Magnetically assisted SERS-based immunoassay for monitoring human stress levels

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Cortisol is a steroid hormone that plays a key role for regulating anti-stress responses of human body. For this reason, cortisol is also called the 'stress-hormone'. Even though cortisol response to stress is essential for survival, its abnormal levels represent serious health risks (e.g. Cushing's and Adison's disease for higher and lower cortisol levels, respectively) [1]. Since the determination of cortisol levels in biological fluids can serve as a biomarker for these diseases, an accurate analysis represents a crucial task for human health care [1]. Additionally, as a consequence of the increasing levels of physiological stress (because of globalization, living style and competition), the development of reliable, sensitive (concentrations in the order of ng/mL) and selective (biological complex matrices) methods capable to quantify cortisol at physiological relevant concentrations are required [2]. In this work, we have developed a novel cortisol biosensor by combining a highly sensitive analytical technique (surface-enhanced Raman spectroscopy, SERS) and a highly specific assay (competitive immunoassay). To fabricate the gold nanotags, three types of nanoparticles shapes were tested (spheres, rods and stars) and compared in terms of sensitivity using three different lasers (532, 633 and 785 nm). The Raman reporter used was 4-mercaptobenzoic acid combined with SH-PEG-COOH (M_v=5000) to modify the nanosurface in order to obtain stable nanoparticles for further surface modification. BSA liked to cortisol was covalently linked onto the nanoparticles surface via EDC/NHS reaction with the -COOH groups. Since stars shaped nanotags showed the greatest sensitivity, they were the ones used in all the immunoassay experiments. Regarding to the capture agent, magnetic beads (MBs) of ~800 nm diameter, with –COOH groups onto the surface, were used to enable a simple and fast separation. Cortisol monoclonal antibody was covalently liked to the MBs beads via EDC/NHS reaction with the -COOH groups, thereby promoting well-oriented linkage of the antibodies. The experimental conditions to carry out the immunoassay probe were optimized to maximize the sensitivity and the reproducibility. Under the optimized conditions, the proposed method was applied to quantify cortisol in biological fluids, particularly urine and serum. The percent of cortisol recoveries in these samples were ranged between 85 and 94%, thus demonstrating a good accuracy. Additionally, there were no significant differences between these recovery values and those obtained using a well-developed analytical technique (high performance liquid chromatography coupled to mass spectrometry, HPLC-MS). Nevertheless, the proposed immunoassay exhibited some advantages over HPLC-MS, such as: (1) better limit of detection, (2) minimal sample preparation, and (3) lower duration of sample analysis. Therefore, the proposed method is suitable for monitoring human stress levels and presents a great potential for point of care applications.

References

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