New phage-protein biointerfaces for electrochemical paper-based bacteria sensors

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This work presents the development of novel biointerfaces for electrical paper-based biosensors.

Paper-based sensors such as lateral flow assays (LFA) provide inexpensive platforms for fabrication of simple, portable and disposable tools for environmental monitoring, in particular, in resource-limited settings. Common LFA tests rely on the use of antibodies as a specific bioreceptors and nanoparticles for colorimetric pathogen detection. However, the porous nature of paper offers the possibility for more precise electrical detection of pathogens in the volume of the paper, overcoming the limitations experienced by colorimetric tests (semi-quantitative detection) and usual planar electrochemical biosensors (low number of bacteria attached to the biosensor).

In this work, we first propose the use of innovative phage-proteins to be alternative bioreceptors of the expensive antibodies commonly used in LFA applications. Phage proteins show strong affinity and high specificity towards target bacteria. In particular, new phage proteins encoded by Deep-Blue, a bacteriophage targeting *B. thuringiensis*, were recently discovered [1]. Protocols for biofunctionalization of the nitrocellulose membrane and for the conjugation of the gold nanoparticles composing the LFA with the proteins encoded by Deep-Blue were developed and optimized. We then show successful capture of whole-cell *B. thuringiensis* in nitrocellulose (Figure 1), and specific detection of the bacteria by gold nanoparticles (Figure 2), suggesting the use of phage proteins to develop innovative, low-cost and highly specific LFA.

Then, we accommodated innovative electrical probes to nitrocellulose membranes to allow for the development of paper-based detection platforms allowing joint volumetric electrical or optical measurements. On the one hand, interdigital electrodes were deposited directly on the nitrocellulose membranes (Figure 3). They are characterized and validated to detect cell *B. thuringiensis* captured in the membrane volume by means of electrochemical impedance spectroscopy. On the other hand, we validate and demonstrate the relevance of simple, external electrodes to detect *B. thuringiensis* cells captured in the membranes. This last plug-and-play electrode setup eliminates the unpractical need to integrate electronics components directly onto the paper substrate. Future works are conducted to miniaturize the electrodes and accommodate their geometry to LFA, ultimately leading to the development of electrical LFA detection scheme for efficient and reliable point-of-care quantitative detection of pathogens in the environment.

References

[1] Hock L., Leprince A., Tournay M., Gillis A., Mahillon J. 2019. *Food Control*.

Figures

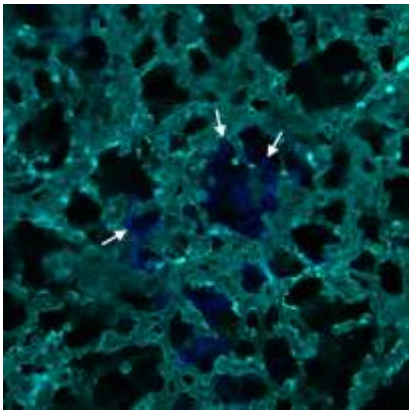

Figure 1. Confocal fluorescence image of immobilized B. thuringiensis cells (blue, indicated by white arrows) on a phage protein-biointerfaced nitrocellulose membrane (turquoise).

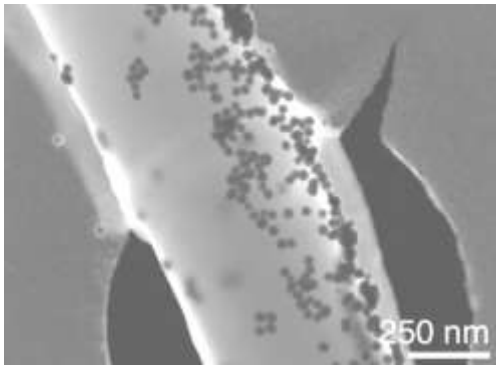

Figure 2. SEM images of bacteria incubated with conjugated gold-nanoparticles (AuNPs). AuNPs were covered by phage proteins to specifically detect the target bacteria.

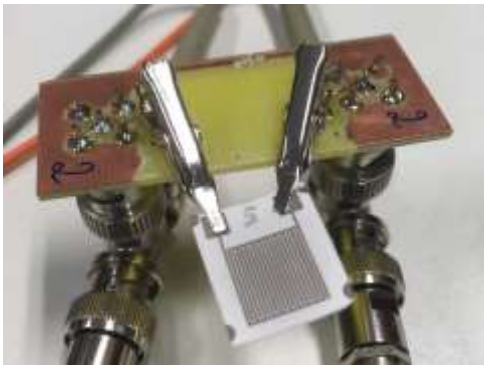

Figure 3. Electrical detection setup consisting in interdigital electrodes directly applied on the nitrocellulose membrane. Combining fringing-field electrodes and porous membrane allows for volumetric electrical detection of bacteria.