

Milk pathogens trapping with nanoparticles and detection by QCM

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The dairy sector can be faced with contamination of raw materials and transformed products by pathogenic bacteria [1]. Existing methods take a long time to detect the absence of contamination by these bacteria, ranging from one to several days depending on the case. In addition, these methods must have very low detection thresholds, regarding the microbiological criterion for these micro-organisms of “absence of pathogen in 25 g of product”.

Therefore, and in order to release safely the dairy production or in the contrary to stop production or distribution of contaminated products, it is essential to set-up a method that can quickly confirm the absence of these pathogenic germs in the products.

In this context, the overall aim of the DEPLASP-BAAG project (**DE**veloppement d’une **PL**ateforme de détection **rA**pide et **SP**écifique de **BA**ctéries sur matrices complexes **AG**roalimentaires) is to significantly reduce the time to obtain the first negative result by improving the two main stages in the analytical process: the enrichment phase that must be reduced by optimizing the microbiological protocols and the detection by quartz crystal microbalance (QCM) that must be improved.

The joint optimization of these two stages will, in one hand, significantly reduce the time of enrichment by optimizing the growth conditions, and in a second hand, lower the QCM limit of detection by weighing down the bacteria thanks to a complex formation with heavy nanoparticles.

To achieve this, we will use two types of nanoparticles, functionalized with anti-*Listeria monocytogenes* antibodies: magnetic iron oxide particles (MNPs) and gold nanoparticles (GNPs). MNPs will be used for the capture and the ‘artificial’ concentration of the targets in the sample, using a magnet, to obtain a target-concentrated sample. [2] Then, GNPs, will be used as amplifiers in a second capture step, to weigh down the bacteria-MNP complex, making the bacteria-MNP-GNP complexed easier to be detected with the QCM than a bacterium on its own ($\rho_{\text{bacteria}} = 1.4 \text{ g/cm}^3 - \rho_{\text{GNP}} = 19.7 \text{ g/cm}^3$). [3,4] Trapping the bacteria-MNP-GNP complexes using the functionalized QCM surface will lead to the creation of a ‘sandwich’ type device, Figure 1.

In this presentation, I will show you the first experiments and results obtained to improve each step mentioned above:

Surface functionalization:

The aim is to capture efficiently targets in a sample. For that, we used polyclonal anti-*L. monocytogenes* antibodies grafted on flat gold surfaces (i.e. electrodes on piezoelectric quartz for QCM measurements). We transposed it then to functionalized GNPs and we adapted it for MNPs that are already coated with -COOH groups. Characterization was performed for each functionalization step by FT-IR and XPS spectroscopy to validate the protocols.

Capture on flat surfaces:

Bacteria capture was performed on flat functionalized gold surfaces in static (contact tests) and dynamic (capture with QCM) conditions. To show the specificity and the efficacy of the ligand used for the capture, crystal violet dying and optic microscopy observations were done.

Capture on nanoparticles:

Target trapping with functionalized nanoparticles (MNPs and GNPs) with anti-*L. monocytogenes* antibodies was carried out, and the bacteria-NP complexes were observed by scanning electron microscopy.

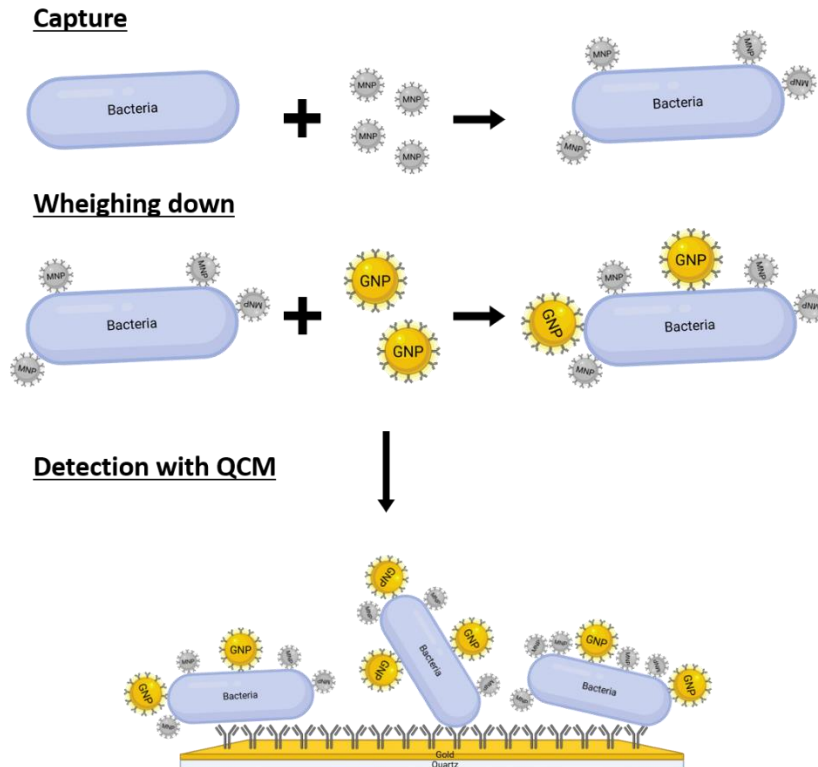


Figure 1: Sandwich type device for pathogens-particles complexes trapping on functionalized QCM quartz

References :

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