The QCM Detection of Bacillus atrophaeus spores enhanced by magnetic particles

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Detection of bio-aerosols is very important especially in relation to the threat of attacks using biological warfare agents (BWAs). The possibility for using anthrax spores as a BWA is quite high, as was shown in the attacks in 2001. The anthrax spores are serious threat for people health and security. The early detection is thus extremely relevant for the immediate warning and deployment of treatment. The detection of BWAs in the form of aerosols is challenging due to the low concentration of microbes; usually, a suitable air-sampling system - cyclone is used to collect all particles from air into a small volume of liquid. The main goal of this study is improvement of limit detection by further preconcentration of target agents with using magnetic (nano)particles (MPs).

Methods

The bacterial strain used in this study was Bacillus atrophaeus (ATCC 9372) – also called Bacillus subtilis subsp. niger or B. globigii (BG). Bacillus atrophaeus is Gram-positive endospore-forming bacterium. Very often it is used as non-pathogenic surrogate of Bacillus anthracis. The endospores are dormant form and extremely resistant to stresses.

All measurements were done in home-made flow-through cell. Phosphate buffered saline (PBS) was used as carrier of samples. After stabilization of the baseline, the samples were flown through and signal was measured in real time. The magnetic particles were used as mass enhancers by QCM measuring as mass label [1]. Here is presented direct capture and preconcentration of spore targets. 1 mL of spore suspension (10^7 CFU) was incubated with 10 µl of MPs for 30 minutes. The MPs were magnetically separated and suspended in 0.4 mL.

Fabrication of QCM based immunosensor

Polyvalent antibodies were immobilized on the SAM (self-assembled monolayer) and MPs for 30 minutes. The MPs were magnetically separated and suspended in 0.4 mL.

Synthesis of magnetic particles (PEG-MPs)

Magnetic particles were prepared by co-precipitation method at pH value 11.0. PEG-2.000 was added in solution as emulsifier. The black product was separated by magnetic field and aggregated during preconcentration procedure.

Preparation of particles conjugates

Streptavidin coated MPs (Strep-MPs) were conjugated with biotinylated polyclonal antibodies. Amine terminated MPs (NH₂-MPs) and PEG-MPs were conjugated with antibodies via glutaraldehyde. The Schiff bases were reduced with sodium cyanoborohydride. The protein A and polyclonal antibodies were cross-linked using DMP (dimethyl pimelimidate). To reduce nonspecific reactions of remaining free aldehyde groups, the surface was finally incubated with ethanolamine.

Preparations

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References


Acknowledgements

Fig. 1: The atomic force microscopy (AFM) images of MPs. Scan of (A) PEG-MPs with a mean diameter about 70 nm; (B) NH₂-MPs mean diameter about 75 nm; (C) Strep-MPs mean diameter about 90 nm. Scan size 3 µm × 3 µm.

Fig. 2: Schematic of QCM sensor with bio-recognition layer and bacterial target pre-concentrated with using immuno-MPs (not in the scale).

Fig. 3: The real time response of relative frequency shifts during the injection of calibration spore suspensions and MPs with captured spores. NH₂-MPs are not shown because strongly aggregated during preconcentration procedure.

Conclusions

The application of immuno-magnetic particles for preconcentration of model samples of bacterial spores was presented. The capture procedure was simplified and advanced. The suspensions near to limit of detection were concentrated and analyzed by QCM immunosensor.