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Silica Nanoparticles Assisted Electrochemical Biosensor For The Detection And Degradation Of *Escherichia Coli* Bacteria

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Abstract

An electrochemical biosensor, based on an amplification method using nanoparticles, is being developed for bacteria detection. Firstly, silica nanoparticles with different sizes were synthesized and studied in interaction with *Escherichia coli* cells for their potential toxicity, by atomic force microscopy and viability tests. A critical diameter in the range of 50-70 nm was found under which nanoparticles triggered drastic membrane damage even leading to cell lysis. Secondly, harmless nanoparticles were used in combination with polyelectrolytes as a transducer. A two steps spin coating protocol enabled (i) to immobilize a polyelectrolytes multilayer and (ii) to adsorb a 100 nm – NPs solution at 1g.L⁻¹. Lastly, we plan to conjugate these nanoparticles with antibodies specific to *Escherichia coli* for their selective and sensitive detection through an electrochemical sensor.

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Keywords: bacteria; nanoparticles; toxicity; biosensor; AFM.

1. Introduction

Rapid and selective detection of pathogenic bacteria, eventually followed by their degradation, is of great importance for biomedical, environmental and defense issues. Compared to conventional methods, biosensors integrating nanomaterials, offer rapid, ultrasensitive and cost-effective detection [1]. Though, for the safe

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development of such technique, it is necessary first to understand, at the cellular level, the interactions between bacteria and nanoparticles (NPs).

Among widely used biological systems, *Escherichia coli* (*E. coli*) are the most thoroughly studied Gram-negative bacteria. Along with the cytoplasmic membrane and a peptidoglycan layer, they possess an outer membrane (OM), containing lipopolysaccharides (LPS) molecules and proteins. Diverse nanomaterials (metal oxides, silver...) proved to be bactericidal towards *E. coli* through mainly disruption of the membrane integrity and reactive oxygen species production [2]. Surprisingly, their interactions with silica NPs (SiO_2 -NPs), which proved to be good candidates for biomedical applications (biocompatible, low toxicity) [3] are not well documented despite some toxicity studies relying only on the percentage of dead cells [4].

This work presents the development of a miniaturized electrochemical biosensor (a “lab on chip”) for the detection of *E. coli* bacteria, based on their recognition and elimination by selective functionalized SiO_2 -NPs, which can both detect the specific antigen and induce bacteria stress, even lysis, if necessary. It will first investigate the influence of the size and charge of SiO_2 -NPs on *E. coli* to distinguish between the bactericidal and harmless NPs for the future biosensor. Atomic force microscopy (AFM) is a well suited technique for such study as it allows (i) to observe, both in air and in physiological environment, in real time, biological systems at a nanometer scale, and (ii) to probe their nanomechanical properties. Morphological and rheological AFM studies allow thus to probe the effect of antibacterial agents such as NPs on the cell behavior, the membrane elasticity and integrity [5].

2. Results

2.1. Existence of a size threshold in the action of SiO_2 -NPs⁻ on *E. coli*

In a fundamental study, we first optimized the size of SiO_2 -NPs needed for (i) *E. coli* bacteria detection and (ii) antibacterial activity.

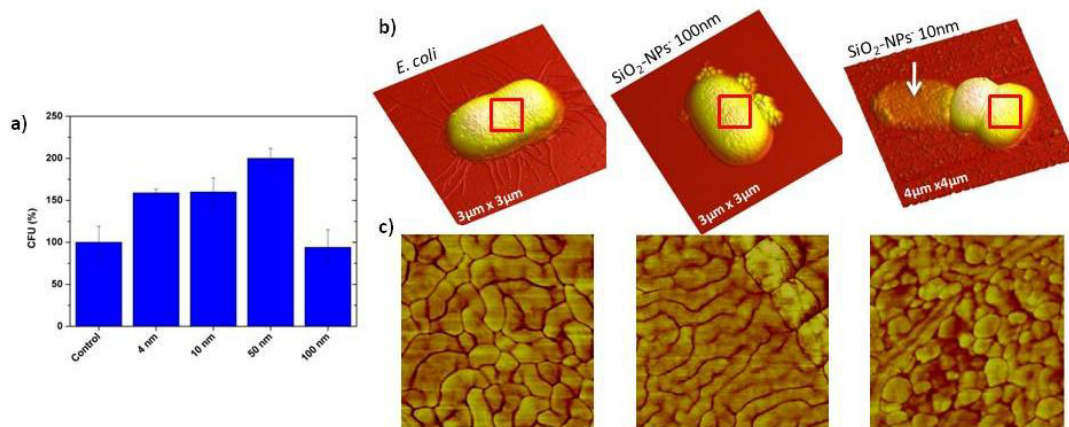


Fig. 1. (a) Viability test of *E. coli* bacteria incubated with SiO_2 -NPs⁻ of different diameters during 2h; (b) AFM height images of *E. coli* without and with SiO_2 -NPs⁻; (c) corresponding zoom-in, in phase images, of the OM structure of *E. coli*.

Antibacterial activity experiments (Fig. 1a) revealed that negatively charge NPs (SiO_2 -NPs⁻) smaller than a critical diameter ϕ_c (50 nm – 70 nm) induce a significant increase in the colony forming units (CFU) capacity of *E. coli*, notably due to cells cluster splitting. Larger SiO_2 -NPs⁻ do not change CFUs nor bacteria population organization, implying their harmless activity, though a potential balance between toxic and splitting effects could be considered. Viability tests offered an overview of the bacteria population but appeared to be insufficient to determine the potential toxicity of SiO_2 -NPs⁻. Consequently, AFM observations allowed for a more detailed study, from hundreds of microns to the nanometer scale. In air, *E. coli* bacteria exhibit a typical rod shape with an average length, width and height of 2.0 μm , 1.2 μm and 300 nm, respectively. Their surface exhibit a domain separation pattern with the presence of nanodomains, referred as “ripples” thereafter, mainly emphasized in phase images (Fig.

1b-c). This separation is attributed to molecules present in the OM of such bacteria, probably LPS [6]. The influence of $\text{SiO}_2\text{-NPs}^-$ size was then investigated on both the morphology and rheology of *E. coli*. Whatever is their size, all $\text{SiO}_2\text{-NPs}^-$ tend to aggregate in a non specific way around cells and on the substrate. $\text{SiO}_2\text{-NPs}^-$ larger than ϕ_c ($\text{SiO}_2\text{-NPs}^-$ 70 and 100 nm in diameter) neither change *E. coli* morphology nor its OM organization, keeping the ripples structure (Fig. 1b-c). Conversely, $\text{SiO}_2\text{-NPs}^-$ smaller than ϕ_c ($\text{SiO}_2\text{-NPs}^-$ 4, 10 and 50 nm in diameter) induce an unusual spherical shape, suggesting the disruption of the peptidoglycan layer integrity. In addition, such small $\text{SiO}_2\text{-NPs}^-$ induce membrane damage: the OM exhibited spherical aggregates (Fig. 1c) as observed in the early ageing of *E. coli* bacteria [6], invaginations and protrusions (results not shown), leading, in turn, to leakage of cellular compounds and cell lysis (Fig. 1b white arrow).

2.2. *E. coli* morphology degradations induced by $\text{SiO}_2\text{-NPs}^+$

Unlike large positively charged NPs ($\text{SiO}_2\text{-NPs}^+$ 100 nm in diameter), the small $\text{SiO}_2\text{-NPs}^+$ ($\text{SiO}_2\text{-NPs}^+$ 30 nm) lead to a strong decrease of CFUs, associated with a strong aggregation of cells (Fig. 2a). Again, viability test do not lead to any conclusion on the toxicity of $\text{SiO}_2\text{-NPs}^+$ as compared to $\text{SiO}_2\text{-NPs}^-$. Electrostatic attractions observed in dark field microscopy are also emphasized in AFM, as shown in Fig. 2b for $\text{SiO}_2\text{-NPs}$ 100 nm: at equivalent diameter, $\text{SiO}_2\text{-NPs}^+$ exclusively accumulate on and around cells, suggesting an increased affinity with *E. coli*. For both $\text{SiO}_2\text{-NPs}^+$ under and above ϕ_c ($\text{SiO}_2\text{-NPs}^+$ 30 and 100 nm in diameter), the OM structure remains organized in ripples domains (Fig. 2c) but drastic membrane damage are observed : extra-membrane aggregates circling cells and membrane invaginations such as pore-like lesions (Fig. 2d arrows) are even more frequent than with small $\text{SiO}_2\text{-NPs}^-$. Consequently, we showed that the size threshold is not the same when changing the charge of $\text{SiO}_2\text{-NPs}$ and that similar cell damage could be due to different mechanisms discriminating the charge and size effects.

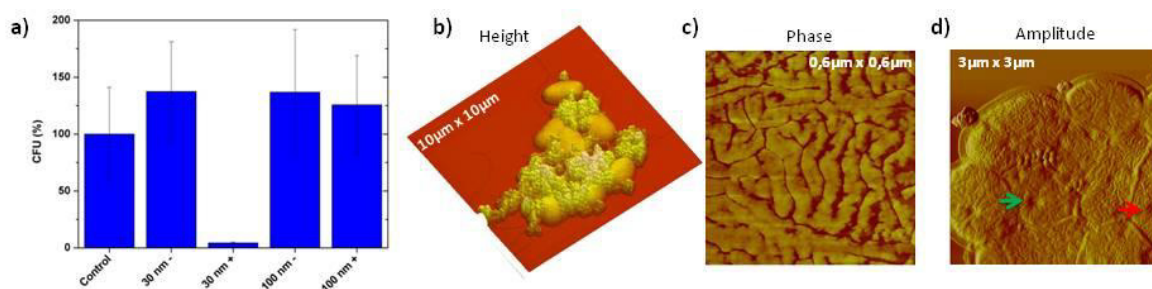


Fig. 2. (a) Viability test of *E. coli* bacteria incubated with $\text{SiO}_2\text{-NPs}^+$ of different diameters during 2h; (b-d) AFM images of different samples of *E. coli* incubated with $\text{SiO}_2\text{-NPs}^+$ 100 nm.

2.3. Conception of the bacteria biosensor

Based on this fundamental work, we integrated harmless $\text{SiO}_2\text{-NPs}$ ($\phi > \phi_c$) in a biosensor for bacteria detection. First, we optimized the functionalization procedure of the transducer on a mica substrate, used for its very low roughness compatible with AFM. Positive (PAH) and negative (PSS) polyelectrolytes were alternatively deposited to immobilize $\text{SiO}_2\text{-NPs}$ used for the further detection, both by spin-coating allowing for highly reproducible results (Fig. 3). The influence of spin speed and spinning time were investigated to achieve (i) the lower roughness for the polyelectrolytes multilayer (PEM) and (ii) the best homogeneous coverage for NPs. Increasing time and speed lead to higher roughness and lower hydrophilicity; optimal PEM is obtained for [45 s; 100 rpm; 200 s^{-2}]. For NPs coverage, the lower the speed, the higher the aggregation; the best coverage is observed for [60 s; 1000 rpm; 200 s^{-2}]. This optimized protocol was successfully applied to functionalize our working nanoelectrodes. Then, antibody-antigen cross linking allowed cells detection. A protocol has been established but still need to be approved: mixing for 2-4 h, at 200 rpm and room temperature, an amino-functionalized $\text{SiO}_2\text{-NPs}$ solution and an anti-*E. coli* antibody solution with a 1000:1 ratio should led to a good conjugation rate. Detection of *E. coli* cells will finally be estimated

through cyclic voltametry and QCM-D measurements by respectively following oxydo-reduction reactions and frequency shifts (mass deposition and dissipation), as in our previous work [7].

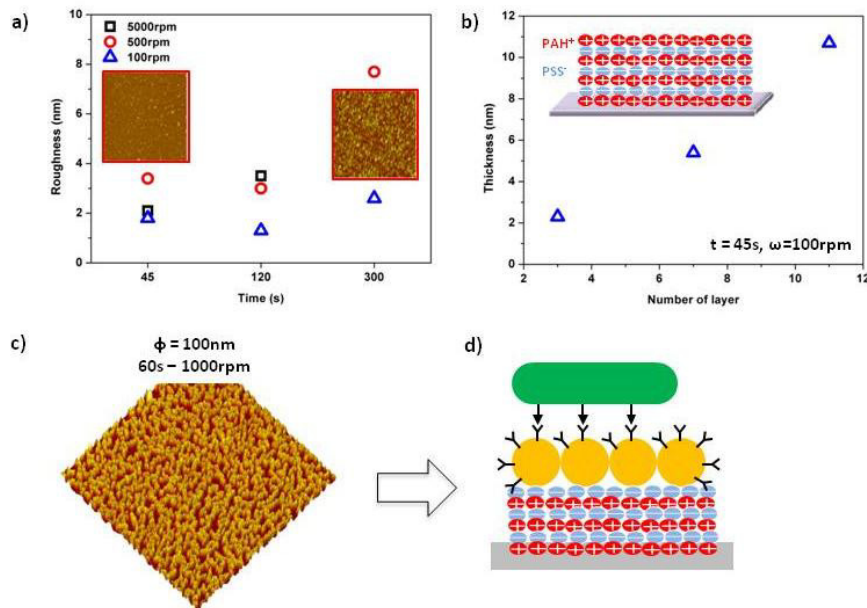


Fig. 3. (a) Influence of spinning time and speed on the roughness of the PEM, part of the biosensor transducer. (b) Influence of the number of polyelectrolyte layers on the thickness of the transducer. (c) AFM height image of the immobilisation of SiO₂-NPs 100 on a PEM (7 layers) ended by PAH. (d) Scheme of the final transducer enabling the specific detection of *E. coli* cells.

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