An antibacterial surface coating composed of PAH/SiO₂ nanostructurated films by Layer by Layer

<u>A. Urrutia</u>, P. Rivero, L. Ruete, J. Goicoechea, I.R. Matías, F.J. Arregui. Public University of Navarra (UPNA), Campus Arrosadía S/N, Pamplona, Spain <u>aitor.urrutia@unavarra.es</u>

In this article we propose a novel antibacterial coating composed of SiO₂ and the polymer Poly(allylamine hydrochloride) (PAH) on glass slides by the technique Layer-by-Layer (LbL), see Figure 1. This technique has already used in previous works [1-4], and it has the advantage that it allows to control the construction of nanosized and well organized multilayer films. In other works, it has been studied the antibacterial behaviour of the silica [5]. In this work, the new nanotexturized LbL SiO₂ surface acts as antibacterial agent. The fabricated coatings have been tested in bacterial cultures of genus *Lactobacillus* to observe their antibacterial properties.

LbL coatings were performed by dipping the substrates (glass slides) into alternatively charged solutions, PAH as polycation and SiO_2 as polyanion. This dipping cycle was repeated until a total of 50 (PAH/SiO2) bilayers were constructed. AFM images were obtained from the substrates (see Figure2) in order to characterize the tickness and the surface morphology of the LbL coatings.

Those new surfaces were tested in *Lactobacillus Delbrueckii* bacteria culture to observe their antibacterial activities. *L. Delbrueckii* was inoculated in a "MRS Broth" aqueous medium and incubated at 37° C for 24 hours. The obtained bacterial suspension was shaken and diluted 100000 times. "MRS Agar" was autoclaved at 121° C for 30 min and cooled in sterile Petri-dishes to form a 3mm thick slab. Then 0.1ml bacterial dilution suspension was spread uniformly on the surface of the nutrient agar slab. The substrates coated with PAH/SiO₂ were then placed on the agar slab. Also, clean glass slides like reference were placed (disinfected by dipping in isopropanol and treated by contact flame). Then Petri-dishes were introduced into an incubator for 24 hours at 37°C.

The antibacterial activities against *L. Delbrueckii* of the multilayer (PAH/SiO₂) were carefully measured using an optical method. Figure 3 shows the results in two substrates placed on agar slabs after 24 h. The first one shows one reference substrate where we can observe lots of *L. Delbrueckii* colonies that grow up randomly in the whole agar slab surface. The second one shows one substrate coated with PAH/SiO₂. Here it is possible to see a clear difference between the coated area (there is not growth colonies) and the uncoated area and the rest of the slab (there is growth colonies).

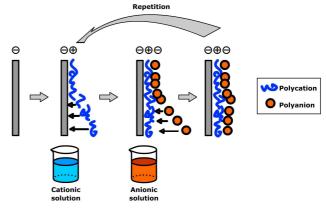
All the experiments were performed in triplicate. The results were analyzed and are represented in average, taking the error as the standard deviation. The treated surfaces reach $90.5 \pm 5\%$ of inhibition effect on the growth of *L. Delbrueckii* after 24 hours. In conclusion, it has been demonstrated these PAH/SiO₂ coating films have a very good antibacterial behaviour against *Lactobacillus Delbrueckii*.

References:

- [1] G. Decher. **277**, (1997), 1232-1237.
- [2] N. I. Kovtyukhova, B. R. Martin, J. K. N. Mbindyo, T. E. Mallouk, M. Cabassi and T. S. Mayer. **19**, (2002), 255-262.
- [3] P. Bertrand, A. Jonas, A. Laschewsky and R. Legras. 21, (2000), 319-348.
- [4] J. W. Ostrander, A. A. Mamedov and N. A. Kotov. 123, (2001), 1101-1110.
- [5] Z. Li, D. Lee, X. Sheng, R. E. Cohen and M. F. Rubner. 22, (2006), 9820-9823.

Figures:

Figure 1: The electrostatic selfassembly or Layer-by-Layer technique



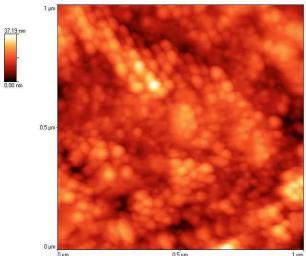


Figure 2: 1x1µm AFM image of the PAH/SiO₂ multilayer.

Figure 3: L. Delbrueckii cultures after 24 hours in: (a) reference substrate and (b) coated substrate.

