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Optimal design for studying mucoadhesive polymers interaction with gastric mucin using a quartz crystal microbalance with dissipation (QCM-D): Comparison of two different mucin origins

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ABSTRACT

The objective of this present study was to develop an efficient and simple method, based on the use of a quartz crystal microbalance with dissipation (QCM-D), to evaluate the mucoadhesive characteristics of cationic polymers; chitosan, thiolated chitosan (chitosan-SH), and polyallylamine hydrochloride (PAH), and anionic polymers; hyaluronic acid (HA) and thiolated hyaluronic acid (HA-SH). The experiments were carried out at pH 4 to assess the interaction between mucoadhesive polymers and a mucin-coated gold surface. A key point in the QCM-D protocol development was to evaluate two sources of mucin: native porcine gastric mucin (NPGM) and commercially available porcine gastric mucin (CPGM). QCM-D has shown its potential as a highly sensitive technique that provides information about the interaction of mucoadhesive polymers with gastric mucin. The technique would allow the classification of these polymers in order to further assess their application as base materials for nanocarriers, designed to interact with the mucosal barrier which represents a stumbling block for drug adsorption.

1. Introduction

Mucus is a viscoelastic and tenacious gel barrier that covers and protects the body by quickly trapping foreign substances, including macromolecular drugs, via steric and adhesive forces [1]. Most of mucoadhesive bonds are attained by either physical or secondary chemical bonds, the predominant interaction being hydrogen bonding. Consequently, several functional groups such as hydroxyl, carboxyl and other hydrogen bond-forming functional groups can contribute to mucoadhesion [2]. The concept of mucoadhesive polymers was introduced in pharmaceutical technology in the early 1980s and recently it has been accepted as a promising strategy to prolong the residence time of polymers used in drug formulations on the mucosal membranes in the body, including the gastrointestinal (GI) track [3]. The development of novel mucoadhesive drug delivery systems has been of great interest as drug carriers capable of adhering to the mucus layer and may help the trapped particles avoid removing by mucus clearance mechanism [4,5].

Mucus is composed mainly of water (up to 95% weight) lipids, inorganic salts, and glycoproteins called mucins [6]. Mucins consist of large macromolecular monomers with a protein backbone with one or more heavily glycosylated domains, rich in serine and threonine residues which serve as anchoring points for the oligosaccharide side chains [7,8]. The oligosaccharide chains have sugar residues such as galactose, fructose, N-acetylglucosamine, N-acetylgalactosamine and sialic acid. At pH > 3, both sialic acid and sulphated sugars are fully ionized and this confers a net negative charge to the molecules [1]. Mucins are responsible for viscoelastic gel-like properties of mucus [7]. Due to their importance the relevance for mucoadhesion, adsorption of mucins has been considerably studied to a variety of surfaces and by applying different techniques [9]. Here, we focused on the methods and techniques *in vitro* to evaluate the interaction between the mucoadhesive properties of polymers and the mucin layer.

Commercially available porcine gastric mucin (CPGM) has been widely used for mucus-relevant investigation because it is simple and inexpensive to purchase. However, Kocevar-Nard et al. [10] compared the rheological properties of CPGM obtained from Sigma Aldrich (Type II) and isolated native porcine gastric mucus. It was demonstrated that the usage of rehydrated CPGM for the preparation of *in vitro* membranes is limited because CPGM is

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isolated after enzymatic hydrolysis treatment that can affect its primary structure. After this study, several groups have reported the differences in the gel behaviour (rheology) within native gastric mucus and mucin [11–13]. In this study we would like to evaluate how the differences in structure can affect the ability of the different mucins to interact with the described mucoadhesive polymers.

A quartz crystal microbalance with dissipation (QCM-D) is a high sensitive and versatile instrument for real-time study of the dynamic behaviours of a layer on the crystal surface [14–17]. QCM-D technique provides information of both the mass and structural changes occurring to the layer by simultaneous measurements of the frequency (f) and dissipation factor (D) [18]. From these changes it is possible to calculate the mass/thickness of the adsorbed layer and also its viscoelastic properties [19]. Therefore, QCM-D is a powerful technique to elucidate interactions or reaction on various surfaces.

Chayed and Winnik [3] studied the interaction between mucin (bovine submaxillary mucin, a sigma preparation that is unlikely to be native) and mucoadhesive polymer-based nanoparticles by means of QCM-D, and demonstrated that QCM-D was a promising technique for studying the mucoadhesive properties of biopolymers. Recently, Pedersen et al. [20] showed QCM-D can be used as a screening method of biodurability of toxic nanoparticles. They suggested that the interaction of nanoparticles with CPGM could be a good model to understand the impact of nanoparticles in the body. However, as far as we know, all the experiments described in the literature have been carried out using CPMG, and no comparison with mucins with different origins has been reported yet.

The objective of the present study was to develop and evaluate an *in vitro* method based on the QCM-D technique to assess the mucoadhesive properties of various polymers; chitosan, thiolated chitosan (chitosan-SH), and polyallylamine hydrochloride (PAH), and anionic polymers; hyaluronic acid (HA) and thiolated hyaluronic acid (HA-SH). Positively charged chitosan polymer forms polyelectrolyte complexes with negative charged mucins, whereas negatively charged HA polymers have mucoadhesive properties due to hydrogen bonding with the mucus layer. Both chitosan and HA exhibit excellent mucoadhesive properties [21–23]. Recently, it has been shown that polymers with thiol groups provide much higher adhesive properties [24–26], resulting from covalent bonds with cysteine-rich subdomains of mucins, than polymers generally considered to be mucoadhesive. PAH composed of a large number of primary amine groups and can also improve mucoadhesion [27] via electrostatic interactions between negative charges on the mucin and positively charged amino groups on the polymer.

In our study, two types of mucin have been used: NPGM and CPGM. We will compare the mechanism of the interaction between polymers and either NPGM or CPGM layers. Thus, it is proposed to use the QCM-D protocol developed to study the behaviour of the different mucins and their interaction with the different polymers analysed. In this study, the results show the ionic interaction of charged polymers with the negative charged mucins as well as the permeation of the polymers through the mucins, which can be characterized by measuring at multiple frequencies and applying a viscoelastic model (e.g. the so-called Voigt model) incorporated in Q-Sense software QTools.

2. Materials and methods

2.1. Sample preparation

Commercially available porcine gastric mucin (CPGM, Type III) containing 0.5–1.5% bound sialic acid was purchased from Sigma Aldrich. All chemicals were purchased from either Sigma Aldrich or Panreac and used as received, unless otherwise mentioned. All reagents were of analytical grade and used without further purification. The procedure of purification of native porcine gastric mucin (NPGM) was described in the previous paper in detail [28]. Briefly, the mucus gel, from freshly slaughtered pig stomach, was scraped from the mucosal surface of the gastric fundus using a glass microscope slide. The mucin was purified by equilibrium density gradient centrifugation in CsCl (1.42 g/mL starting density). The final NPGM sample has been freeze-dried and kept at −20 °C until use. Two buffer solutions were prepared depending on the type of mucin used: For NPGM, the citric acid/phosphate buffer was prepared by mixing a solution of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate. NPGM was dissolved in 150 mM buffer with gentle stirring at least for 1 h at room temperature. For CPGM, succinate/phosphate buffer was obtained from a mixture solution of 0.01 M H_3PO_4/succinic acid and 0.01 M Na_3PO_4/sodium succinate dibasic. CPGM was dissolved in 30 mM NaCl buffer and left under stirring for 1 h at room temperature. A buffer solution was prepared to optimal isoionic conditions with mucin at appropriated concentrations of NaCl [20,29]. The polymers used in this study were supplied by Kainz, B. of Croma GmbH (Austria).

2.2. QCM-D

The QCM technique is well suited for measuring adsorption of mass on a thin quartz sensor surface, which induces a decrease in f which for a rigid substance translates into the increase in mass according to the Sauerbrey relation [30]. However, in the case of a non-rigid or viscoelastic film like mucins, the Sauerbrey relation is not valid and thus we developed the QCM technique using the D factor (dimensionless unit, 1E−6), of the oscillator (the "damping"), and the comparable Q factor [31–33] to fully characterize the viscoelastic properties of the adsorbed layers. The QCM-D technique is able to simultaneously detect changes in f and D of quartz crystal (that can be coated with different materials) produced when the materials are adsorbed onto it [34–37]. These changes provide the mass/thickness of the adsorbed layer and its viscoelastic properties [32,35]. Moreover, sequential multi-frequency measurement (the frequencies corresponding to the harmonic frequencies of the crystal) is carried out in order to record the different overtones of the oscillating system. It is important to point out that each overtone has a specific penetration depth, and they are measuring the behaviour at different depths of the attached film [38]. The sensoŕs overtones have higher f, so the penetration depth (sensitivity range) will be smaller. This means that, as measurements are at different depths of the deposited film, they can be used to record the film response in several points at the same time. This characteristic has been used to study how the polymer interacts with the mucin film. If the polymers are only absorbed on the mucin film surface, the overtones response would be different, however a penetration that affects the whole mucin would lead to similar overtones behaviour.

2.3. Preparation QCM-D sensor

Gold-coated QCM-D sensors were cleaned prior to use or re-use by immersion in 1:1:5 mixture of H_2O_2 (30%), NH_3 (25%) and Milli-Q water for 10 min at 75 °C, followed by rinsing with Milli-Q water, dried with nitrogen, rinsing with ethanol ($\geqslant$99%), and dried with nitrogen. This cleaning procedure was repeated at least three times. The procedure followed a standard of protocol supplied by Q-Sense.

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