The Modified Fe_3O_4-NH_3^+ with RGD-4C Ligand for Cancer Cell Targeting MR Contrast Agent

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Abstract

A comprehensive totally aqueous phase synthesis of NTA-Ni modified superparamagnetic Fe_3O_4 nanoparticles was presented and the particles diameter is 6.2 ± 1.1 nm. The Fe_3O_4-NTA-Ni particles may act as a modular designed unit rendering molecular orientation control, which was demonstrated in the receptor mediated targeting for cancer cells expressing specific integrins using RGD-4C-6-His fusion peptide as the ligand to self-assemble on the nanoparticle through surface immobilized Ni-NTA. In MR imaging study, we found that the Fe_3O_4-NTA-Ni-RGD-4C-6-His particles exhibited an efficient tumor targeting and contrast enhancement. Furthermore, the results of Pearls' iron stain on the tumor cells also provided the Fe_3O_4-NTA-Ni-RGD-4C-6-His particles as the tumor-targeting probe.

Introduction

Nanoparticles have recently been extensively investigated for their potential biomedical applications in both diagnostics and therapeutics due to their distinctive physical and chemical properties compared to their bulk status. Among these, magnetic nanoparticles presented prominent advantages as they were usually superparamagnetic in nature suitable for both magnetic force driven molecular manipulation and as a reporter system in MR imaging [1]. Integrins are the main cell surface receptors mediating cell adhesion to extracellular matrices [2], and the αvβ3 integrin was found to play very significant roles in tumor angiogenesis [3]. Furthermore, cyclic RGD-4C peptide presents specific affinity to $\alpha_3\beta_v$ and $\alpha_3\beta_5$ integrins that usually selectively expressed in the tumor tissues. Enhanced tumor gene therapy has been demonstrated using RGD-4C modified adenovirus [4]. In most applications, the cross-linkage of the proteins onto the nanoparticles has utilized traditional chemical modifications, which may result in unpredictable orientation of the molecule and thus lead to compromised efficacy. Recently, we synthesized the aqueous Fe_3O_4-NH_3^+ nanoparticles displayed excellent *in vitro* biocompatibility. On the other hand, Fe_3O_4-NH_3^+ showed the excellent performance as the negative contrast agent for the *in vitro* or *in vivo* MRI measurements, and showed significantly reduced water proton relaxation times of both T_1 and T_2 [5]. In this report, we modified the aqueous Fe_3O_4-NH_3^+ nanoparticles with RGD-4C peptide, and the results show a prominent selectively targeting to cancer cells in both *in vitro* and *in vivo* experiments.

Materials and Methods

For preparing the aqueous Fe_3O_4-NH_3^+ nanoparticles, two-stage additions of protective agent and chemical co-precipitation were employed in the process [5]. We then we modified the Fe_3O_4-NH_3^+ nanoparticles with NTA (N^{α},N^{α}-Bis(carboxymethyl)-$_L$-lysine) and Ni ion followed by conjugation of RGD-4C-6His. For *in vitro* targeting assay, the oral cancer cell line HCDB-1 with high level of αvβ3 and the control normal oral keratinocytes (HNOK) were analyzed. For the *in vivo* study, the male Syrian golden hamsters purchased from National Laboratory Animal Center (Taipei, Taiwan) were induced to present oral cancer orthotopically by inoculation of 10^7 tumor cells in 100μl normal saline. All animals were received humane care in compliance with the institution's guidelines for maintenance and use of laboratory animals in research. The nanoparticles were given by jugular vein injection with a dose of 5mg/kg in 20μL volume and the *in vivo* targeting imagings of the magnetite nanoparticles were acquired on a 3T MRI Biospec system (Bruker, Germany). The images were taken at 24 hrs after the hamster was injected with the contrast agent by T_2* weighed MR acquisition sequence: grading echo with TR/ TE = 743 ms / 5 ms, and flip angle (FA) = 30^0.

Results and Discussions

Nanoparticles with RGD-4C tag were shown to present a prominent selectivity for targeting the cancer cells, while only few non-specific stainings were detectable in the normal keratinocytes (Figure 1a and b). On the other hand, nanoparticles alone failed to target HCDB1 cells and showed only background staining, as depicted in Figure 1c. The synthesized RGD-4C surface modified nanoparticles without control molecular orientation by 1-ethyl-3-(3-dimethylaminopropyl)-car-bodiimide (EDC) conjugation presented significantly lower efficiency for tumor targeting (Figure 1b). In MR imaging study, we found that the imaging signal of tumor was decreased after administration of the RGD-4C modified iron oxide nanoparticles (Fig 2b). Furthermore, Pearls' iron stain on the frozen section revealed that most nanoparticles targeted on the tumor cells, as shown Figure 2c and d.

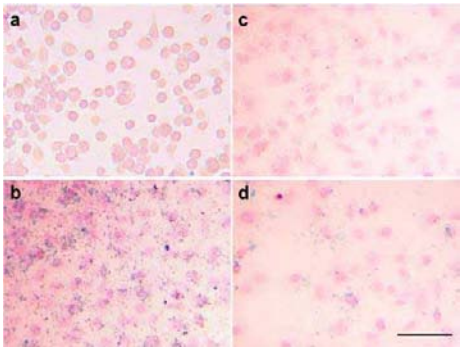

Figure 1: The normal oral keratinocytes HNOK (a) compared to Fe_3O_4-NTA-Ni nanoparticles presented selectively targeting to oral cancer cell HCDB1 (b) after incubation with RGD-4C-6-His cyclic peptides to self-assembled on the particle surface via specific affinity between 6-His tag and the nickel ion. The nanoparticles patched on the cell surface by the RGD-4C tagged nanoparticles showed blue color after Pearl's stain. Nanoparticle alone failed to target HCDB1 cells and presented only background staining (c). Nanoparticles with randomly conjugated RGD-4C by EDC showed significantly lower targeting efficiency to HCDB1 cells (d) as compared to the self-assembly strategy.

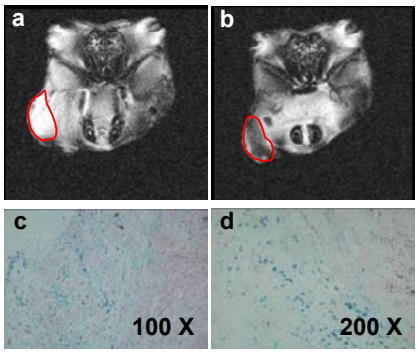

Figure 2: T_2*-weighed MR images of a representative hamster were acquired before (a), and 24 hrs (b) after tail vein administration of 5mg/Kg modified iron oxide nanoparticles. (c) and (d) were ron stain of the tumor frozen section 24 hrs after tail vein administration of 5mg/Kg modifie iron oxide nanoparticles. The nanoparticles targeted on the tumor cells, which presented blue color.

Conclusions

We have demonstrated the aqueous Fe_3O_4-NH_3^+ nanoparticles modified with Ni-NTA for auto-assembly of RGD-4C-6-His as the MR targeting probe for $\alpha_3\beta_v$ and $\alpha_3\beta_5$ integrins. Furthermore, *in vitro* and *in vivo* targeting applications were evaluated. The current platform holds a great potential as an universal module for functionalization of magnetite nanovehicles with controlled peptide orientation, which can be applied to a broad spectrum of affinity based protein-protein interaction biosensing and imaging.

References

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