique, recently termed chromovitrectomy, consists on the use of vital dyes or crystals to improve the visualization of intraocular tissues during vitrectomy, thereby improving specific procedures such as anterior capsule (AC) and complete vitreous (V) removal, as well as the surgical outcome [4]. By staining the intraocular membranes, vital dyes make an otherwise optically transparent structure easier to identify and remove [5].

The ideal macular vital dyes should have the following characteristics: (i) safety profile for intraocular use; (ii) ability to reliably and selectively stain the intraocular membranes and specific intraocular microstructures; and (iii) fast elimination from the eye [6]. There are many dyes available for chromovitrectomy, such as Brilliant Blue (BB), Indocyanine Green (ICG) and Infracyanine Green [7]. However, all of them are synthetic and present some degree of toxicity that may limit their use in ocular surgery. Recent publications have shown affinity between BB and the ILM [8,9], and it is nowadays the most used dye worldwide. However, some cases of presumed toxicity after intraoperative use of BB have been recently reported and all of them are dose-dependent [10]. It is known that ILM removal is an important procedure to improve the results in macular hole surgery [11]. ILM peeling guided by ICG staining is also a surgical technique worldwide performed. However, it is known that retinal pigment epithelium (RPE) atrophy may occur after ILM peeling guided by this dye, which may be partly explained by the nature of the interaction with which ICG presents with ILM as well as by the toxicity profile of ICG molecule components, such as the iodine and carbolinic complex. Additionally, the final osmolarity of the solution may be involved in this toxicity profile [11,12]. Different research lines have been in place to find a dye with optimal safety and efficacy in staining intraocular membranes and structures [4,13].

Lutein and zeaxanthin (L/Z) are lipophilic pigments belonging to the group of carotenoids traditionally found in fruit and vegetables [14,15]. These two carotenoids are also physiologically present in the macula lutea, an integral part of the retina in humans [16]. Additionally, they are structural isomers with a hydroxyl group at the

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http://dx.doi.org/10.1016/j.colsurfb.2013.01.076

terminal part of the molecule, which in part explain their different polarity and tropism for certain biological structures, such as the macula [17–20]. Both are considered dyes and are associated with the prevention of age related maculopathies, due to its antioxidant effect and their exclusive distribution in the macula [16,21,22].

Published peer-reviewed studies have reported association of L/Z with prevention of age-related maculopathies due to an antioxidant and blue light filtering mechanisms [22–25]. This potential ability to prevent progress of macular degeneration may be explained by its exclusive distribution in the macula [26]. Recently, it was published that L/Z-based dyes, alone or combined with BB, could also be used with efficacy as intraocular dyes to stain different membranes and microstructures in ophthalmic surgery, including ILM peeling [27].

The nature of the interaction of an L/Z-based dye is herein investigated using a Langmuir monolayer model. This methodology is justified since monomolecular films at the air–water interface are widely recognized to be systems able to mimic biological membranes and other biological surfaces [28–32].

The hypothesis is that L/Z could interact with the components of ILM that would explain its staining efficacy as well as safety associated with its physiological role in macula lutea and its antioxidant effect. Furthermore, information on dye-membrane interaction can be reached at the molecular level by using Langmuir monolayers. Techniques such as vibrational spectroscopy and surface tensiometry are able to bring information on specific interactions and may be useful to evaluate the action of L/Z on ILM models [33].

This present study aims to create an experimental model of human ILM using biointerfaces that may be helpful to analyze the mechanisms that dyes stain the ILM as well as to evaluate the intermolecular interactions of the L/Z-based dye in such experimental model.

2. Methods

For all monolayers, dipalmitoyphosphatidylcholine™ (DPPC) (Sigma–Aldrich, USA) was employed as lipid model. Surface pressure (π)–area (A) (π–A) isotherms were obtained through a mini Langmuir trough (KSV Instruments Ltd., Finland), equipped with a surface pressure sensor based on the Wilhelmy method. Movable barriers that sweep the air–water interface with a rate of 5 Å^2 molecule^{-1} min^{-1} were employed to compress the monolayers. Initially, the Langmuir trough was filled with a buffer solution (phosphate 1.0 mM, NaCl 100 mM, pH 7.2), and then a DPPC solution at a 0.5 mg/mL concentration was carefully spread on the air–water interface drop-by-drop. After 20 min elapsed for chloroform evaporation, compression of the monolayer was then carried out, and the surface pressure (π) – defined as γ_0–γ, being γ_0 and γ the surface tension of the subphase without and with the covering of the monolayer, respectively, was followed as long as the average molecular area (A) of DPPC decreased. The π–A curves were obtained at least three times to test the reproducibility of the experiments.

Other biological components present in ILM were also evaluated in this study, and several combinations of DPPC with a second component were carried out in order to better investigate the role of each one. For that, aliquots of 5 μL of a 0.5 mg/mL solution of each component were co-spread with DPPC. The following components were tested: (i) collagen type IV from human placenta, dissolved in 0.25% acetic acid, phosphate 0.1 mM, NaCl 100 mM, pH 7.2; (ii) laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane, dissolved in phosphate 1.0 mM, NaCl 100 mM, pH 7.2; and (iii) proteoglycan from bovine nasal septum, dissolved in phosphate 1.0 mM, NaCl 100 mM, pH 7.2. All these materials were purchased from Sigma–Aldrich (USA). After that, all the related components were mixed together and co-spread with DPPC in order to observe the overall effect of the dye on the ILM model.

The Retidyne-Plus™ (Kemin Pharma, USA) dye, an L/Z-based dye associated with BB (0.3:0.025) for intraocular use in vitreoretinal surgery, was then inserted in the films, with all possible combinations, in aliquots of 5 μL. After dye incorporation in the monolayer, the measurements were performed only after 30 min in order to allow stabilization of the film in terms of lateral diffusion and homogenization.

Additionally, polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) measurements were carried out with a KSV PMI 550™ (KSV Instruments Ltd., Finland). The Langmuir trough was set up so that the light beam was able to reach the monolayer at a fixed incidence angle of 80°. At this angle, the light beam intensity was the maximum and the noise level was the lowest. The incoming light was continuously modulated between s- and p-polarization at a high frequency, which allowed for the simultaneous measurement of the spectrum for the two polarizations. The difference between the spectrum provided surface-specific information, and the sum provided the reference spectrum; using the simultaneous measurements, the effect of water vapor was largely reduced [34].

All the experiments were carried out at a controlled room temperature (25.0 ± 0.1 °C). All the other reagents were the highest purity available. Water employed was previously purified with Milli-Q® system, with resistivity of 18.2 MΩ cm and pH of 5.4.

3. Results and discussion

3.1. Analysis of each components in DPPC monolayers

First of all, it is important to say that the behavior of xanthophylls on the air–water interface is previously reported [35]. In this present paper, however, a specific mixture of two xanthophylls with Brilliant Blue is employed as dye. Obviously, this mixture will have different properties from that for pure xanthophylls. Also, it is important to emphasize that, when spread alone on the air–water interface, this dye does not form regular Langmuir monolayers, forming visible aggregates with no reliable surface pressure–area isotherms. Reproducible results are obtained only when the dye is mixed with pure DPPC or with DPPC with the other proteins employed in this work. This effect may be therefore attributed to a cooperative effect of the dye with the lipid monolayer, even at high DPPC molecular areas, indicating high molecular affinity.

The effect of each substance on DPPC monolayers as well as the π–A isotherms for pure DPPC and for its binary mixture are shown (Fig. 1). At the highest DPPC molecular area achieved by the experimental conditions (i.e. 126 Å^2/molecule), only collagen was not able to increase the surface pressure of pure DPPC. An increase of the values was observed from 1.8 mN/m to 4.8 mN/m (after proteoglycan addition) and to 3.5 mN/m (with laminin addition) as a consequence of the molecular adsorption.

With the presence of these three substances, the isotherms present a decrease of the surface pressure values corresponding to the plateau that characterizes the transition between the liquid-expanded and the liquid-condensed phases. This is a consequence of the incorporation of each biomolecule in the lipid monolayer, which reduces the energy necessary to provide the phase transition. This phenomenon is already described in the literature for other components [36–38] and is associated to the lower repulsion between polar heads, which is therefore induced by favorable interactions with other components coming from aqueous subphase.

In lower molecular areas, with the presence of collagen and proteoglycan, a shift of the isotherms to higher molecular areas is