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Study on the endocytosis and the internalization mechanism of aminosilane-coated Fe_3O_4 nanoparticles in vitro

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Abstract In this study, the endocytosis and the internalization mechanism of aminosilane-coated Fe_3O_4 nanoparticles into human lung cancer cell line SPC-A1 was studied compared with human lung cell line WI-38 in vitro. The particle endocytosis behavior was studied by using Transmission Electron Microscope (TEM) and Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). It was found that aminosilane-coated Fe_3O_4 nanoparticles could be greatly taken up by SPC-A1 human cancer cells (202 pg iron/cell) but not by WI-38 human lung cells (13 pg iron/cell). The particles could be retained in SPC-A1 cells over a number of generations in vitro. Different endocytosis was observed by TEM after SPC-A1 cells were treated with different temperature or with/without Cytochalasin B (Inhibitor of phagocytosis) at 37 °C. No nanoparticles were taken up by SPC-A1 after the endocytosis inhibited in low temperature. Restoring the endocytosis activity at 37 °C, the process of nanoparticles from coated pit to endosomes and lysosomes was observed by TEM. Endocytosis activity was effectively inhibited by the presence of Cytochalasin B at 37 °C, while a lot of nanoparticles were uptaken to the cytoplasm of SPC-A1 cells in the control group. Our results suggest that the process of endocytosis of aminosilane-coated Fe_3O_4 nanoparticles can efficiently takes place in lung cancer cells and nanoparticles can be kept in cancer cells for generations. Phagocytosis may be involved in the internalization process of aminosilane-coated Fe_3O_4 nanoparticles.

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Introduction

Magnetic nanoparticles have been used for cancer therapy and diagnosis, such as drug delivery, magnetic mediated hyperthermia, cell labeling, magnetic resonance imaging and magnetofection [1–9]. The above-mentioned application is based on the endocytosis of magnetic particles into cells. According to some reports [4, 10], different endocytosis was in dependence of nanoparticles and cell types. For example, aminosilane-coated Fe_3O_4 nanoparticles are taken up by prostate carcinoma cells but not by normal prostate cells, endothelial cells or fibroblasts in vitro [11]. The uptake of dextran-coated nanoparticles into HeLa cells is pinocytosis mediated, while the process, known as phagocytosis, mainly performs the uptake of anionic maghemite nanoparticles [10].

Jordan et al. [4] reported the low hydrodynamic size and the high surface charge of aminosilane-coated Fe_3O_4 nanoparticles facilitated endocytosis efficiency. Similar experiments have not been conducted on human lung cancer cells and human lung cells before. So the aim of this study was to examine whether the uptake of aminosilane-coated Fe_3O_4 nanoparticles in the cell culture will also be applicable for human lung cancer cell and what mechanism mainly performs the uptake of nanoparticles? It is important to study the endocytosis and internalization mechanism of magnetic nanoparticles for further clinical application of magnetic label, hyperthermia or chemotherapeutics on tumor.

Materials and methods

Magnetic fluid preparation

The magnetic fluid used in this study was aminosilane-coated Fe_3O_4. The nanoparticles were synthesized by

coprecipitation, according to Molday [12]. The chemical reaction equation was:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 \downarrow + 4H_2O$$

The particles were surface modified by stirring with 3-aminopropyltrimethoxysilane in ethanol for 6 h at 70 °C, then washed three times by water. After sonification for 30 min, a stable dispersion of the silane coated particles was finally prepared. By using a dynamic light scattering (DLS, Malvern HPPS 5000), the diameter of aminosilane-coated Fe_3O_4 was determined to be approximately 30 nm [polydispersity index (PDI), 0.15].

Cell cultures

Human lung cancer cells SPC-A1 (Chinese Academy of Science) were grown in RPMI 1640 medium (Invitrogen Co., USA), supplemented with 10% fetal calf serum (Invitrogen Co., USA). Human lung cells WI-38 (ATCC, USA) were grown in Eagle's MEM medium (Invitrogen Co., USA), supplemented with 0.1 mM non-essential amino acids (Invitrogen Co., USA), 10% fetal calf serum and 1 mM sodium pyruvate (Invitrogen Co., USA). Both cell lines were cultured at 37 °C in a humidified and 5% CO_2 atmosphere. 1×10^6 cells were seeded in 25 cm^2 culture flasks and grown to the exponential phase before changing the culture medium to aminosilane-coated Fe_3O_4 containing medium. The final concentration of ferrite in the medium was 0.5 mg/mL.

Iron uptake assays

At the end of designed incubation time with aminosilane-coated Fe_3O_4 for up to 24 h, these cells were harvested and washed 5 times with PBS buffer (pH 7.4). A small portion of suspended cells was aliquot for cell counting while the remaining cells were spun down. Iron content was measured by ICP-AES (TJA Co., USA) after dissolving the pellet. The iron content per cell indicated cellular iron.

TEM observations

After being grown in nanoparticles containing medium for 1, 6, 12 or 24 h, SPC-A1 and WI-38 cells were washed 3 times with PBS. Then these cells were harvested in 0.25% trypsin/EDTA (Invitrogen Co., USA) and fixed with 4% (v/v) glutaraldehyde solution (Merck Co., Germany) before the process for TEM observations.

After 24 h culture in nanoparticles containing medium, SPC-A1 cells were washed and grown in the fresh medium up to the 10th subpassage. TEM was employed to observe the intracellular distribution of nanoparticles in each subpassage.

To compare the different uptake results, SPC-A1 cells were observed by TEM after 24 h incubation with nanoparticles at 4 °C and 37 °C, respectively.

SPC-A1 cells were treated with cytochalasin B (50 μg/mL) for 12 h, washed three times with culture medium, then changed the culture medium to aminosilane-coated Fe_3O_4 containing medium(0.5 mg/mL). The cells were cultured for 24 h before harvest and fixing. Then the cells were divided into two parts. One part was used for TEM observation, and the other part was used for ICP-AES measurement.

In order to observe the internalization process of nanoparticles, SPC-A1 cells were cultured at 4 °C for 1 h with nanoparticles. After diverting the cells from 4 to 37 °C,culturing additional 1 h, 3 h and 6 h, respectively.

Results and discussion

As shown in Fig. 1, the great difference in intracellular iron content between SPC-A1 with WI-38 was observed. After 1 h of incubation, intracellular iron content was increased in SPC-A1 cells but not in WI-38 cells. Intracellular iron content was increased in a time dependent manner in SPC-A1 cells. It almost reached a plateau after 12 h of incubation. After 24 h of incubation, SPC-A1 intracellular accumulated to 202 pg iron per cell, which was 16-fold higher compared with WI-38 (13 pg iron per cell). These results suggest that, compared with the human lung cell line WI-38, aminosilane-coated Fe_3O_4 uptake into human lung cancer cell line SPC-A1 is kinetically much more efficient

After loading cells with aminosilane-coated Fe_3O_4, the nanoparticles content in the cytoplasm was reduced from generation to generation in the medium free of nanoparticles. From Fig. 2B we can see a lot of nanoparticles in the cytoplasm of the 2nd generation cells. However, it was still retained in the cytoplasm of SPC-A1, for up to 7th generation. After 7th generation, we could not observe nanoparticles in the cytoplasm. In addition, these cells can also

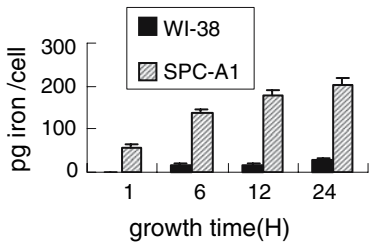

Fig. 1 Increase of intracellular iron of SPC-A1 cells or WI-38 cells after growth in aminosilane-coated Fe_3O_4 containing medium

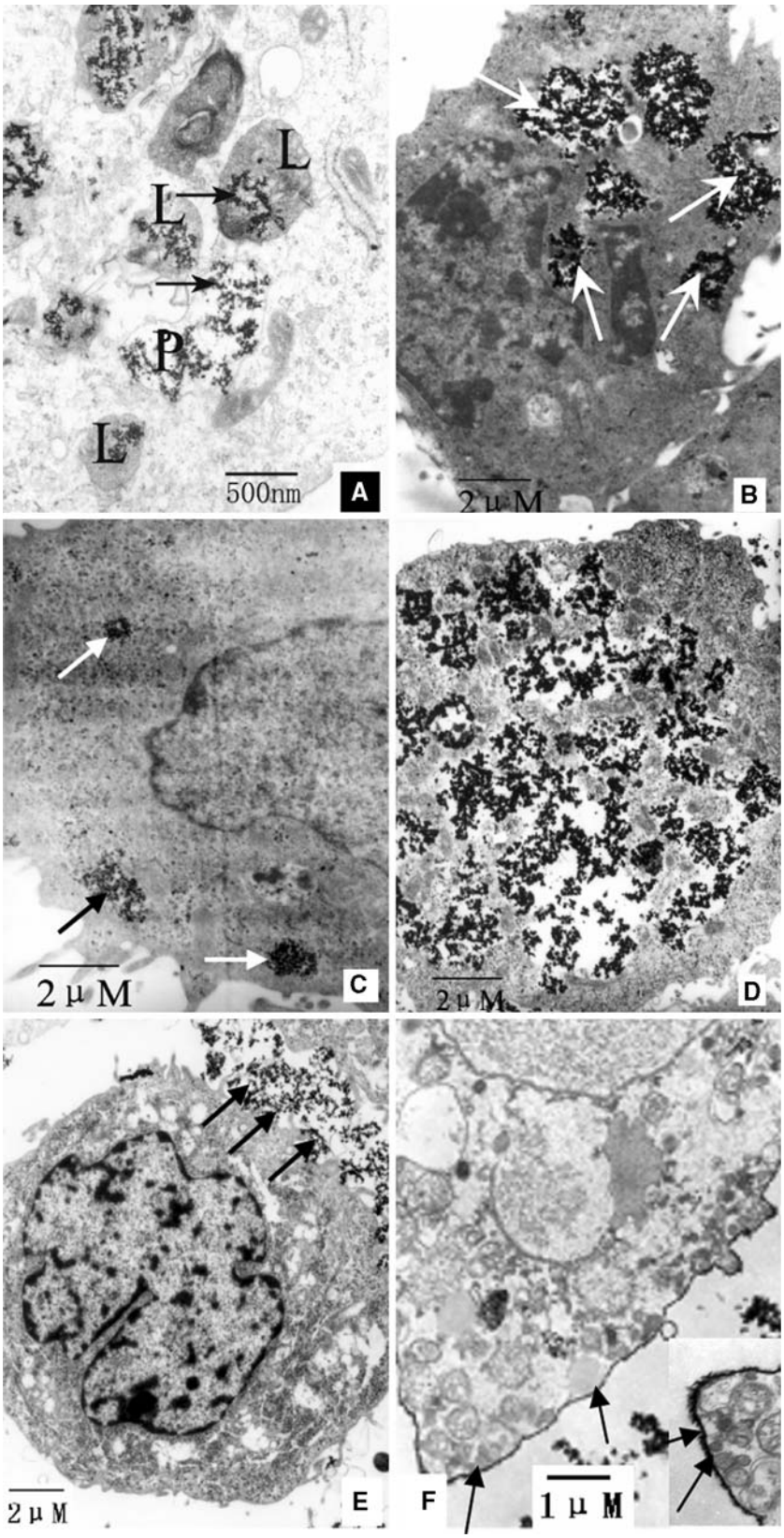

Fig. 2 (**A**) TEM micrographs of aminosilane-coated Fe_3O_4 in phagosomes(P) and lysosomes(L); (**B**, **C**) TEM micrographs of the 2nd and 7th subgeneration of SPC-A1cells after taking up of aminosilane-coated Fe_3O_4; (**D**) TEM micrographs of a SPC-A1 cell fixed after 24 h incubation with aminosilane-coated Fe_3O_4 at 37 °C; (**E**) TEM micrographs of a SPC-A1 cell fixed after 24 h incubation with aminosilane-coated Fe_3O_4 at 4 °C; (**F**) After treated with cytochalasin B, SPC-A1 cells were cultured for 24 h in aminosilane-coated Fe_3O_4 containing medium. (Arrow: magnetic nanoparticles)

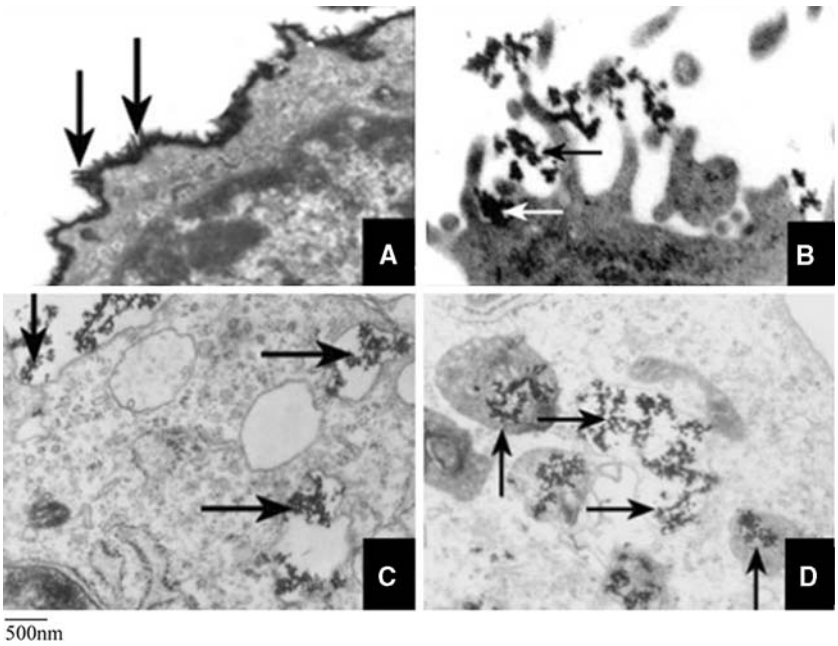

Fig. 3 TEM micrographs of a SPC-A1 cell fixed after 1 h incubation with aminosilane-coated Fe_3O_4 at 4 °C (**A**). After diverted from 4 to 37 °C, the cells were cultured additional 1 h (**B**), 3 h (**C**) and 6 h (**D**), respectively. (Arrow: magnetic nanoparticles)

survive in the presence of particles for up to 10 generations. This suggests that aminosilane-coated Fe_3O_4 may be distributed during mitosis, and it may have low cytotoxicity.

In this study, we demonstrated that endocytosis of nanoparticle occurred in lung cells. Endocytosis of aminosilane-coated Fe_3O_4 nanoparticles took place in human cancer cell lines such as: glioblastoma cells and prostate carcinoma cells [11]. The different uptake of aminosilane-coated Fe_3O_4 nanoparticles is shown in human glioblastoma cell and human cortical neuronal cell [11].

In order to understand the high level of internalization of aminosilane-coated Fe_3O_4 nanoparticles in tumor cells, uptake assays were performed at 4 °C (endocytosis inhibited). For the interaction between the positive charges of amino groups and negative charges on the cell membrane, we can observe adsorption of nanoparticles on the plasma membrane mainly on the form of clusters (Fig. 2E). So the high efficiency of aminosilane-coated Fe_3O_4 nanoparticles cell uptake seems to be related to the non-specific adsorption mediated by electrostatic interaction. Adsorptive endocytosis of anionic superparamagnetic nanoparticles has been reported [13].

The mechanism of endocytosis of nanoparticles by lung cells in this study remains to be elucidated. Endocytosis was effectively inhibited by the presence of Cytochalasin B at 37 °C (11.1 ± 2.6 pg iron per cell, Fig. 2F), while a lot of aminosilane-coated Fe_3O_4 nanoparticles were uptaken to the cytoplasm of SPC-A1 cells in the control group (189.4 ± 7.6 pg iron per cell, Fig. 2D). Cytochalasin B, known as a functional inhibitor of actin assembly and cytoskeletal reorganization, prevented nanoparticles uptake by inhibiting the phagocytosis pathway [13, 14]. The results suggest that phagocytosis may be involved in internalization process of aminosilane-coated Fe_3O_4 nanoparticles. No nanoparticles were taken up by SPC-A1 after the endocytosis inhibited by the low temperature (Fig. 2E, 3A), restoring the endocytosis activity at 37 °C, the process of nanoparticles from coated pit (Fig. 3B) to endosomes (Fig. 3C) and lysosomes (Fig. 3D) was observed by TEM. These results might show the process of phagocytosis and were in accordance with the reports [4, 10], which argue that the mechanism of endocytosis is in dependence on the cell types and nanoparticles.

Conclusion

In conclusion, we have demonstrated that aminosilane-coated Fe_3O_4 nanoparticles may be endocyted into the cytoplasm and was efficiently taken up by lung carcinoma cells. Furthermore, the uptaken nanoparticles can retain in the cytoplasm of SPC-A1 for several generations. This may be implied for the possible future application of aminosilane-coated Fe_3O_4 nanoparticles in intracellular hyperthermia, drug delivery system for lung cancer therapy or magnetic tracer for cell implantation. Phagocytosis may be involved in the internalization process of aminosilane-coated Fe_3O_4 nanoparticles, but it remained to be elucidated.

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References

1. Z. M. SAIYED, S. D. TELANG and C. N. RAMCHAND, *Bio-Magn. Res. Technol*. **I2** (2003) 1
2. P. MOROZ, S. K. JONES and B. N. GRAY, *Int. J. Hyperthermia* **18** (2002) 267
3. M. H. FALK and R. D. ISSELS, *Int. J. Hyperthermia* **17** (2001) 1
4. A. JORDAN, R. SCHOLZ, P. WUST, H. SCHIRRA, T. SCHIESTEL, H. SCHMIDT and R. FELIX, *J. Mag. Mag. Mat.* **194** (1999) 185
5. J. W. BULTE and D.L. KRAITCHMAN, *NMR Biomed.* **17** (2004) 484
6. I. H. LEE, J. W. BULTE, P. SCHWEINHARDT, T. DOUGLAS, A. TRIFUNOVSKI, C. HOFSTETTER, L. OLSON and C. SPENGER, *Exp. Neurol*. **187** (2004) 509
7. C. PLANK, U. SCHILLINGER, F. SCHERER, C. BERGEMANN, J. S. RÉMY, F. KRÖTZ, M. ANTON, J. LAUSIER and J. ROSENECKER, *Biol. Chem*. **384** (2003) 737
8. J. A. FRANK, B. R. MILLER, A. S. ARBAB, H. A. ZYWICKE, E. K. JORDAN, B. K. LEWIS, L. H. BRYANT and J. W. BULTE, *Radiology* **228** (2003) 480
9. J. V. FRANGIONI and R. J. HAJJAR, *Circulation*. **110** (2004) 3378
10. C. WILHELM, C. BILLOTEY, J. ROGER, J. N. PONS, J. C. BACRI and F. GAZEAU, *Biomaterials* **24**(2003) 1001
11. A. JORDAN, R. SCHOLZ, K. MAIER-HAUFF, M. JOHANNSEN, P. WUST, J. NADOBNY, H. SCHIRRA, H. SCHMIDT, S. DEGER, S. LOENING, W. LANKSCH and R. FELIX, *J. Mag. Mag. Mat.* **225**(2001) 118
12. R. S. MOLDAY, US Patent 4452773(1984)
13. T. SUZAKI, M. ARIKAWA, A. SAITO, G. OMURA, S. M. MOSTAFA KAMAL KHAN, M. SAKAGUCHI and K. HAUSMANN, *Acta Protozool*. **42** (2003) 7
14. N. A. Compbell and J.B. REECE, "*Biology"* (6th edn, Benjamin-Cummings Publishing Company, 2002) chap. 8, p. 138