

MULTILAYERED FILMS OF POLYSACCHARIDES: INFLUENCE OF IONIC STRENGTH ON THE BUILDUP AND ON BACTERIAL ADHESION

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INTRODUCTION: Creating biomimetic thin films could allow to give a biological activity to an inert substrate. A new kind of supramolecular assembly based on polyelectrolyte multilayer¹ has been proposed for the nanometer scale modification of material surfaces. Preparation of multilayer entirely made of natural polysaccharides has emerged. Chitosan (Chi) and Hyaluronic Acid (Ha) are two polysaccharides with interesting biological properties. The aim of our study was to investigate the formation of thin film of these polyelectrolytes in different conditions and their properties with respect to bacterial adhesion.

METHODS: Chitosan (Pronova, 120 kDa) and Hyaluronic Acid (Bioiberica 400 kDa) were solubilized in NaCl solution (0.5, 0.15, 10⁻² and 10⁻⁴ M) at pH: 5. Quartz Microbalance (QCM-D) and Optical Waveguide Lightmode Spectroscopy (OWLS) were used to analyze *in situ* the film growth. The conformation and topography of the multilayer films were examined with Confocal Laser Scanning Microscopy (CLSM) and Atomic Force Microscopy (AFM). The bacterial adhesion tests are carried out with *E.Coli* transformed with a plasmid bearing the GFPmut3.

RESULTS: The stepwise adsorption of chitosan and hyaluronic Acid was monitored by OWLS and QCM experiments. At 0.15 M NaCl, the growth of the film was exponential and after some layers the raw NTE signal obtained by OWLS became cyclic. This suggests a diffusion of a polyelectrolyte in the film². Observations performed by CLSM using Chitosan-Fitc confirms that Chi diffuses within the film while Hyaluronic Acid-(Texas Red) is deposited as a uniform layers. The QCM experiments indicate a progressive passage from an exponential to a linear regime when the ionic strength decreases from 0.15M to 10⁻⁴M (Fig. 1). The thickness (OWLS) of the film decreased of 300 nm to 10 nm for (Chi/Ha)₁₀ in 0.15 M and 10⁻⁴ M NaCl. The study of bacterial adhesion show that multilayer buildup in 0.15 M were three times less adhesive that films built in 0.01 M NaCl and five times less that glass. (Fig. 2)

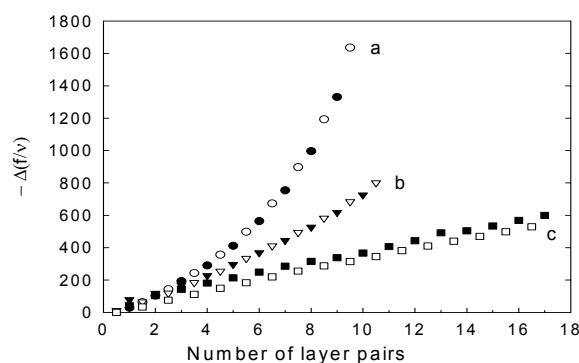


Fig. 1: Frequency shift measured by QCM for the (Chi/Ha) films in the presence of NaCl: 0.15 M (a), 10⁻² M (b) and 10⁻⁴ M (c). The open and closed symbols represent the Chitosan and Hyaluronic Acid depositions.

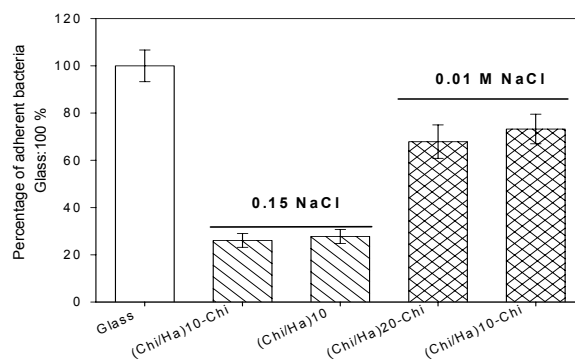


Fig. 2: Bacterial Adhesion (*E.Coli*) after 30 min on (Chi/Ha) films built in 0.15 M or 0.01 M NaCl versus bare glass.

DISCUSSION & CONCLUSIONS: The growth of thin films made of two polysaccharide –Chitosan and Hyaluronic Acid is strongly influenced by the ionic strength: -exponential growth associated to chitosan diffusion in 0.15 M NaCl, - linear at low salt concentration. The films built in 0.15 M NaCl may be used as anti-bacterial coating.

REFERENCES: ¹G. Decher, (1997) *Science*. **227**: 1232-1237. ²C. Picart, J. Mutterer, L. Richert, and al (2002) *PNAS*. **99**: 12531-12535