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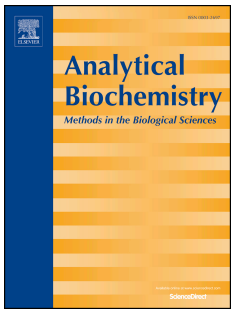

Label-free and simple detection of endotoxins using a sensitive LSPR biosensor based on silver nanocolumns

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Label-Free and Simple Detection of Endotoxins Using a Sensitive LSPR Biosensor Based on Silver Nanocolumns

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This paper describes the construction of a silver-based LSPR biosensor for endotoxin detection. We used GLAD method to procure reproducible silver nanocolumns. In this work, the silver nanostructures were considerably stabilized by a SAM of MPA, and the limit of detection of biosensor was measured to be 340 pg/ml for endotoxin *E.coli*. Considering endotoxin *B.abortus* as the second type of endotoxin contamination in our target samples (HBs-ag produced in Institute Pasteur, Iran), we investigated selectivity of the biosensor in various experiments. We showed that this biosensor can selectively detect both types of endotoxins compared to other biological species. Overall, this study proposes that LSPR biosensing can be considered as a sensitive, simple, and label-free method for endotoxin detection in the quality control laboratories.

Keywords: LSPR Biosensor, Endotoxin, Label-Free, Silver Nanocolumns, GLAD Method

Abbreviations: LSPR; Localized Surface Plasmon Resonance, GLAD; Glancing Angle Deposition, SAM; Self Assembled Monolayer, MPA; Mercaptopropionic Acid.

Introduction

Lipopolysaccharide (LPS), also known as endotoxin, is a highly toxic component that exists in the outer membrane of gram negative bacteria. It is released into the environment during every phase of bacterial growth cycle(1), so it causes contamination of a wide range of biopharmaceutical products. Even small quantities of endotoxin injected to human body can result in fever, septic shock, and death(2). Therefore, it is highly important to detect and also quantify endotoxin of biopharmaceutical products in quality control laboratories. The most validated method used for endotoxin detection is Limulus Amebocyte Lysate (LAL) test. Although this method is sensitive, it has some unavoidable drawbacks such as highly dependency on temperature, long incubation times to access the results, hard multistep preparation procedures and being expensive(3). These problems forced research groups all around the world into working on novel methods of endotoxin detection based on biosensors.

Biosensing is a process in which a biological element interacts with an analyte, and then the result of the interaction is sent to a transducer as a response. There are several published papers which used biosensors for endotoxin detection. The researchers have used all the electrochemical (4-16), optical (3, 17-21) and mass-based(22) approaches of biosensing. Biosensor miniaturizing, improving sensitivity, lowering expenses and increasing simplicity are the most important objectives that have been investigated by these researchers. In order to reach these objectives, we suggested using an LSPR biosensor for endotoxin detection.

In recent years, LSPR biosensors are gaining importance due to their interesting detection characteristics. LSPR is a hallmark of noble metal nanoparticles that caused by collective oscillation of the conduction electrons at nanoparticles surface. In LSPR biosensors, interaction of biomolecules at the surface of sensor leads to a change in the refractive index of surrounding medium. As a result, a change occurs in the LSPR peak wavelength of the extinction spectra. Therefore, the wavelength shift ($\Delta\lambda_{max}$) represents the detection of analytes in these biosensors.

In this paper, we constructed an LSPR biosensor based on silver nanocolumns for detection of endotoxin. For deposition of silver nanocolumns, we used GLAD method due to its low cost and ability to control shape of nanoparticles (23). In addition, as expected, nanostructures produced by this method are notably reproducible. A polycationic peptide, Polymyxin B (PmB), has been used as a biorecognition element to interact with endotoxin. We chose PmB, because it is more stable, more homogeneous, and less expensive (˜2000*less) than other conventional peptides and aptamers used as biosensing element(7,24). Moreover, due to its high positive charge, PmB has considerable affinity to endotoxin molecules and can neutralize endotoxin toxicity. SAMs of MPA and EDC-NHS was performed on the surface of silver nanoparticles in order to stabilize the silver nanoparticles. Furthermore, SAMs assist efficient immobilization of PmB molecules onto the biosensor surface.

We chose LSPR method for many reasons: 1.LSPR based biosensors can be used in the extreme miniaturized scale(25), because in LSPR biosensors, every sensing site is as big as the spectrometer light emitted to the surface of nanostructures (which is a circle with 2mm radius in the present work). 2.Despite electrochemical biosensors, these sensors are not affected by ionic properties of sample solutions, so they are convenient for biological samples and clinical usages(26). 3.In LSPR biosensors, the interaction between sensing element and analyte, per se, leads to a signal transduction, so we do not need any labeling procedure which is cumbersome and time consuming. 4.To our knowledge, this would be the first step in using refractive index LSPR biosensors for detection of endotoxin. Therefore, the feasibility and advantages of this method for endotoxin detection would be explored for the first time.