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Peptide-assembled graphene oxide as fluorescent turn-on sensor for ultrasensitive Lipopolysaccharide (Endotoxin) detection

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ABSTRACT

Introduction: Lipopolysaccharide (LPS), or endotoxin, a major component in the outer cell membrane of Gram-negative bacteria is a very powerful and toxic inflammatory stimulator, resulting in sepsis or septic shock, a significant medical problem affecting about 700 000 patients and causing 250 000 casualties annually in the United States itself. The detection of LPS is highly importance. However, the currently used enzymatic limulus amebocyte lysate assay is highly susceptible to changes in temperature and pH, interference factors, and requires cumbersome sample preparation. A more cost-effective, sensitive and robust detection method is needed.

Objective: To design and develop biosensor for LPS detection by assembling a LPS-binding peptide (as LPS receptor) with graphene oxide (GO, as fluorescence quencher).

Methods: GO was synthesized using a modified Hummer's method. A synthetic LPS-binding peptide was designed, fluorescent labelled, and assembled with GO in PBS buffer solution. The fluorescence recovery of the peptide-GO was measured upon addition of LPS from Gram negative bacteria: *E. coli*, *K. pneumoniae, Samonella Thyphosa, P. aeruginosa*, as well as living pathogenic bacteria. Specificity tests were conducted with various biological molecules to evaluate the sensing performance.

Results & Discussion: Specific binding of LPS with peptide release the peptides from GO, resulting in fluorescence recovery, allowing ultrasensitive detection of LPS with the limit of detection of 130 pM, the most sensitive synthetic LPS sensors to-date. The LPS sensor is highly selective to LPS than other biological species.

Conclusion: We developed a peptide-GO assembled fluorescence sensor for ultrasensitive and specific LPS/endotoxin detection. This is the most sensitive synthetic LPS sensor reported in the world.

Identification of foetal DNA in maternal plasma using semi-nested PCR: A possible approach for non-invasive prenatal diagnosis

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ABSTRACT

Introduction: Prenatal diagnosis using foetal DNA is essential for detection of the foetal genotype in life threatening genetic disorders such as thalassaemia. Individuals with β-thalassaemia major require regular blood transfusions for survival. Epigenetic studies have revealed differences in methylation status in CpG sites between foetal and maternal DNA. The objectives of this study are to identify informative CpG sites in the β-globin gene and develop a semi-nested methylation-specific PCR for amplification of chorionic villi (CV) DNA (trophoblasts that contribute to foetal DNA).

Methods: Extracted DNA from 15 maternal blood and their respective CV samples were subjected to bisulfite conversion. The β-globin gene containing three selected CpG sites and the β-gene mutation at codon 41/42 were amplified using a pair of primers (F1 and R1). Amplicons were sequenced and the methylation status of each CpG site of the converted maternal (cM) was compared to the converted CV (cCV) DNA. Three rounds of semi-nested PCR using three methylation-specific reverse primers (R2, R3 and R4) were amplified in succession with a common forward primer (F1).

Results & Discussion: Sequencing results confirmed the three CpG sites were hypermethylated in cM DNA and only partially methylated in cCV DNA. The semi-nested PCR showed amplification in 11 cCV DNA while no band was observed in all maternal DNA. The sensitivity and specificity of this approach is 73% and 100% respectively.

Conclusion: Three rounds of semi-nested PCR on cM and cCV DNA have allowed the identification of circulating foetal DNA in maternal plasma. This technique may be utilised for non-invasive prenatal diagnosis of specific β-globin gene mutations from foetal DNA in maternal plasma.