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An Aptasensor Based on Magnetic Relaxation Switch and Controlled Magnetic Separation for the Sensitive Detection of Pseudomonas aeruginosa

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Abstract : Pseudomonas aeruginosa is a Gram-negative, aerobic, opportunistic human pathogen that is present in the soil, water, and food. This microbe has been recognized as a representative food-borne spoilage bacterium that can lead to many types of infections. Considering the casualties and property loss caused by P. aeruginosa, the development of a rapid and reliable technique for the detection of P. aeruginosa is crucial. The whole-cell aptasensor, an emerging biosensor using aptamer as a capture probe to bind to the whole cell, for food-borne pathogens detection has attracted much attention due to its convenience and high sensitivity. Here, a low-field magnetic resonance imaging (LF-MRI) aptasensor for the rapid detection of P. aeruginosa was developed. The basic detection principle of the magnetic relaxation switch (MRSw) nanosensor lies on the 'T_2-shortening' effect of magnetic nanoparticles in NMR measurements. Briefly speaking, the transverse relaxation time (T_2) of neighboring water protons get shortened when magnetic nanoparticles are clustered due to the cross-linking upon the recognition and binding of biological targets, or simply when the concentration of the magnetic nanoparticles increased. Such shortening is related to both the state change (aggregation or dissociation) and the concentration change of magnetic nanoparticles and can be detected using NMR relaxometry or MRI scanners. In this work, two different sizes of magnetic nanoparticles, which are 10 nm (MN_{10}) and 400 nm (MN_{400}) in diameter, were first immobilized with anti- P. aeruginosa aptamer through 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS) chemistry separately, to capture and enrich the P. aeruginosa cells. When incubating with the target, a 'sandwich' (MN_{10}-bacteria-MN_{400}) complex are formed driven by the bonding of MN400 with P. aeruginosa through aptamer recognition, as well as the conjugate aggregation of MN_{10} on the surface of P. aeruginosa. Due to the different magnetic performance of the MN_{10} and MN_{400} in the magnetic field caused by their different saturation magnetization, the MN_{10}-bacteria-MN_{400} complex, as well as the unreacted MN_{400} in the solution, can be quickly removed by magnetic separation, and as a result, only unreacted MN_{10} remain in the solution. The remaining MN_{10}, which are superparamagnetic and stable in low field magnetic field, work as a signal readout for T_2 measurement. Under the optimum condition, the LF-MRI platform provides both image analysis and quantitative detection of P. aeruginosa, with the detection limit as low as 100 cfu/mL. The feasibility and specificity of the aptasensor are demonstrated in detecting real food samples and validated by using plate counting methods. Only two steps and less than 2 hours needed for the detection procedure, this robust aptasensor can detect P. aeruginosa with a wide linear range from 3.1×10^2 cfu/mL to 3.1×10^7 cfu/mL, which is superior to conventional plate counting method and other molecular biology testing assay. Moreover, the aptasensor has a potential to detect other bacteria or toxins by changing suitable aptamers. Considering the excellent accuracy, feasibility, and practicality, the whole-cell aptasensor provides a promising platform for a quick, direct and accurate determination of food-borne pathogens at cell-level.

Keywords : magnetic resonance imaging, meat spoilage, P. aeruginosa, transverse relaxation time
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