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Interaction of chlorhexidine with biomembrane models on glass ionomer by using the Langmuir–Blodgett technique

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ABSTRACT

The antimicrobial property of chlorhexidine is believed to be associated with its interaction of bacterium membrane, which calls for research on the identification of membranes sites capable of drug binding. In this study, we investigated the interaction of chlorhexidine digluconate, a known agent with bactericidal and bacteriostatic activities employed in the treatment of periodontal diseases, with bacteria cell model systems by using Langmuir monolayers. The insertion of the drug caused the surface pressure–area isotherms for a mixed protein–lipid monolayer to be shifted to higher lipid molecular areas, which was the first indication of the action of chlorhexidine in the membrane model. Surface infrared spectroscopy pointed to intrinsic interactions of the drug with the hydrophobic part of the lipid, leading to a disruption of the lipid organization at the interface. Also, the secondary structure of the polypeptide model employed in this work has been changed, as a consequence of the drug interactions. Such change in the lipid–protein models could be confirmed when the membrane was transferred to glass ionomer cement as a solid support, which can be considered a model for dental surfaces. Therefore, chlorhexidine interacts with lipid and protein moieties supposed to be present in lipid membranes. This may have important implication in understanding how the drug acts on specific sites of the bacteria membrane.

1. Introduction

Mechanical removal of dental biofilm is the key to prevent dental disease such as caries, gingivits and periodontitis [1,2]. However, despite the emphasis on mechanical methods of plaque control, the incidence of gingival inflammation is high. For this reason other oral hygiene agents such as mouthrinses with anti-plaque properties may have clinical value not only in post surgical situations, but also for handicapped and elderly patients, who are less able to perform adequate oral hygiene procedures [3].

Chlorhexidine digluconate is an antimicrobial agent employed by dentists and is known to be an effective anti-plaque and anti-inflamatory agent. This action does not involve inactivation of the enzyme ATPAse, as thought previously, but is related to interactions with membrane of microorganisms, avoiding microbial proliferation [2,4].

The benefits of chlorhexidine, a cationic biguanide, are based on its high intra-oral substantivity. In addition, this drug exhibits a large spectrum of antibacterial activities that target both Gram-negative and Gram-positive bacteria. However, some local adverse effects, including staining of the teeth, restorations and dentures and temporary taste disturbances are related [4].

Because of its toxicity in human beings in higher concentrations, its safe use as mouthwash solutions involves the water soluble chlorhexidine digluconate at very low concentration (0.12%, w/v). It is also defended by dental industries that the products may be employed to prevent gingival diseases.

In this sense, it is interesting to employ biomembrane models in order to understand at the molecular level the mechanism of action involving the interaction between the antimicrobial agent and the membrane components. Regarding some existing models, lipids monolayers formed at the air–water interface (Langmuir films) have been performed as a functional system to mimic half a membrane and to investigate in detail interactions between biomolecules [5–8]. It has been reported in the literature the interaction between proteins [9–12], polysaccharides [13,14], and drugs [15–17] with lipid monolayers investigated mainly with tensiometry associated to other relevant techniques, such as spectroscopic and microscopic ones. This film, with monomolecular thickness, can be transferred from the liquid interface to solid supports by vertical deposition, originating the so-called Langmuir–Blodgett (LB) films [18]. For this purpose, several kinds of solid substrates can be employed. For dental applications, it is of interest to employ matrices that mimic dental or restorative dental surfaces, such as

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glass ionomer cements (GIC). Glass ionomer cement is a restorative dental material widely used by clinics in treatment and prevention of caries disease. Albeit the extensive study in dental research about the ability of chlorhexidine to cure gum and dental diseases associated to proliferation of bacteria, no report on the molecular mechanism of action on dental surfaces has been previously reported to the best of our knowledge. In this sense, the use of ultrathin films, whose properties and process can be investigated at the molecular level, seems to be scientifically appealing.

In this present work, we studied the interaction of chlorhexidine digluconate with Langmuir and Langmuir–Blodgett films constructed out of phospholipids and proteins, mimicking the microbial membrane. For that, dipalmitoylphosphatidylcholine (DPPC) and glucose oxidase (GOx) forming a hybrid monolayer as a simple membrane model was used. Chlorhexidine digluconate (CHX) aqueous solution was employed as subphase. Interaction of the antimicrobial agent with the monolayer was investigated with surface pressure, and Polarization Modulation Infrared Reflection Adsorption Spectroscopy (PM-IRRAS), which is a vibration spectroscopy technique able to analyze changes in chemical groups at surfaces. The lipid–protein hybrid monolayer was transferred to the GIC substrate, mimicking a dental surface covered with microorganisms. The novelty of this paper lies in the fact that monolayers of CHX were investigated only in the 1970s [19] and up to now, to the best of our knowledge, no additional paper was reported in the literature on this issue. Moreover, this paper is the first report on the CHX acting on glass ionomers covered with bacteria cell membrane models.

2. Material and methods

Dipalmitoylphosphatidylcholine (DPPC,) purchased from Sigma–Aldrich, was dissolved in chloroform (Synth) to render a concentration of 0.5 mg/mL. Langmuir monolayers were formed spreading DPPC solutions on the surface of pH 7.3, 0.01 mol/L phosphate buffer. The water employed was previously purified by a MilliQ-Plus System (resistivity 18.2 MΩ cm, pH 5.5). Glass ionomer cement was prepared according to manufacturer instructions and placed in a plastic mold in order to obtain a solid support after 24 h. Surface pressure–area (π–A) isotherms were obtained by means of a mini-KSV Langmuir trough, equipped with a surface pressure sensor (Wilhelmy method), and movable barriers that compress the air–water interface with a speed of 5×10^{17} Å^2 min^{-1}. Initially, the Langmuir trough was filled with the buffer solution and then a DPPC solution was carefully spread, drop-by-drop, on the air–water interface. After 20 min allowed for chloroform evaporation, glucose oxidase (Sigma–Aldrich) was injected in the subphase to render a final concentration of 1 μg/mL and allowed for stabilization for 1 h. This protein was used as simple model to understand the action of CHX (aqueous solution 0.12%, w/v) on polypeptide structures existing in bacteria membranes. Compression of the monolayer was then carried out, and the surface pressure (π), defined as γ_o–γ, being γ_o 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, and the results were practically coincident. For clarity, only one curve is shown.

PM-IRRAS measurements were taken with a KSV PMI 550 instrument (KSV instrument Ltd, Helsinki, Finland). The Langmuir trough is set up so that the light beam reaches the monolayer at a fixed incidence angle of 80°. At this angle, the intensity is maximum and the noise level is the lowest. The incoming light is continuously modulated between s- and p- polarization at a high frequency, which allows for the simultaneous measurement of the spectra for the two

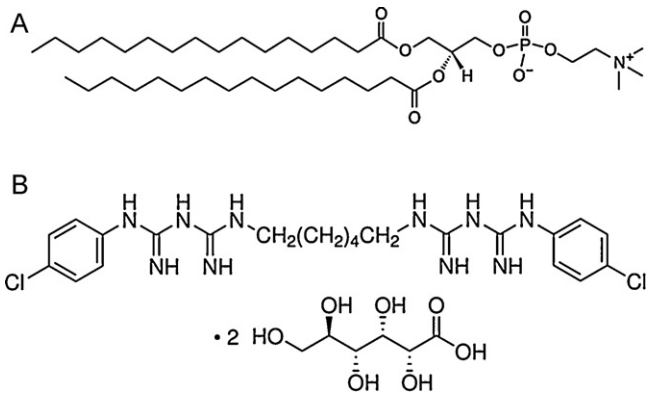

Fig. 1. Chemical structures of DPPC (A) and CHX (B).

polarizations. The difference between the spectra provides surface-specific information, and the sum provides the reference spectrum. With the simultaneous measurements, the effect of water vapor is largely reduced. All the experiments were carried out at a controlled room temperature (25 °C). Fig. 1 shows a scheme of chemical structures of DPPC (A) and CHX (B).

3. Results and discussion

Initially, it is important to emphasize that the reason for the choice of DPPC to analyze CHX acting on a cell membrane model was the relative amount of lipids with PC heads on several cells [20–22], including those ones forming bacteria membranes [23,24]. Also, DPPC has been employed as a lipid model for preliminary studies involving simple models for diverse kinds of cell membranes [25,26]. In the attempt of improving our model, and aiming to investigate the role of CHX not only in lipid structuring, but also in polypeptide moieties present in biomembranes, the enzyme glucose oxidase was additionally employed as protein model in this study. This protein was firstly chosen because of the availability of data in Langmuir monolayers. This present work at first tried to be a proof of concept on how the drug may interact with hybrid monolayers of lipids and proteins. Further, it is intended to extend the work to more complex models of cell membranes.

Fig. 2 shows the action of CHX on protein–DPPC monolayers. First of all, it is important to emphasize that CHX was spread at the buffer–air interface (in the absence of DPPC and protein), and compressed, and with the quantity employed in this work, the surface pressure did not increase more than 1 mN/m,

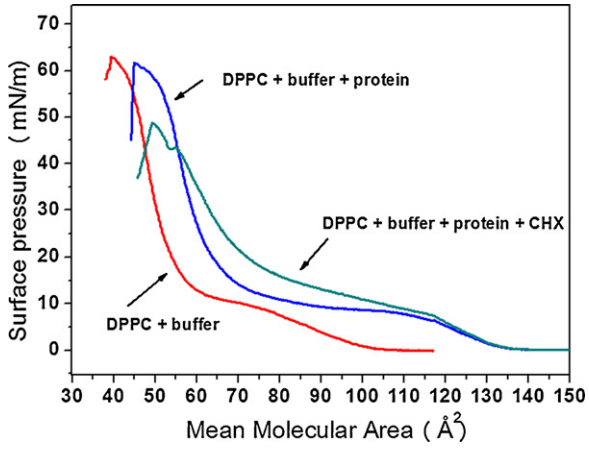

Fig. 2. Surface pressure–area isotherm for DPPC on different subphase conditions (as indicated in the inset). Protein concentration was 1 μg/mL, and CHX 0.12% (w/v).