Targeting Multifunctional nanomedicine delivery system for pancreatic cancer

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ABSTRACT SUMMARY
RGD-grafted magnetic mesoporous silica nanoparticles were developed for targeting imaging of pancreatic cancer and gemcitabine delivery. This efficient multifunctional magnetic drug delivery system would achieve targeting delivery gemcitabine to the pancreatic cancer and evaluate the therapeutic effects using MRI simultaneously.

INTRODUCTION
Pancreatic cancer is a highly malignant tumor and the 5 year-survive rate is very low. The low sensitivity and resistance to gemcitabine, which has been used as the standard therapy of patients with advanced pancreatic cancer for more than one decade, is an urgent problem need to be solved. Great efforts have been made to increase the intracellular delivery of gemcitabine. The development of targeting multifunctional nanomedicine delivery system will increase the intracellular delivery of gemcitabine. Magnetic mesoporous silica nanoparticles (MMSNs) loaded with gemcitabine would increase the concentration in the tumors due to the enhanced permeability and retention (EPR) effect1. The RGD peptides would be used as targeting molecules to achieve the targeting delivery of gemcitabine. RGD peptides could combine with the integrin αvβ3 overexpressed on the pancreatic cancer cells and then the RGD-grafted MMSNs (RGD-pMMSNs) could be internalized via both EPR effects and integrin-mediated endocytosis. The targeting delivery of gemcitabine would be enhanced. These RGD-pMMSNs can also serve as MRI contrast agents to observe the biodistribution and accumulation of the nanoparticles, evaluate the therapeutic response and predict the prognosis. The purpose of this study is to develop an efficient multifunctional nanomedicine delivery system to achieve targeting delivery of gemcitabine and evaluating the therapeutic effects using MRI simultaneously.

EXPERIMENTAL METHODS
The magnetic nanoclusters (MNCs) were synthesized using a modified hydrothermal reaction2. These MNCs would serve as the magnetic core. The mesoporous silica shell with amino-groups was then coated to the surface to form the MMSNs. RGD peptides were conjugated onto MMSNs-NH2 through condensation reaction. The surface would also be modified with PEG to reduce the uptake of the nanoparticles by RES and prolong the circulation time in vivo. Gemcitabine was then loaded into the synthesized RGD-pMMSNs and the release study was observed in vitro. BxPC-3, Panc-1 and CFPAC-1 were chosen to evaluate the targeting and cellular uptake of RGD-pMMSNs in vitro. Immune fluorescent (IF) and TEM imaging were used to confirm the cellular uptake of RGD-pMMSNs. The therapy efficacy was evaluated in three cell lines treated with RGD-pMMSNs loading with GEM for different time intervals.

RESULTS AND DISCUSSION
The average diameter of MMSNs was around 50 nm with a mesostructured silica shell of about 15nm (Fig.1a). The X-ray diffraction pattern (XRD) of the synthesized MMSNs showed the ultra-fine nature and small crystallite size of the magnetic cores. The saturation magnetization of MMSNs was 27emu/g. Surface modification of MMSNs was verified with measuring zeta potential. The strong negative zeta potential of the magnetic
clusters was changed to +20 mV by amine modified mesoporous silica coating. Further, COOH-PEG modification and functionalization with RGD peptides were confirmed with zeta potential changes. TEM images showed the enhanced cellular uptake of RGD-pMMSNs in BxPC-3 cells (red arrow Fig. 1).

The drug release curve in vitro (Fig. 2a) showed the typical two-phase drug release behavior. The drug release in the first phase (0-8h) was 40%, then the release became slower and sustained in the second phase (8-72h) and about 90% of GEM was released within 72h. With the increasing incubation times (1h, 8h and 24h), the cell viability decreased (p=0.0005, 0.0013 respectively) in BxPC-3 and CFPAC-1 cells. While there was no significant decrease observed in Panc-1 cells (p = 0.7) (Fig. 2b).

This result was strongly related to the expressions of integrin αvβ3 and sensitivities to gemcitabine in these cell lines. Integrin αvβ3 was highly expressed in BxPC-3 cells, therefore, with the increasing incubation time, more and more RGD- MMSNs could be internalized due to the targeting effects of the RGD peptides. For the cell line Panc-1, the concentration of the gemcitabine released was so far less than the IC50 value. Besides, the expression of the integrin αvβ3 was low in Panc-1 cells. The endocytosis would not be enhanced with the incubation time.

CONCLUSION
RGD could enhance the cellular uptake by pancreatic cancer cells. The synthesized RGD-pMMSNs could achieve the targeting delivery of gemcitabine to pancreatic cancer cell lines.

REFERENCES

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