Conjugation of titania and silver nanohybrid: a new label for enhancing lateral flow biosensors

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Paper-based lateral flow (LF) biosensors are a very significant tool, providing a low-cost and rapid diagnostic evaluation of the result by the naked eye, with high sensitivity, good selectivity and low amount of sample volume being required [1]. Ongoing research seeks to utilize novel and innovative nanomaterials able to achieve a better limit of detection (LOD), thus improving the analytical performance of traditional lateral flow immunoassays, typically based on gold nanoparticles [2]. For this purpose, a novel nanohybrid system was developed through the direct conjugation of commercially available titania anatase nanoparticles with silver nanoparticles (TiO_2-AgNPs). This nanoplatform has great potential to be used, for the first time in LF, to detect human immunoglobulin (HIgG), which serves as a model protein for the study. To obtain the nanohybrid, first the surface of 25 nm-sized TiO_2NPs was covalently functionalized with the linker 3-(mercaptopropyl)trimethoxysilane (MPTMS), a bifunctional ligand that provides terminal -SH groups suitable for attachment to the silver (Ag) surface. The quantification of free -SH groups on titania surface was quantified by using a rhodamine-based dye. Then, *in-situ* formation of silver nanoparticles occurred in the presence of TiO_2NPs bearing -SH groups. In this process, different amounts of Ag^+ precursor were used and just one TiO_2NPs/Ag weight ratios was chosen based on the best size, stability and darker colour for the final lateral flow application, i. e., TiO_2-AgNPs = TiO_2NPs:Ag 1:2.5 wt/wt. Thus, silver nitrate interacted with sodium borohydride as reducing agent and 3MPS (3-mercapto-1-propanesulfonate) as a stabilizer. The 3MPS imparted negative surface charges to the nanoparticles preventing their aggregation, resulting in a hydrophilic nanohybrid. Extensive characterization techniques were employed to investigate structure-property correlations, colloidal stability, and the extent of titania surface decoration, including UV-vis, ATR, SEM-EDX, DLS, and ζ-potential. The obtained results demonstrate the successful silanization of TiO_2NPs and the subsequent *in-situ* decoration of AgNPs-3MPS on their surfaces. Lastly, the interaction between TiO_2-AgNPs and goat anti-human IgG-HRP (HIgG-HRP) was investigated before considering its potential as a new label in LF assays. A colorimetric reaction between the enzyme horseradish peroxidase (HRP) and the substrate 3,3',5,5'-tetramethylbenzidine (TMB) was used to evaluate this interaction. The substrate oxidizes from TMB to TMB^+ in the presence of HRP, turning from colorless to blue. To stop the HRP-mediated oxidation reaction, the oxidative conversion of TMB^+ to $TMB2^+$ was then induced by H_2SO_4 (0.1M), again changing the color from blue to yellow. Different concentrations of HIgG-HRP, ranging from 2 to 30 ug/mL, were tested to optimize the conjugation process. The performance of TiO_2-AgNPs nanohybrid in LF assays is currently under evaluation.

References

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