Optimization and Characterization of Nanoparticles for Nanotechnology-Enhanced ELISA in FKBPL Detection and Preeclampsia Monitoring

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Preeclampsia is a major pregnancy disorder that is characterized by the new onset of hypertension, proteinuria, or another organ damage, including the placenta. It is the leading cause of mortality and morbidity in pregnancy, yet no reliable monitoring exists for all phenotypes of preeclampsia, especially late-onset preeclampsia. FKBPL, an emerging predictive and diagnostic biomarker for preeclampsia, is a critical regulator of angiogenesis and immune modulation [(1)]. However, FKBPL is tightly regulated during pregnancy and lowly secreted in the blood. Previous work has increased the limit of detection of FKBPL in the point of care setting by one order of magnitude using upconversion nanoparticles compared to commercially available ELISA[(1)]. In this study, we aim to develop and validate a nanotechnology-enhanced ELISA that employs liposome nanoparticles. The high sensitivity ELISA utilizing liposomes is enabled through signal amplification attributed to the encapsulated enzymes or fluorophores in their carriers that improves detection limits. Larger surface area created with liposomes facilitates higher binding capacity between antibodies and antigens circulating in plasma, thus enhancing the sensitivity and specificity of the assay. Liposome-based signal amplification techniques thus allow very specific measurement of biomarkers at lower concentrations. To assess its potential for improving the sensitivity of ELISA for the detection of FKBPL, liposome nanoparticles were synthesized and characterized. A response surface methodology (RSM) termed the Box-Behnken design (BBD) was applied to optimize the formulation of liposomes by assessing the impact of numerous independent variables on critical formulation characteristics. Three independent variables were optimized for formulation: the phospholipid-to-cholesterol ratio, lipid concentration, and sonication time. Formulation sizes varied between 95 and 200 nm, the PDI between 0.2 and 0.5 and the zeta potential between -30 and -60 mV. The optimal formulation was achieved with a zeta potential of -41 mV, a PDI of 0.26, and a particle size of 100 nm. Dynamic light scattering (DLS) and Nanoparticle Tracking Analysis (NTA) were employed to evaluate their physicochemical characteristics, including size, polydispersity index (PDI), and zeta potential. The morphology of liposomes was confirmed by scanning electron microscopy (SEM). The favourable physicochemical features of these nanoparticles are highly suitable for applications in ELISA through efficient antibody conjugation, prevention of aggregation, and amplification of sensitivity. With bioconjugation efficiency, size control, and stability of these nanoparticles, they are well suited for early diagnosis of preeclampsia and perinatal health monitoring that will enhance detection of FKBPL.

Keywords: Preeclampsia, FKBPL Elisa, Liposome, Box-Behnken

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