Targeted new peptide based nanoparticles toward high EGFR expressing cancer cells for MRI

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Abstract

In this study, the various core sizes of manganese ferrite nanoparticles ($MnFe_2O_4$) conjugated with D4 peptide ($MnFe_2O_4$-PEG-D4) were synthesized. The high relaxivity $MnFe_2O_4$ nanoparticles were obtained by thermal decomposition of Iron acetylacetonate and manganese acetylacetonate in hydrophobic solution at high boiling process. The surface of $MnFe_2O_4$ nanoparticles were coated with polyethylene glycol (PEG) and EGFR peptide ligand (D4: Leu-Ala-Arg-Leu-Leu-Thr) to improve their dispersion and ability to target EGFR. The negative signal enhancement of EGFR expressing cancer cells (SKBR-3 and PC-3) were significantly higher than that of low EGFR expressing cells (HEK-293).

Introduction

Molecular Imaging has recently been developed very rapidly and extensively in biotechnology [1]. Tumor-targeted drug delivery can enhance the effectiveness of therapy while decreasing the systemic toxicity of these drugs. We synthesized $MnFe_2O_4$ nanoparticles for MR imaging are coated with polyethylene glycol, which has been frequently used as a drug carrier because of its biocompatibility, water solubility and their ability to escape capture by macrophages. $MnFe_2O_4$-PEG conjugate with D4 peptide substrate (Fig 1.) were synthesized to examine the binding affinity of EGFR using in vitro MR imaging.

Methods

Iron acetylacetonate ($Fe(acac)_3$) and manganese acetylacetonate ($Mn(acac)_2$) precursors were mixed in a molar ratio of 2:1, and reacted in benzyl ether solvent containing oleic acid, oleylamine and, 1,2-hexadecanediol at 300°C for 1 hour to produce manganese ferrite nanoparticles ($MnFe_2O_4$). The $MnFe_2O_4$ nanoparticles have various core sizes were synthesized through the same procedure while the amount of solvent was controlled. We tested two cancer cell lines with different levels of EGFR expression: SKBR-3 and PC-3. In addition, we had chosen HEK-293 cells as negative cell for control which lacks of EGFR receptors. All cells were incubated with $MnFe_2O_4$-PEG-D4 (0.5 mM Fe), washed by PBS buffer and scanned by 3.0 T MRI.

Results and Discussion

The manganese ferrite nanoparticles ($MnFe_2O_4$) was synthesized and characterized by TEM (Fig 2.), SQUID, and FT-IR. Moreover, the TEM results showed that the well-dispersed nanoparticles which coated with PEG. The detection of saturation magnetization by SQUID magnetometry demonstrated that $MnFe_2O_4$-PEG-D4 has high saturation magnetization (84 emu/g) and small coercivity (about 5 G). The internalization of $MnFe_2O_4$-PEG-D4 nanoparticles into positive cells was confirmed by in vitro MR imaging study (Fig 3.). With the $MnFe_2O_4$-PEG-D4 conjugates, the detection of the SKBR-3 cell line occurred with a noticeable negative enhancements (T_2-weighted MR images). As the relative EGFR expression level increased, the MR contrast increased consistently. The signal intensity of positive cells in the present of $MnFe_2O_4$-PEG-D4 is significantly lower than that of negative cells. No signal intensity change was observed for the negative cells in the presence and absence of $MnFe_2O_4$-PEG-D4.

Conclusion

We have successfully synthesized and characterized $MnFe_2O_4$-PEG-D4 nanoparticles, which was shown well-dispersed, high relaxivity and high saturation magnetization. Moreover, we have demonstrated the D4 peptide substrate that was conjugated to $MnFe_2O_4$ nanoparticles and shown to enhance binding to and entry into EGFR expressing cells by MR imaging studies.

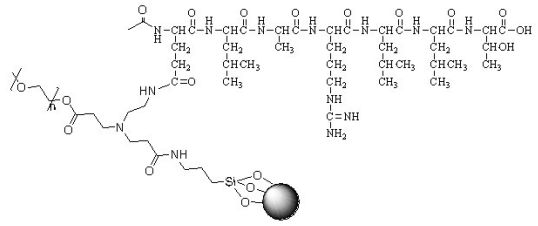

Figure 2. Structure formula of $MnFe_2O_4$-PEG-D4

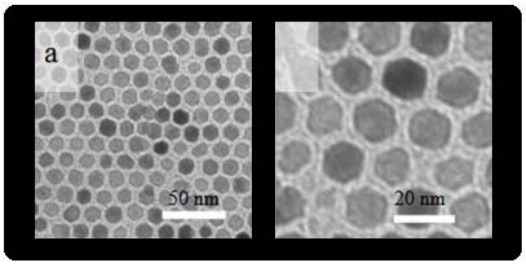

Figure 1. The TEM images of $MnFe_2O_4$ nanoparticles (A), enlarged images of $MnFe_2O_4$ nanoparticles 12.4 ± 0.9 nm (B).

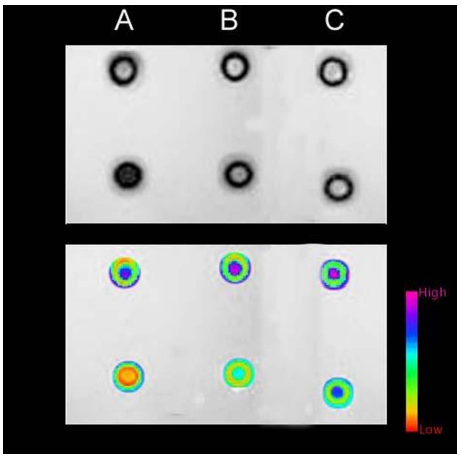

Figure 3. T2-weighted images of positive and negative cells after the treatment with or without o.5 mM $MnFe_2O_4$–PEG-D4. (a) SKBR-3 cells (b) PC-3 cells (c) HEK-293 cells. Upper: cells treatment without contrast agent. Lower: cells treatment with contrast agent.

References

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