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

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Here we developed a fluorescent probe for *in vivo* colorectal cancer detection using Cy5-doped silica nanoparticles (Cy5-SiNPs) conjugated to monoclonal antibody (mAb) with controlled orientation. Monoclonal antibody specific to anti-epithelial cell adhesion molecule (EpCAM), a cell surface protein overexpressed in colorectal carcinoma, was conjugated on the Cy5-SiNPs coated with protein G layer. The site specific interaction between protein G and constant domains (Fc) of the antibody allowed for oriented immobilization of the antibody with binding sites (Fab) facing outward. As a result, the target binding affinity of the antibodies is maintained. The targeting efficiency of the Cy5-SiNPs with oriented mAb conjugation demonstrated 8 times higher sensitivity than Cy5-SiNPs with randomly conjugated mAb for *in vitro* detection of HT-29 cells using confocal fluorescence imaging and flow cytometry. *In vivo* targeting efficiency of the Cy5-SiNPs with oriented mAb conjugation was further investigated on HT-29 tumor xenograft model. Fluorescent signal was only observed at the tumor site of the mouse injected with Cy5-SiNPs with oriented conjugation of anti-EpCAM mAb and the fluorescent signal remained up to 14 days post injection. Whereas the mouse injected with control probe demonstrated weak fluorescent signal at all timepoints. In conclusion, this study demonstrated that the Cy5-SiNPs with oriented antibody conjugation has enhanced tumor targeting efficiency *in vitro*, and is applicable for targeted *in vivo* imaging. This make them a promising candidate to be developed into a new class of effective fluorescence contrast agents for cancer diagnostics.

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