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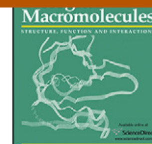

Galactomannan thin films as supports for the immobilization of Concanavalin A and/or dengue viruses

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ABSTRACT

The immobilization of the glucose/mannose-binding lectin from *Concanavalia ensiformis* seeds (ConA) onto a monolayer made of a galactomannan extracted from *Leucaena leucocephala* seeds (GML), which was adsorbed onto – amino-terminated surfaces, was investigated by means of ellipsometry and atomic force microscopy. The mean thickness of GML monolayer, which polysaccharide consists of linear $1 \rightarrow 4$-linked β-D-mannopyranosil units partially substituted at C-6 by α-D-galactopyranosyl units, amounted to (1.5 ± 0.2) nm. ConA molecules adsorbed onto GML surfaces forming (2.0 ± 0.5) nm thick layers. However, in the presence of mannose the adsorption failed, indicating that ConA binding sites were blocked by mannose and were no longer available for mannose units present in the GML backbone. The GML film was also used as support for the adsorption of three serotypes of dengue virus particles (DENV-1, DENV-2 and DENV-3), where DENV-2 formed the thickest film (4 ± 2) nm. The adsorbed layer of DENV-2 onto ConA-covered GML surfaces presented mean thickness values similar to that determined for DENV-2 onto bare GML surfaces. The addition of free mannose units prevented DENV-2 adsorption onto ConA-covered GML films by ~50%, suggesting competition between virus and mannose for ConA binding sites. This finding suggests that if ConA is also adsorbed to GML surface and its binding site is blocked by free mannose, virus particles are able to recognized GML mannose unities substituted by galactose. Interactions between polysaccharides thin films, proteins, and viruses are of great relevance since they can provide basis for the development of biotechnological devices. These results indicate that GML is a potential polysaccharide for biomaterials development, as those could involve interactions between ConA in immune system and viruses.

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1. Introduction

Polysaccharides thin films have important functions, such as cellular recognition and intracellular adhesion, due to the binding sites that can be found on the surfaces of different cells, making them important tools for the immobilization of biomolecules and for the development of biosensors. Fabrication of multilayer films utilizing carbohydrate polymers has been reported for xyloglucan [1], cashew gum [2], chitosan [3], carrageenan [4], alginate [5], amylose [6], and oxidized galactomannan [7].

Galactomannans are storage or structural heteropolysaccharides from plants, with a basic structure consisting of linear $1 \rightarrow 4$-linked β-D-mannopyranosil units partially substituted at C-6 by side chains of α-D-galactopyranosyl units. The galactose distribution in the main chain varies with different botanical sources and controls properties such as viscosity, solubility and stability of solutions [8]. Natural polymers have been object of investigation by researchers all over the world, owing to their large field of applications, particularly as substitutes of synthetic polymers, whereby they present several advantages, such as low cost, low toxicity, ready availability and biodegradability [9,10].

In nature, most of the microorganisms, viruses, and many proteins have either carbohydrate or carbohydrate-binding sites at their surface. For example, lectins are a family of proteins that can bind monosaccharides and oligosaccharides with high specificity [11], and they play an important role in cell adhesion and pathogen recognition. Therefore, the adsorption of proteins onto polysaccharide films is of great relevance, since it can provide the basis for the development of probes in studying carbohydrates on cell surfaces [12]; the specific protein binding can be applied for further studies in cell adhesion and pathogen recognition by specific cell surface carbohydrates of the immune system.

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Lectins generally exhibit strong binding to specific carbohydrate moieties (glycans), a property that has been extensively exploited as the basis for biosensor design. The carbohydrate–lectin interactions are particularly promising, as lectin-based immunosensor techniques have been routinely used for identifying pathogen and viral species expressing particular carbohydrates on their surface. For example, immobilized lectins based ELISA methods were developed for the detection of the human immunodeficiency virus (HIV) [13]. Such techniques rely upon the selectivity of particular lectins, such as Concanavalin A (ConA), for capturing the carbohydrate antigens of the envelope glycoproteins displayed on the surface of the virus [13,14]. ConA, a jack-bean lectin, is classified as part of the mannose/glucose-specific binding group, as these are the monosaccharides to which ConA exhibits the highest binding affinity [15,16]. This association requires Ca^{2+} and Mn^{2+} ions as co-factors. Favourable interactions between ConA and sugar-containing polymers, also, contribute for multilayer film construction [17]. Recent studies showed the irreversible adsorption of ConA monolayer onto a blend of xyloglucan and alginate [1]. In another study, the adsorption onto cashew gum amino-terminated films [2] demonstrated that although gum is predominantly composed of galactose, the presence of glucose as a side-chain favoured the interaction with ConA. Moreover, strong adhesion forces between colloidal probes of poly(methyl methacrylate)/carboxymethyl cellulose (PMMA/CMC) and ConA films were measured by means of atomic force microscopy [18].

Recent studies revealed that ConA is specifically recognized by the envelope protein (Eprot) located at the surface of dengue virus [1,19,20]. Dengue virus is an arthropod-born virus and a member of the family *Flaviviridae* that cause infections with a wide spectrum of clinical illness, which is important for public health. Dengue virus is predominant in urban and semi-urban areas of tropical and sub-tropical regions around the world [21]. Dengue hemorrhagic fever, a potentially lethal complication, is responsible for approximately 500,000 hospitalizations each year and approximately 2.5% of affected individuals die [22]. One of the key issues for the development of therapeutics is the diagnosis of dengue fever, which should be a low tech, low cost and rapid screen designed principally for the recognition of Dengue hemorrhagic fever. There are four serotypes of dengue virus, type 1 (DENV-1) to type 4 (DENV-4), whose are closely related genetically and have similar clinical manifestations and epidemiology. Additionally, all of them present glycoproteins on their surfaces, and these proteins are responsible for the major steps of the viral infection process, in which receptor recognition and fusion between viral and cellular membranes are involved [23]. Thus, the interactions between polysaccharides, proteins and viruses are important for the development of biomedical devices. One way to achieve this goal is to examine the characteristics of adsorbed proteins and other biomolecules on solid surfaces.

In the present work, a partially substituted galactomannan from *Leucaena leucocephala* seeds (GML) was deposited as monolayer films onto amino-terminated Si wafers, which were subsequently used as supports for the immobilization of ConA and/or dengue virus particles. The films were characterised by ellipsometry and atomic force microscopy (AFM), important tools for monitoring thin films growing.

2. Experimental

2.1. Materials

The purified galactomannan (GML), extracted from *Leucaena leucocephala* seeds, is composed of mannose:galactose (Man:Gal) at a ratio of 1.7, has an average molar mass of 713,600 g/mol, and carbohydrate and protein composition of 83% and 5% (w/w), respectively [24]. $MnCl_2$ and $CaCl_2$ were analytical grade (LabSynth, São Paulo, Brazil) and used without further purification ConA, type IV, from *Concanavalia ensiformis* with molar mass of 25.583 g/mol, was purchased from Sigma (C2010, St. Louis, USA) and used without any purification.

Dengue virus types 1 (DENV-1; Mochizuki strain), 2 (DENV-2; New Guinea strain) and 3 (DENV-3; H87 strain) were grown in C6/36 cells cultured in L15 Leibowitz medium supplemented with heat-inactivated fetal bovine serum (2%) and antibiotics. Viruses were added to infect confluent cell monolayers and incubated for 1 h at 28 °C with periodic rocking every 15 min. Infected cell culture supernatants were collected 7 days after virus infection, centrifuged to clear remaining cells and cell debris, aliquoted, and frozen at −80 °C. The virus infection was then determined by Reverse transcription polymerase chain reaction (RT-PCR) using serotype-specific primers [25]. To prepare high titres of dengue virus, supernatants of infected cell cultures were obtained by centrifugation in a Millipore Centricon® Centrifugal Filter Unit with a 100-kDa cut-off (Amicon; Millipore).

Silicon wafers purchased from Silicon Quest (USA), with a native oxide layer approximately 2-nm thick, were used as substrates. Si wafers with dimensions of 1 cm × 1 cm were rinsed in a standard manner [26], dried under a stream of N_2, and functionalizated by reacting with aminopropyltriethoxysilane (APS, Fluka, Switzerland), as described elsewhere [26]. Briefly, the freshly cleaned silicon wafers were dipped into a 1 wt% solution of 3-(aminopropyl)triethoxysilane, in toluene for 4 min at 60 °C. After 4 min the wafers were washed five times with toluene and dried by a stream of N_2. This method yields a flat and homogeneous amino-terminated monolayer covalent bound on silicon wafers and the substrate become cationic at pH < 4, since pK_i value for propylamine groups at 20 °C is reported to be 3.3 [27].

2.2. Adsorption of polysaccharides onto functionalized Si wafers

GML was dissolved in 0.01 M NaCl so that final concentration amounted to 1.0 g/L. The pH of the salt solution was adjusted with HCl to pH 4.0 prior to polymer dissolution. Amino-terminated wafers were dipped into solutions of GML at 25 ± 1 °C. After 4 h, the wafers were removed from the solutions, washed with distilled water, dried under a stream of N_2 and characterised by means of ellipsometry and AFM [28].

2.3. Adsorption of ConA onto the polysaccharide layer

ConA was dissolved in 10 mM NaCl in the presence of 0.01 mol/L $MnCl_2$ and 0.01 mol/L $CaCl_2$, at final lectin concentration of 0.20 mg/mL. The medium was adjusted to pH 5.0 adding 0.1 mol/L HCl. Polysaccharide covered amino functionalized wafers were dipped into lectin solution for 4 h at 25 °C. In another set of experiments, mannose (50 mM) was added to the lectin solution, so that both ConA and mannose were in the same solution at the same time. Thereafter, all samples were removed from the solution, washed with distilled water, dried under a N_2 stream and characterised by means of ellipsometry and AFM.

2.4. Adsorption of dengue virus onto polysaccharide or polysaccharide-ConA layer

Virus dispersions were prepared at 4 mg/mL and pH 7.2 (PBS buffer). Polysaccharide or polysaccharide-ConA covered amino functionalized wafers were dipped into virus dispersions and allowed to interact for 4 h at 36 °C. In another set of experiments, mannose (50 mM) was added 5 min prior to virus adsorption onto polysaccharide-ConA film. Subsequently, all wafers were removed

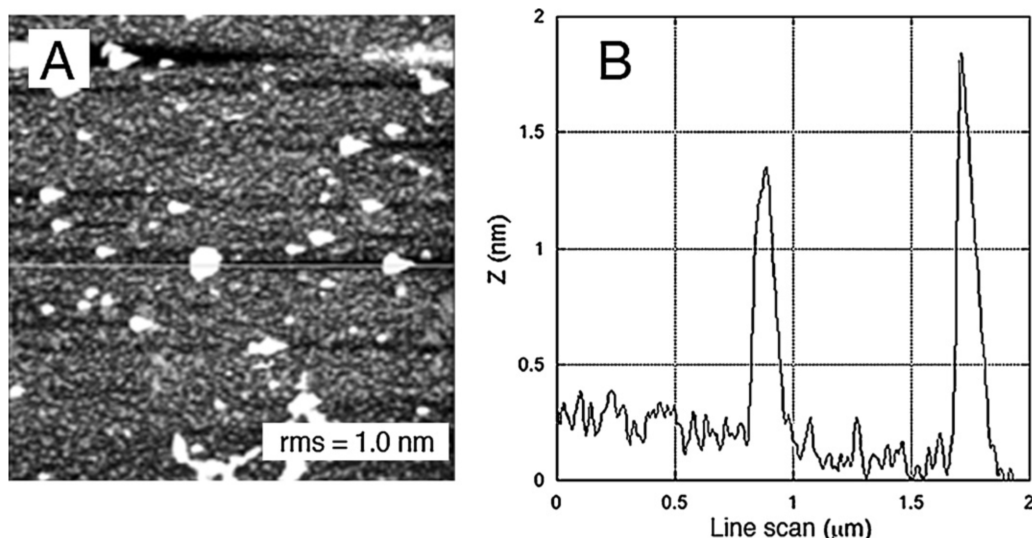

Fig. 1. AFM topographic image obtained for galactomannan adsorbed onto the Si-amino terminated layer (A) and with the corresponding line scan (B). The scan area was 2.0 μm × 2.0 μm.

from dispersion, washed with distilled water, dried under a N_2 stream and characterised by means of ellipsometry and AFM.

2.5. Desorption experiments

Desorption experiments were performed by dipping the (lectin and/or virus)-covered substrates into pure solvent. After 24 h, they were removed from the solutions, dried under a stream of N_2 and characterised by means of ellipsometry.

2.6. Ellipsometric measurements

Ellipsometric measurements [29] were performed in air using a vertical computer-controlled DRE-EL02 ellipsometer (Ratzeburg, Germany). The angle of incidence was set at 70° and the wavelength, λ, of the He–Ne laser was 632.8 nm. For data interpretation, a multilayer model composed by the substrate, the unknown layer and the surrounding medium should be used. The thickness (D) of the unknown layer can be calculated from the ellipsometric angles, Δ and Ψ.

In this study, multilayer models were composed of silicon, silicon dioxide, the adsorbed layers (polysaccharides, lectin or viruses) and the surrounding medium (air). Initially, the thickness of the SiO_2 layers was determined in air, assuming the refractive index for Si as $n = 3.88 - i0.018$ [30] and its thickness as infinite; for the surrounding medium (air), the refractive index was considered to be 1.00. Because the native SiO_2 layer is thin, its n was set as 1.462 [30], and the mean thickness value D was calculated for all Si wafers as 1.9 ± 0.1 nm. The thickness of amino terminated monolayer was determined in air, considering the refractive index of the silane as $n = 1.424$ and the average thick calculated for the amino-terminated layer was 1.0 ± 0.1 nm. It should be noted that ellipsometry calculates the average thickness based on a model that considers the adsorbed layer as a homogeneous isotropic smooth layer. The mean thickness is an average over the peaks and valleys in a spot area of about 3 mm^2.

The thickness of the polysaccharide, ConA or dengue virus layer was determined in air, assuming the indices of refraction as 1.50, 1.52 [18] and 1.50 [1,19], respectively. Ellipsometric analyses, in triplicate, were performed in three different areas of the sample to test for film heterogeneity and were ultimately averaged.

2.7. AFM measurements

Atomic force microscopy (AFM) measurements were performed on a PicoPlus Molecular Imaging microscope in the intermittent contact mode of air at 20 °C, using silicon cantilevers with oscillating amplitude of 50–100 nm and a resonance frequency close to 300 kHz. Scan areas were 2 μm × 2 μm with a resolution of 512 × 512 pixels. Image processing and determination of the root mean square (rms) roughness values were performed using Gwyddion software (free version).

3. Results and discussion

3.1. Adsorption behaviour of ConA onto GML thin films

The adsorption of GML (1 mg/mL) onto amino-terminated Si wafers led to the formation of (1.5 ± 0.2) nm thick layers. In the literature the pKb for aminopropyl group is reported as 3.3 at 20 °C [27]. Therefore, at experimental condition of pH 4.0 the interactions observed might be due to ion-dipole type between the GML hydroxyl groups and positively charged amino-terminated surfaces. Fig. 1 shows a typical AFM topographic image obtained for GML layers onto the amino-terminated substrate. The presence of tiny aggregates (~1.5 nm high) on the surface is probably due to polysacharide chains collapse upon drying process.

In the present study, ConA molecules adsorbed onto GML-covered amino terminated silicon wafers at pH 5.0, for which the AFM topographic image shows layer formation with an average thickness of 2.0 ± 0.5 nm (Fig. 2), as indicated by the cross section, which is consistent with ellipsometric thickness of 1.6 ± 0.2 nm. As GML has a mannose main chain branched with galactose residues, it is reasonable to conclude that at pH 5.0, specific and non-specific hydrogen interactions between the GML segments and lectin positively charged residues might account for this adsorption. The recognition of specific sugar moieties has a binding constant for mannose of 2.2 × 10^3 1/M [31], which also accounts for the attachment. The AFM topographic image (Fig. 2) shows that GML is totally covered by lectin, which appears as densely packed small globules. Similar morphological features have been observed for ConA adsorbed onto polysaccharide surfaces, such as carboxymethyl cellulose (CMC) [18], cashew gum [2], xyloglucan-amino terminated films [1] and bare Si wafers [19] where the average thickness was

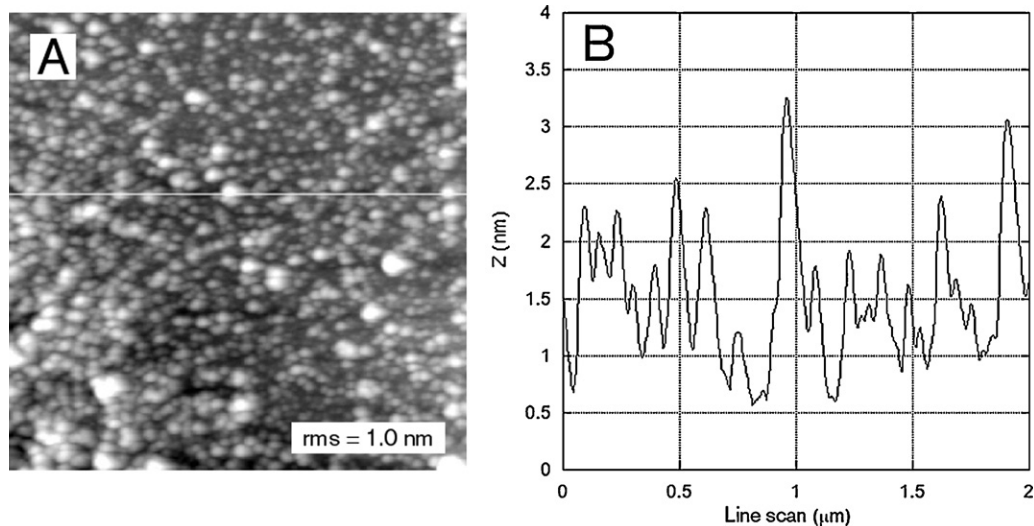

Fig. 2. AFM topographic image obtained for ConA adsorbed onto the GML-amino terminated layer (A) and with the corresponding line scan (B). The scan area was 2.0 μm × 2.0 μm.

4.0 ± 1.0 nm, 2.5 ± 0.5 nm, 2.5 ± 0.5 nm and 2.5 ± 0.5 nm, respectively.

Desorption experiments were performed by dipping GML – ConA-covered wafers into pure water for 24 h. After desorption experiments, ellipsometric measurements were again performed on three different areas of the same sample. Negligible changes in the average thickness values were observed indicating that ConA adsorbed irreversibly onto GML-amino terminated films.

In order to check if the adsorption of ConA onto GML-amino terminated films is driven by specific or unspecific binding, adsorption experiments were performed in the presence of 0.050 M mannose, which is enough to block the specific binding sites on ConA [1]. The lectin affinity to sugars was attributed to the multivalent and spatial organization of the molecule [32]. In our experiments, the mannose solution inhibits the adhesion of ConA onto GML-amino terminated surfaces as determined by means of ellipsometry. The GML–ConA-recognition binding did not occur due to the protein blocked binding sites by the mannose residues dispersed in the solution and therefore suggesting a specific binding from to ConA to GML.

Spectroscopic studies [32] revealed that the molecular recognition of ConA for mannose and glucose residues, whose possess specific hydroxyl configurations at the 3, 4 and 6 carbons. X-ray crystal structures of the ConA-methyl-α-D-mannopyranose complex [33,34] showed that the carbohydrate oxygen atoms O-3, O-4, O-5 and O-6 are directly hydrogen bonded to the protein. Moreover, studies suggested that modifications at C-3, C-4, and C-6 positions of the D-mannopyranose deterred the binding to ConA [35]. In particular, the loss in the hydroxyl groups in the C-6 position as in 6-deoxy-D-mannose and 1,6-anhydro-β-D-*manno*-pyranose result in complete loss of activity [35], unless the substitution of one of the hydrogen atoms in the C-6 position is a hydroxymethyl group [36], where the presence of a water molecule located near the C-6 hydroxy group of the nonreducing mannose helps mediate the interaction of the sugar with the lectin, stabilizing the nonreducing sugar to the protein (Asp 16) [35,37,38].

So, based in these information and because the GML backbone is β-D-mannose 1 → 4 linkage and some units at the C-6 position carries α-D-galactose units, one can propose that the recognition of ConA might be for OH groups at the C-3 and C-6 position for

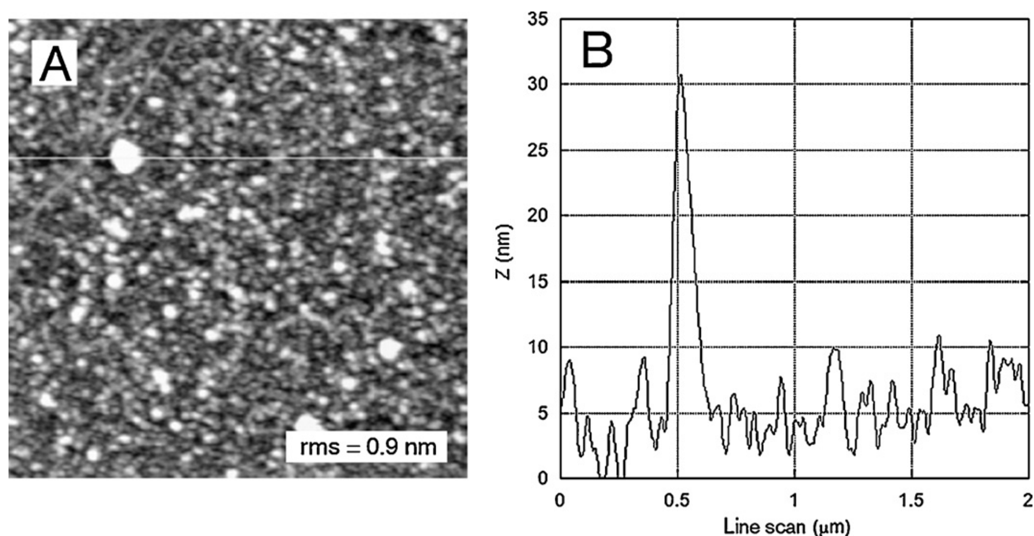

Fig. 3. AFM topographic image obtained for dengue virus (DENV-2) adsorbed onto the GML-amino terminated layer (A) and with the corresponding line scan (B). The scan area was 2.0 μm × 2.0 μm.

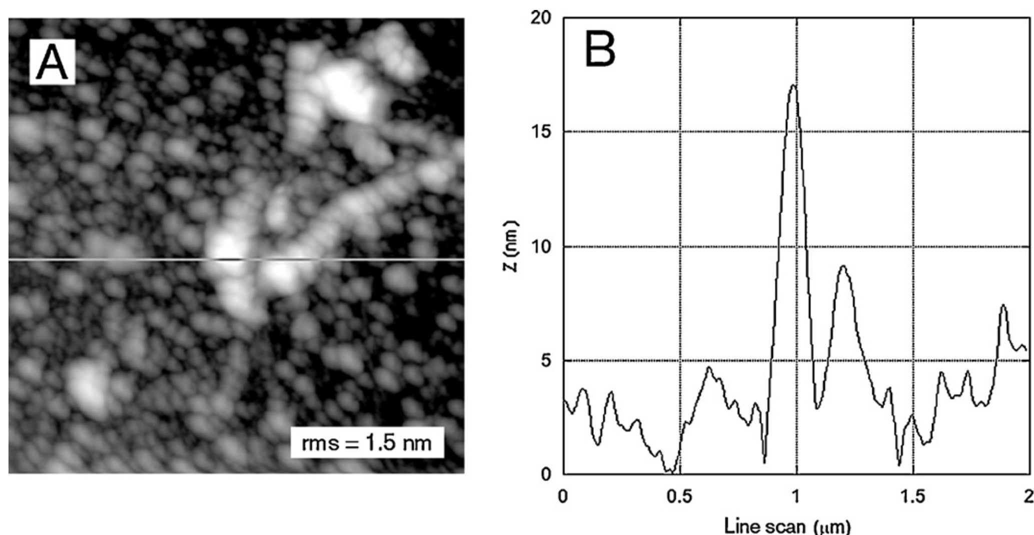

Fig. 4. The AFM topographic image obtained for dengue virus (DENV-2) adsorbed onto the GML–ConA-amino terminated layer (A) and with the corresponding line scan (B). The scan area was 2.0 μm × 2.0 μm.

the mannose units not substituted [39]. It is known that the OH-3 of the mannose accepts a hydrogen bond from the backbone NH group of the Arg-228 in ConA and OH-6 makes hydrogen bonds with Tyr-100 and to the side chain of Asp-208 [40] besides extensive van der Waals contacts formed between OH-3 and OH-6 with the surface of the binding pocket [41]. Thermodynamic analyses in the literature has shown that protein and carbohydrate interactions are driven by enthalpic terms [42,43], so it could be suggested that the carbohydrate–lectin interactions are occurring through weak monomeric bindings affinities such as hydrogen bonds and van der Waals interactions. Consequently, this lectin–polysaccharide interaction could lead to further studies to investigate cellular processes and uncover binding mechanisms, i.e., cell adhesion and pathogen recognition by specific surface carbohydrates in the immune system; the information can also be useful in developing a probe for carbohydrates in cell surfaces studies [18].

Interestingly, the particular structural profiles of glycans and their recognition by lectins are thought to be involved in disease progression in dengue fever, making the saccharide–lectin binding analysis an important step in designing a diagnostic tool [44].

3.2. Binding affinity between dengue virus particles and galactomannan thin films

The capacity for glycoprotein adsorption onto polysaccharide-coated wafers motivated the investigation of more complex molecules, such as dengue virus particles. Therefore, we studied the adsorption of dengue virus particles DENV-1, DENV-2 and DENV-3 onto GML-amino terminated layers. Dengue virus has a capsid composed of three structural proteins, including Eprot, responsible for attachment to host cells and recognition by specific antibodies [45]. Moreover, studies showed that the presence of mannose residues in the Eprot structure is important for the receptor binding function during the viral entry process [46].

DENV-1, DENV-2 and DENV-3 adsorbed onto GML films presented mean ellipsometric thickness values of (1.3 ± 0.6) nm,

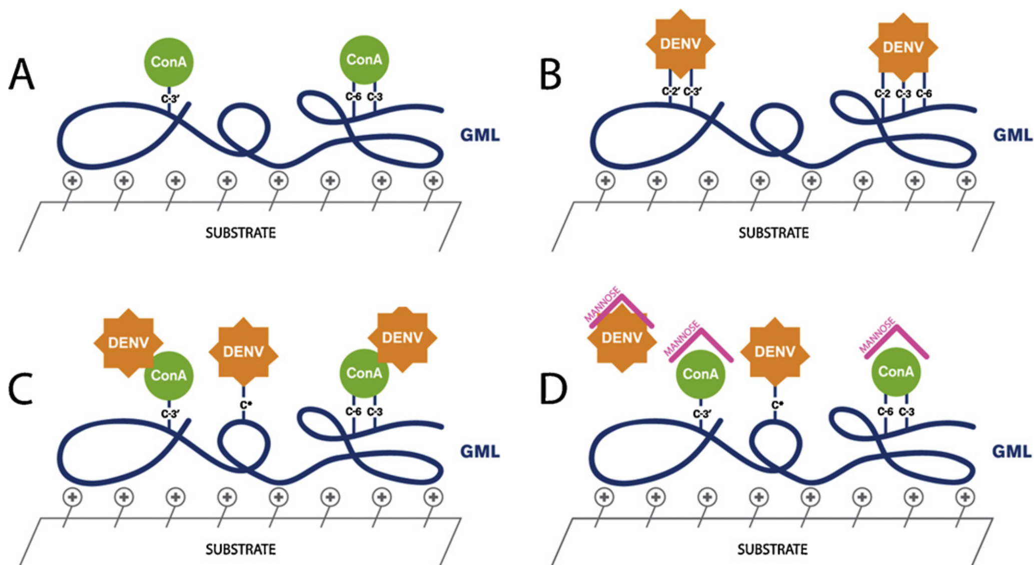

Fig. 5. Schematic representation of (A) the adsorption of ConA onto the GML-amino terminated layer, (B) the adsorption of dengue virus onto the GML-amino terminated layer, (C) the adsorption of ConA and dengue virus onto the GML-amino terminated layer and the adsorption of dengue virus onto mannose-ConA – GML amino terminated layer. C* represents C-2, C-3, C-6, C-2′and C-3′.

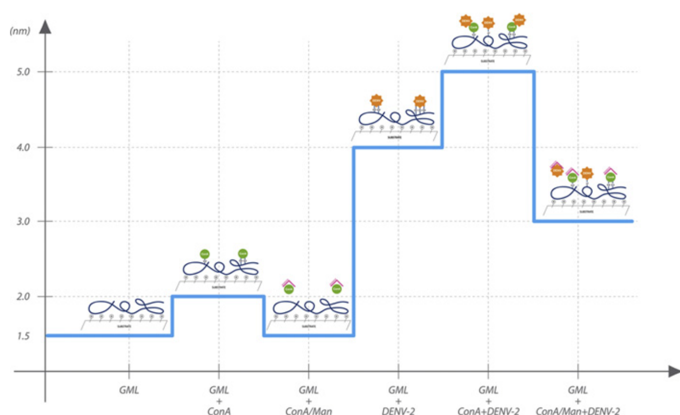

Fig. 6. Schematic representation of mean thickness values determined after the immobilization of protein and/or dengue virus onto GML-amino terminated layer.

(4 ± 2) nm and (0.8 ± 0.5) nm respectively. DENV-2 presented an adsorption, the highest adsorption of the three serotypes. Thus, the attachment of dengue particles onto GML films might be mediated by H-bonding between Eprot polar residues and the surface composed of hydroxyl groups present on the main chain of the polysaccharide, where at C-2 and C-3 of mannose units are more exposed. Differences in adsorption correlated with different serotype could be related to the differences in Eprot composition for which dengue virus serotypes.

The ellipsometric data were obtained by the average of the measures from different areas in the film surface. These values showed that even with dengue virus adsorbed onto its surface, the GML layer was not fully covered by the virus particles; this result accounts for the large error among distribution measurements and that could be, probably, justified by random distribution of side chains formed by galactose units at some C-6 position of mannose main chain. So, C-2, C-3 and C-6 of mannose not substituted and, probably, C-2′ and C-3′ of mannose substituted could be a receptor to virus particle. But, the addition of free mannose units to the system composed of GML and virus has no significant effect on the adsorption process [1].

The AFM topographic image (Fig. 3) obtained for DENV-2 adsorbed onto the GML-amino terminated layer with the corresponding line scan confirms this data. It was previously shown by transmission electron microscopy [47] that DENV-2 virus presented two typical diameters: a 50 nm particle (comprising an electron dense core of 30 nm and a lipid envelope) and a 14 nm particle (corresponding to the virus particle and sedimentation haemagglutinin (SHA) particles, respectively). Dengue virus dispersions used for the adsorption experiments contain DENV, particles secreted by dengue virus and additional proteins from culture medium. The presence of the spherical entity can be observed in the AFM images, which corresponds to a virus particle of approximately 27.3 nm, the size of which might be correlated with the dengue particle core because the lipid envelope was probably collapsed during the drying procedure. All these data are consistent with the results presented by Pereira et al. [1,19], in which a blend of xyloglucan–alginate was used in films for dengue virus support.

Desorption experiments were performed by immersing the covered silicon wafers in water for 24 h; subsequently, the slides were removed from solution, dried and analysed again by ellipsometry. Negligible changes in average thickness values were observed (data not shown), indicating an effectively irreversible adsorption of the dengue virus.

3.3. Galactomannan–ConA layers as a support for dengue virus particles

Tassaneetrithep et al. [48] and Navarro-Sanchez et al. [49] showed that mosquito-grown dengue virus interacts with lectin to efficiently enter the cell. Like all flaviviruses, dengue virus requires a replication step in the arthropod vector prior to its transmission to humans [23]; and in a natural infection, the virus is deposited in the skin by an infected mosquito. Dendritic cells in skin have a lectin that is specific for high-mannose-type carbohydrates that are present in virions produced in insect cells. Therefore, the films of GML–ConA layers were tested to observe the influence of ConA previously adsorbed onto polysaccharide film in the virus deposition.

DENV-2 was chosen for this immobilization experiment for two reasons: first, studies showed that there is higher probability for occurrence of severe symptoms in secondary infections with DENV-2 than in other serotypes [50], and second, thicker films on GML were obtained for this dengue serotype. Virus particles adsorbed onto ConA were in average (5 ± 1) nm thick, as measured by ellipsometry; this result was slightly higher than that observed for GML surfaces, which indicates that the presence of ConA onto GML influences the virus adsorption onto the film. Although, ellipsometric data obtained for films with and without ConA do not differ significantly, comparing the AFM topographic images obtained for DENV-2 onto GML surfaces (Fig. 3) and those for DENV-2 onto ConA/GML films (Fig. 4) one can observe morphological differences between them. In addition to the obvious increase in the number of spherical entities form Fig. 3 to Fig. 4, an increase in the rms values from (0.9 ± 0.1) nm to (1.5 ± 0.1) nm denotes a more disordered arrangement of dengue particles on ConA/GML surfaces than onto GML films. The line scan (Fig. 4B), which corresponds to the AFM topographic image (Fig. 4A), shows particles with height ranging from 8 nm to 17 nm. Because the type of particle adsorbed cannot be distinguished, we can reasonably conclude that the particles might correspond to SHA particles secreted by the dengue virus, which present a typical diameter of 14 nm.

Similar to previous reports, ConA binds to mannose; therefore, the adhesion of virus particles onto GML–ConA layers was evaluated in the presence of 50 mM mannose. The mean thickness of (3 ± 1) nm showed that the mannose prevented approximately 50% of the virus adsorption, suggesting that the mannose presence competes with the virus for binding to ConA. We hypothesized that when lectins are bound mainly by C-3 and C-6 mannose Eprot cannot recognize the binding sites on the lectins and the adsorption will be reduced. Previous results from our group [1] had already shown that the binding between DENV virus particles and polysaccharides are unspecific. The Eprot located at the virus surface recognizes specifically the binding site of ConA, which is blocked by mannose, when it is added to the medium. Mannose binds specifically the binding site of ConA, but has no specific binding to polysacharides like xylogucan, alginate or galactomannan.

Notably, the adsorption was decreased, but some virus adhesion was still present; this result might be related to the recognition of the virus and for the OH exposed mannose units present in the main chain of galactomannan, which are not linked to ConA (C-2′, C-3′, and C-2, C-3, C-6). In comparison to previous studies, which employed xyloglucan (XG) [1] or carboxymethyl cellulose (CMC) [18] as supports for virus and the virus adsorption decreased considerably in the presence of mannose, the present study shows that the mannose units in the polysaccharide chains are attractive binding sites to dengue viruses, even when in the presence of free mannose (monosaccharide).

Fig. 5 represents the results. First, a natural biopolymer was adsorbed onto amino terminated Si wafers. Then, ConA was able to adsorb onto GML exploiting the protein's capacity to binds

mannose residues (Fig. 5A, with C-3′ representing the OH bounded mannose substituted and C-3 and C-6 for the mannose units not substituted by galactose). Not only lectins but also different serotypes of dengue virus were able to adsorb onto GML films (Fig. 5B). Indeed, both lectins and virus were tested onto GML films, with or without the presence of mannose units (Fig. 5C and D with C* representing C-2, C-3 and C-6 of the mannose units not substituted and C-2′ and C-3′ representing the OH bounded mannose substituted).

And finally Fig. 6 links up the film adsorptions with the corresponding thickness. All these results suggest a great potential application for carbohydrate sensors. These results are significant because dengue virus is the most clinically significant mosquito-borne viral disease, and dengue fever represents one of the major public health problems in the world, especially in tropical areas; the World Health Organization (WHO) [22] estimates that approximately 50 million people get infected every year across a hundred countries. As dengue virus is an important disease, more information concerning the attachment of this virus during the host infection process is necessary for the development of techniques aimed to investigate and diagnose such a disease. Therefore, the results showing interactions between proteins and polysaccharides presented here indicated that GML has great potential for utilization in the creation of new devices for biotechnological uses.

4. Conclusions

Native galactomannans obtained from renewable sources might be suitable substrates for the adsorption of ConA, due to its low cost and abundance in nature, creating potential materials for biomedical and biotechnological devices as demonstrated by the adsorption onto amino-terminated surfaces in this study. The ConA adsorption onto specific C-3 (C-3′) and C-6 mannose units of GML films suggests that the surface of the mannose main chain of GML seems to interfere with the adsorption of lectin. Among the different dengue serotypes tested with GML thin films, DENV-2 obtained the highest adsorption value. Its adsorption was also tested against ConA influence on the polysaccharide surface, for which the film thickness did not change significantly. In this case, when the GML–ConA films were previously exposed to a mannose solution prior to virus adsorption, DENV-2 adhesion was reduced by 50%, suggesting a competition between the virus and mannose for ConA binding sites that possibly drives binding of the virus with the C-2 (C-2′), C-3 (C-3′), C-6 of mannose units presents in the GML main chain.

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