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Study of fibronectin adsorption on plasma-enhanced deposition of silver nanoparticles embedded in a silica matrix

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INTRODUCTION: Every year, 1 out of 9 patients in Canada hospitals gets a potentially fatal healthcare-associated infection (HCAI), which makes it the 4th leading cause of death in the country. The development of HCAs are mainly due to the material's surface colonization by microorganisms. Adherent bacteria organize themselves to form a biofilm, once the extracellular polymeric matrix made of proteins, polysaccharides and lipids is formed [1]. The biofilm structure renders it highly resistant to existing antibacterial agents and accordingly, there is a growing need to develop new strategies to inhibit this bacterial structuration. This project takes advantage of the known potential of silver nanoparticles (AgNPs) to deliver Ag⁺ ions to kill bacteria [2]. Protection of the AgNPs from oxidation and homogeneous NPs distribution were ensured through a coating with a plasma-deposited silica matrix [3,4]. This study will focus on the adsorption of fibronectin (FN) on plasma-deposited silica (SiO₂) surfaces containing AgNPs, as protein adsorption is the first step toward bacterial film formation.

METHODS: A customized axially-asymmetric radiofrequency plasma reactor was used to deposit a SiO₂ matrix and AgNPs on silicon wafers. The matrix was obtained using a gas mixture of hexamethyldisiloxane (HMDSO), oxygen and argon, while the AgNPs were synthesized by sputtering of the upper silver electrode in an argon plasma [3,4]. A droplet of 10 µL of human FN solution in HEPES buffer at two different concentrations, 6 and 200 µg/mL, was deposited on the plasma-deposited SiO₂ surfaces. The adsorption lasted for 1h and was followed by 3 successive washings in nanopure water with vortex and drying. AFM, XPS, FTIR analyses and ELISA tests were used to characterize the FN surface density and conformation.

RESULTS: XPS spectra clearly put in evidence the FN adsorption, thanks to the presence of the N1s feature, characteristic of proteins, while no N1s peak was detected on the plasma-deposited SiO₂ matrix (data not shown). The FN adsorption is further confirmed through the decrease of Si surface

concentration with the amount of adsorbed FN (Table 1).

Table 1. Elemental composition obtained by XPS

Sample	Elemental composition (%)			
	O	Si	C	N
FN [6 µg/mL]/SiO ₂ /Si	50.6	25.0	22.0	2.4
FN [200 µg/mL]/SiO ₂ /Si	33.1	14.0	44.0	9.0

AFM images confirmed the presence of FN as round objects on the investigated surfaces. Besides, a higher FN density is observed when using the 200 µg/mL FN solution compared to the one at 6 µg/mL (Fig. 1).

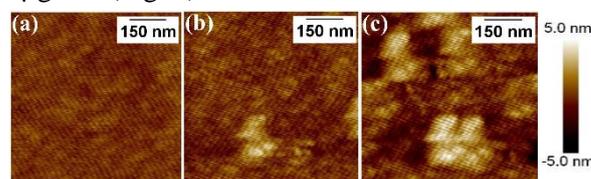


Fig. 1: AFM images (700 nm x 700 nm) of (a) SiO₂/Si, (b) FN [6 µg/mL]/SiO₂/Si and (c) FN [200 µg/mL]/SiO₂/Si.

DISCUSSION & CONCLUSIONS: The FN presence after adsorption was confirmed by XPS and AFM analyses. In addition, the rather circular shape of the protein indicates that FN had a low interaction with the surface and accordingly, adopted a globular conformation. The FN conformation will be further analyzed by ImageJ analyses. In addition, FTIR analyses and ELISA tests will be performed. Finally, FN density, conformation, and bioactivity will be assessed and compared with AgNPs based surfaces in order to evaluate the FN adsorption behaviors on these antimicrobial surfaces.

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