European Cells and Materials Vol. 6. Suppl. 1, 2003 (page 11) ISSN 1473-2262
Session 1: Fundamental Aspects of Polymeric Molecules at Interfaces and Biological Responses

MULTILAYERED FILMS MADE OF POLYSACCHARIDES: INFLUENCE ON CELL AND BACTERIAL ADHESION

C.Picart[1], L.Richert[1], E.Payan[2], Ph.Lavalle[1], P.Schaaf[3] & J.-C.Voegel[1]

[1]*INSERM Unité 595, Université Louis Pasteur, 11 rue Humann, 67085 Strasbourg Cedex, France.*
[2]*Laboratoire de Physiopathologie et Pharmacologie Articulaires, UMR 7561 et IFR 111, Faculté de Médecine, 54 505 Vandoeuvre-les-Nancy, France.*
[3]*Institut Charles Sadron (CNRS-ULP), 6 rue Boussingault, 67083 Strasbourg Cedex, France.*

INTRODUCTION: Polyelectrolyte multilayers (PEM) have emerged as a new and versatile way to coat any type of substrate[1]. In the biomaterial field, their potential applications are wide since they can be used to create biomimetic films and can also be functionnalized by insertion of peptides or proteins [2]. Preparation of films made of natural polysaccharide has emerged. The aim of our study was to buildup films of polysaccharides and to investigate their properties with respect to cell and bacterial adhesion.

METHODS: Chitosan (CHI, 120 kDa), poly(L-lysine) (PLL, 30 kDa), and Hyaluronan (HA, 400 kDa) were dissolved in a 0.15 M NaCl solution at pH = 5 or 6.5. Quartz Microbalance (QCM) and Optical Waveguide Lightmode Spectroscopy (OWLS) were used to analyze *in situ* the film growth. Film topography was examined by Atomic Force Microscopy (AFM) and Confocal Laser Scanning Microscopy (CLSM) allowed to visualize the z-structure of the film and to determine its thickness[3]. Primary chondrocytes adhesion was investigated and bacterial adhesion tests are carried out with *E.Coli* –GFP.

RESULTS: OWLS and QCM exhibits an exponential raise for both CHI/HA and PLL/HA films. OWLS signal begins to cycle after around 9 bilayers have been deposited suggesting the diffusion of the polycation, either CHI or PLL within the film[2]. CLSM images confirm that CHI and PLL diffuse within the film while HA remains as a layer (Figure 1). AFM shows that the surface is first covered by small islets that progressively coalesce toward a uniform film (after 9 bilayers). Primary cell adhesion decreases when the number of bilayers in the films is increased, for both CHI/HA and PLL/HA films whatever the outermost layer of the film is (positive or negative) (Figure 2). But, noticeably, $(CHI/HA)_{10}$ are films lead to a ≈80% decrease in bacterial adhesion as compared to bare glass.

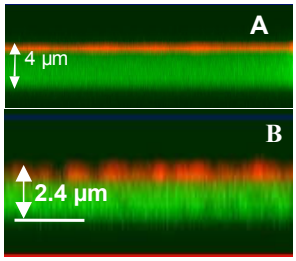

Fig. 1: CLSM images of a $(PLL/HA)_{24}$-PLL-FITC (with HA_{24}-TR) and of a $(CHI/HA)_{36}$ film containing two labeled layers, CHI_{36}-FITC and HA_{36}-TR.

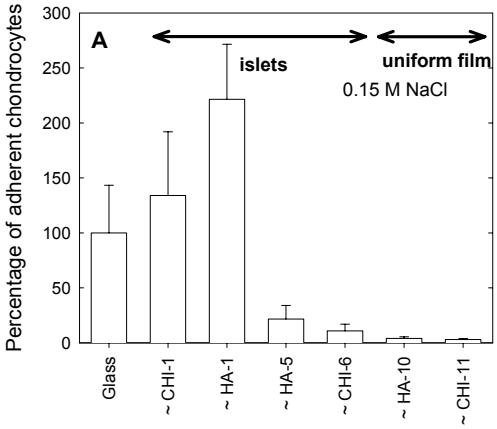

Fig. 2: Primary rat chondrocytes adhesion as measured by ^{3}thimidine after 4 days of contact with $(CHI/HA)_i$ films containing an increasing number of layers (1,5,10) either terminated by ~CHI or ~HA.

DISCUSSION & CONCLUSIONS: Multilayered films made of polysaccharides grow exponentially with the number of deposited layers and can become more than micrometer thick. As these films are highly hydrated, they are cell-resistant but also bacterial resistant. In recent experiments, these films have been crosslinked thereby changing the cell adhesive properties.

REFERENCES: [1]G. Decher. (1997) *Science*. **227** :232-1237. [2]N. Jessel, F. Atalar, Ph. Lavalle, et al. *Adv. Mat.* **15**, 692-694. [3]Picart, C., Mutterer, J., Richert, et al. *PNAS*. **99**:12531-12535.

ACKNOWLEDGEMENTS: This work was supported by an Action Concertée Incitative "Technologies pour la Santé" from the French Ministry for Research.