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Oriented antibody conjugation on fluorescence dye-doped silica nanoparticles for targeted *in vivo* imaging

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Abstract

This work demonstrates the use of Cy5-doped silica nanoparticles (Cy5-SiNPs) with oriented conjugation of monoclonal antibodies against epithelial cell adhesion molecule (EpCAM), a biomarker for colorectal cancer, for targeted *in vivo* imaging. The antibody conjugation was performed on Cy5-SiNPs that were previously coated with a layer of protein G. The Protein G serves as a linker controlling antibody orientation due to their site-specific interaction with the constant domain (Fc) of Immunoglobulin G (IgG). This conjugation method allows the binding sites (Fab) of the antibodies to be facing outward, thus maintaining the conjugates affinity to bind to the target (EpCAM). The affinity of the oriented conjugates was compared with that of conjugates derived from a conventional EDC coupling by *in vitro* analysis using confocal fluorescence imaging and flow cytometry. The result demonstrated that the oriented conjugates provided 12 times higher sensitivity to bind to the target cells (HT-29) than that of the conjugates prepared by conventional EDC method. *In vivo* fluorescence imaging in mice bearing HT-29 tumor xenograft indicated time-dependent accumulation of the oriented conjugates at the tumor site and prolong fluorescence signal retention up to 14 days post-injection. This research demonstrated that the Cy5-SiNPs with oriented antibody conjugation developed herein can improve the sensitivity of *in vitro* analysis and can be successfully applied for targeted fluorescence *in vivo* imaging.

Keywords: antibody conjugation; protein G; fluorescence imaging; *in vivo* imaging; colorectal cancer detection

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