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DEVELOPMENT OF HEPARINASE NANOSENSORS FOR METASTATIC CANCER CELLS DETECTION

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Heparin sulphate is closely related to heparan sulphate that plays a role in extracellular matrix modulation. Heparinase is a bacterial enzyme that breaks down heparin and heparan sulphate into small fragments. Heparanase is the mammalian enzyme that cleaves heparan and heparin sulphate but at more restricted regions. Detection of heparanase is quite important in early diagnosis of metastasis and cancer spreading. Herein, heparin sulphate was attached to the surface of silica nanoparticles (NPs) via intrachain carboxylate conjugation using cabodiimide chemistry. FRET based quenching was utilised to detect the fluorophore fluorescence changes using two different models. Model I adopted the attachment of fluorescein labelled heparin to the surface of black hole quencher 1-incorporated NPs. The achieved quenching efficiency was ~30% compared to fluorescent heparin attached to blank NPs as a control. In model II, BHQ 2 conjugated heparin was attached to the surface of core-shell NPs containing TAMRA in the shell and either blank core or 7-Methoxycoumarin incorporated core. NPs with different shell thicknesses were prepared ranging from 12 nm to 75 nm. The quenching efficiency of the 12 nm shell-NPs was ~ 42% using blank core. The quenching efficiency of 12 nm shell was also tested using Comarin incorporated core as internal standard to achieve more accurate results. In this system ratiometric method was developed, where the quenching efficiency was calculated based on the florescence ratio of TAMRA in the shell to coumarin in the core. The calculated quenching efficiency was ~ 10% which is lower than that predicted by blank core system. The developed system could be further employed as sensing tool for heparinase and heparanase to detect metastatic cancer cells.

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