Passively masked surface charge of SPIO nanoparticles for specific detection of EGFR expressing tumor cells

Yun-Ming Wang[1], Shou-Cheng Wu[2], and Jia-Gong Lin[3]

[1]*Department of Biological Science and Technology, National Chiao Tung University, HsinChu, Taiwan,* [2]*Department of Biological Science and Technology, National Chiao Tung University, Taiwan,* [3]*National Chiao Tung University, Taiwan*

Abstract

In this study, a facile approach for the passive masking of non-conjugated reactive amine groups on the surface of iron oxide nanoparticles is demonstrated. The usefulness of this strategy has been exemplified by EGFR specific MR-optical imaging agent (SPIO-mAb-FITC NPs). The TEM and confocal image shows that the passive masking of reactive groups enhances the targeting efficacy of the imaging agent.

Introduction

In this study, we reported a highly sensitive dual modality MR-optical imaging agent specific to the epidermal growth factor receptor (EGFR). The study was aimed at innovative surface engineering of NPs to reduce nonspecific binding. A novel PEG derivative, mPEG-EA-silane, was strategically designed and synthesized such that the non-conjugated -NH_2 on the surface of NPs remain buried deep inside the entangled mPEG chains. Thus, mPEG can act as an insulator, which in turn can decrease the electrostatic interaction between NPs and negatively charged cell membranes of non-targeted tissue[1]. Erbitux is chimeric monoclonal antibody specific for the EGFR. It has been safely used in human for the treatment of metastatic colorectal cancer (MCRC) and squamous cell carcinoma of head/neck cancers (SCCHN)[2].

Materials and Methods

The monodisperse SPIO NPs were obtained by thermal decomposition method. Post-synthesis ligand exchange reaction was carried out to replace hydrophobic oleic acid and oleylamine surfactants with mPEG-NH_2-silane in order to transform hydrophobic SPIO nanoparticles into hydrophilic ones. In vitro MR imaging was carried out using three EGFR positive cell lines (A-431, SKBR-3 and PC-3) with different levels of EGFR overexpression and COLO-205 cells as negative cell for control which has low level of EGFR expression. All cell lines were incubated with SPIO-mAb (15 µg/mL of Fe), washed by PBS buffer and scanned by TEM and confocal microscope.

Results and Discussion

In this study, we have developed highly specific dual modality T2 weighted MR-optical imaging contrast agents (SPIO-mAb NPs) for early detection of EGFR expressing tumor cell. These cell lines have high level of EGFR expression. Furthermore, substantial variation in the intensity of FITC fluorescence can be easily observed in Fig. 2, in this figure the maximum fluorescence intensity was observed in A431cells and minimum in PC-3 cells, reflecting EGFR expression level on the cell membrane. On the other hand, lower of FITC fluorescence intensity was observed on negative group of COLO-205 cells. These results were similar finding in TEM image (Fig. 1) of cell lines incubated with SPIO-mAb NPs. The quantitative of confocal images was shown in Fig 3. In confocal data showed that the SPIO-(mAb)-NH_2 NPs could induce higher uptake by A431, SKBR-3, and PC-3 cells than SPIO-mAb NPs (data not show). These results clearly show passively masked surface charge can overcome the problem of nonspecific binding effect.

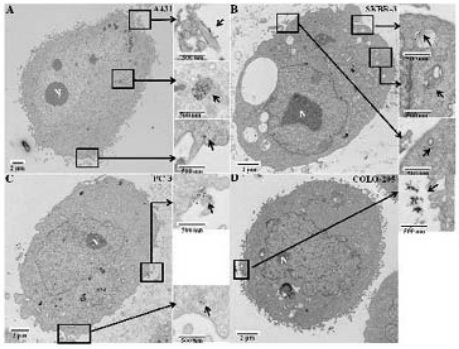

Fig 1. Analyses by TEM for the cellular interaction of MnMEIO-Silane-NH2-(mAb-FITC) -mPEG NPs. Images A-C are for EGFR-positive and image D is for EGFR-negative cell lines. The black dots inside the square are NPs.

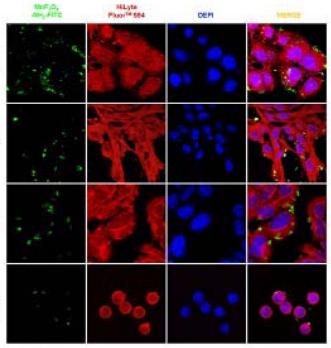

Fig 2. Confocal microscopy images of negative controls cell lines incubated with MnMEIO-Silane-NH_2-(mAb-FITC)-mPEG NPs for 1 hr at 37 °C. Cytoplasm (stained with HiLyte Fluor TM 594 in red), nuclei (stained with DEPI in blue).

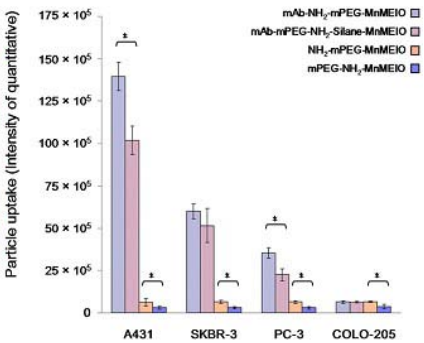

Fig 3. Quantitative of Confocal images. Statistically significant differences ($p < 0.05$) are indicated by asterisks.

Conclusion

We demonstrated the significance of surface chemical properties of NPs. A novel and facile approach was followed to simultaneously achieve functionalization and chemical inertness of NPs surface. MR and optical imaging studies also show that the passively masked surface charges enhance the specificity of EGFR targeted MR optical imaging agent. Consequently, this approach can be potentially applied for the design and development of the next generation targeted nano-scale diagnostic and therapeutic modalities.

References

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