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Lectins and/or Polysaccharide Layers as Supports for Immobilization of Dengue Virus Particles

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Abstract – Adhesion of four serotypes of dengue virus, DENV (1-4), particles to polysaccharides or lectins covered surfaces was observed by ellipsometry and AFM. The attachment of dengue particles onto polysaccharide films might be mediated by (i) H bonding between E protein (located at virus particle surface) polar residues and hydroxyl groups present on polysaccharide surfaces and (ii) electrostatic interaction between E protein positively charged residues and polysaccharides carboxylic groups.. All four DENV particles serotypes adsorbed similarly onto mannose ligand lectin films. Nevertheless, the addition of 0.005 mol/L of mannose prevented dengue particles from adsorbing onto mannose ligand lectin films.

Films of polysaccharides and glycoproteins are potential supports for biomedical applications due to their biocompatibility and their role in many recognition processes. In this work, stable films of mixtures of xyloglucans and alginate (XG-ALG) films were prepared and characterized by means of ellipsometry and atomic force microscopy (AFM). They have been used as matrices for the adsorption of two glucose/mannose-binding seed (*Canavalia ensiformis* and *Dioclea altissima*) lectins, coded here as ConA and DAlt. XG-ALG and lectin XG-ALG covered surfaces were used as supports for the immobilization of dengue virus particles. Dengue is one of the most important infectious diseases affecting tropical urban areas, and is usually characterized by a flu-like disease but, in a few percentages of the cases, may present as a severe disease with hemorrhagic manifestations. Dengue virus might be found as four serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4. XG-ALG negatively charged surfaces were suitable for the immobilization of DENV-1, DENV-2 and DENV-3 particles. The weak affinity of DENV-4 particles for XG-ALG surfaces indicated structural differences in its E-protein structure. All four serotypes adsorbed onto lectin films. However, the addition of 0.005 mol/L mannose prevented dengue virus from adsorbing onto lectin films. Although ConA and DAlt presented similar behavior, this is the first study presenting the formation and application of DAlt thin layers. Most available methods for detecting dengue are based on ELISA assay [1] by measuring IgM or RT-PCR by detecting viral RNA. Although these methods are valuable, they still face limitations due to cost, technical constraints and reliability. Ellipsometry is an optical non-destructive technique able to detect very quickly changes in film thickness in the order of angstroms. The combination of ellipsometry and AFM enhances the reliability for detecting adhesion of small molecules to solid surfaces. Monitoring the immobilization of dengue virus particles on thin films of XG-ALG or of lectin-covered XG-ALG layers by ellipsometry complemented with AFM could be helpful for developing alternative diagnostic methods for dengue.

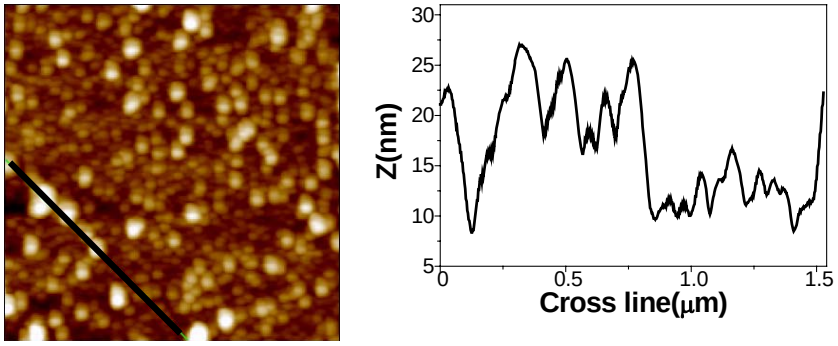

Figure 1. AFM topographic image obtained for DENV-1 adsorbed onto DAlt covered XG-ALG layer with the corresponding cross sections. Scan area of 3 μm x 3 μm.

[1] G. Bock and J. Goode, New Treatment Strategies for Dengue and other Flaviviral Diseases, Novartis Foundation, John Wiley & Sons Ltd. Chichester, UK, 2006.