Nanoparticles for impedimetric biodetection on paper-based substrates

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The recent sanitary crisis has highlighted the need for affordable and rapid biosensors towards pathogen detection and discrimination. In this work, we are combining three tools towards the development of new sensitive and selective biosensor for pathogenic bacteria in water: (1) paper as a valuable substrate that ensures both passive microfluidics and a specific and volumetric capture of the pathogen, (2) impedimetric detection enabling quantification of the analyte concentration, and (3) nanoparticles as labels allowing greater sensitivity towards the target bacteria.

Paper substrates present several assets for biodetection. First, paper has beneficial spontaneous microfluidic properties through capillarity. Second, it facilitates the attachment of bioreceptors which are often proteins (e.g. antibodies), that contribute to specificity towards pathogens. [1] Third, it is low-priced and allows for straightforward manufacturing and disposability. These benefits are already used in lateral flow assays (LFA) such as pregnancy tests and the more recently widespread Covid self-tests [2], in which analytes of interest are passively drained towards the detection zone where they are immobilized by specific bioreceptors. In our work, we extend the use of paper substrates to the handling of complex biological fluids and successfully immobilized bacterial whole-cells thanks to newly discovered specific phage proteins [3]. Figure 1A illustrates the presence of *B. Thuringiensis* in cellulose, while Figures 1B and 1C demonstrate specific immobilization of these bacteria by phage endolysin cell-wall binding domain (CBD).

Impedance measurements applied to paper substrates enable precise assessment of the changes in the electrical properties of the paper volume. To accommodate impedance measurements on paper, we developed two innovative set-ups of detection for *B. Thuringiensis* by applying electrodes on the nitrocellulose membrane surface: either via an external parallel plate setup (Fig. 2A) or the definition of thin-film interdigital electrodes (IDE) on nitrocellulose membranes through metallization with a shadow mask, as shown in Figure 2B. We characterized these new systems by detecting bacterial solutions of different concentrations. Changes in the electrical properties of the volume, induced by the bacteria, enable the differentiation of concentrations down to 10^7 CFU/ml [4].

Gold nanoparticles (AuNP) are used to increase the detection sensitivity in impedimetric measurements since they accentuate changes in electrical properties induced by bacteria [5]. They are synthesized by the Turkevich method [6] (Fig. 3A) and functionalized with phage endolysin receptor binding proteins (RBP) to allow specific binding with the target bacteria (Fig. 3B) [3]. The external parallel plate electrodes set-up was used to characterize the changes induced by the AuNPs presence on the electrical properties of the paper substrate between 1 kHz and 1 MHz. Furthermore, the electrical footprint of biofunctionalization of the nanoparticles was assessed (Fig. 4). The RBP decreases the impedance modulus between 100 kHz and 1 MHz, and has a significant effect on the impedance phase on the whole detection range. When bound to bacteria, gold nanoparticles significantly decrease the phase of the measured impedance between 1 kHz and 20 kHz, highlighting their potential as labels for increasing the system sensitivity over the specified frequency range.

As a result of this work, we highlight that AuNPs offer a significant leverage towards more sensitive and selective impedimetric biodetection scheme in paper-based substrates.

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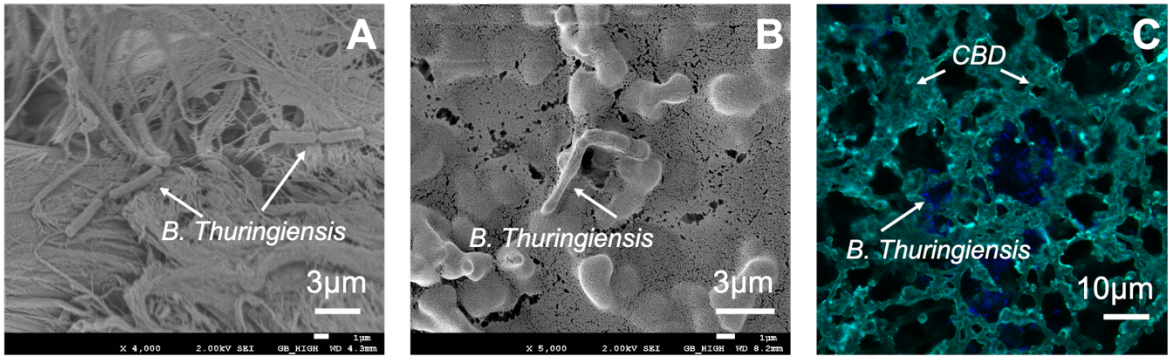


Figure 1. (A) SEM image of *B. Thuringiensis* bacteria attached to a cellulose membrane. (B) SEM image of *B. Thuringiensis* specifically attached to a nitrocellulose membrane. (C) Confocal fluorescence microscopy image of immobilized B. thuringiensis cells (blue) in the pores of a CBD-biofunctionalized nitrocellulose.

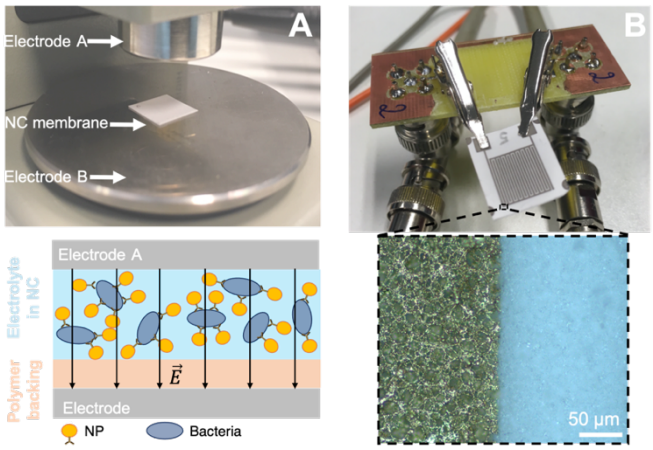


Figure 2. (A) Parallel plate setup: electrode A can be leveled down until contact is made with the nitrocellulose membrane. (B) Gold IDE printed on nitrocellulose through metallization. Optical microscopy showing the Au deposition on the NC membrane.

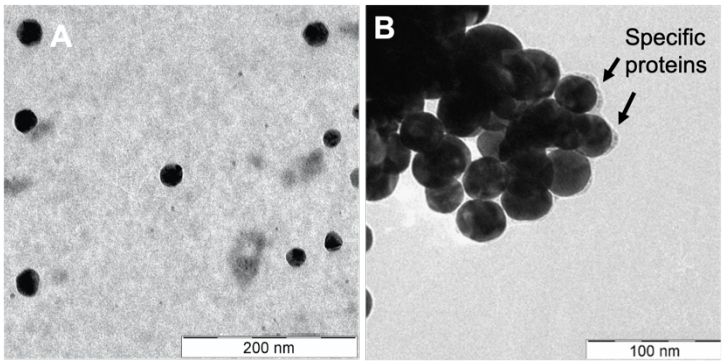


Figure 3. (A) TEM image of AuNP synthesized by the Turkevich method and (B) TEM image illustrating biofunctionalization of NP with specific proteins.

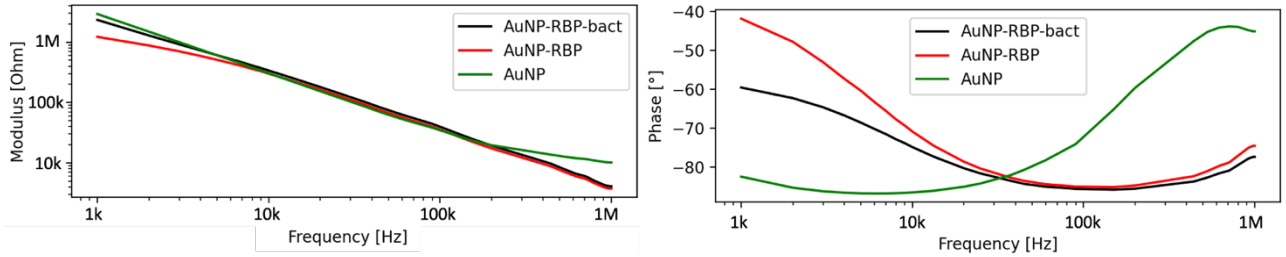


Figure 4. Impedance modulus and phase measured by the parallel plate setup shown in Fig. 2. The presence of bacteria and receptor binding proteins (RBP) significantly alter the impedimetric properties of the paper membrane impregnated with Au nanoparticles solution.