Drug-attached magnetic nanoparticles: Locomotion control and in vivo biocompatibility

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Abstract. Magnetic nanoparticles (MNPs) are playing an increasingly important role in the biomedical fields such as the diagnosis, treatment and monitoring of various diseases. Due to the unique properties of MNPs, MNPs with functionalized non-toxic surface coatings can be used for drug delivery in combination with therapeutic drugs or chemical molecules. A novel type of drug-attached magnetic nanoparticle was presented, where insulin was bound to magnetic Fe₃O₄ nanoparticles (MNPs-Insulin). It was particularly proposed for the future in vivo control to realize the targeted locomotion. The properties of the MNPs-Insulin were characterized by Fourier-transform infrared spectra (FTIR), thermo gravimetric analysis (TGA) and vibrating sample magnetometer (VSM) analysis. The ability of magnetic control was tested in the local motion control system in vitro. The good biocompatibility was certified by Hematoxylin-eosin staining (HE) on mouse.

1. Introduction
Magnetic nanoparticles (MNPs) are a class of non-invasive therapeutic agents that can be used for imaging [1], cell-specific targeting [2], and drug delivery [3]. Recent advances in this field have made MNPs widely used in various biomedical and therapeutic applications. One of the main reasons is their inherent magnetism. Magnetic nanoparticles (MNPs) include metal, bimetal or superparamagnetic iron oxide (SPION) -based nanoparticles [4, 5]. Due to their relatively low toxicity, superparamagnetic iron oxide (SPIONs) have attracted the attention of many researchers. More importantly, their active surfaces can be modified with biocompatible coatings for the combination with imaging, targeting and therapeutic molecules [3, 6].

Here, inspired by the rapid development of nan medicine and nano-robotics[7, 8], we propose a magnetic-field controllable drug-loaded nanoparticles or nan robots, using insulin as a model drug, to show the integration of medicine effects and locomotion control together which has rarely been reported before. The property of the insulin-attached magnetic nanoparticles (MNPs-Insulin) was confirmed by various characterizations including fourier-transform infrared spectra (FTIR), thermo gravimetric analysis (TGA), and vibrating sample magnetometer (VSM). The magnetic hysteresis loop demonstrated the attached insulin did not affect the magnetic property of the MNPs-Insulin. The
ability of magnetic control was tested in the local motion control system in vitro, indicating the locomotion of MNPs-Insulin can be controlled to achieve target site. And for the in vivo experiments, the results showed that the MNPs-Insulin can achieve a good biocompatibility, which means that it has the potential for the in vivo application [9, 10].

2. Materials and Methods

2.1. Materials

500 nm -COOH functionalized magnetic nanoparticles were supplied by Aladdin Biotechnology Co., Ltd, Shanghai, China. Insulin (C_{254}H_{377}N_{65}O_{75}S_{6}) from bovine pancreas was supplied by Macklin Biotechnology Co., Ltd, Shanghai, China. Deionized water in experiments was all produced by water purification system from Shanghai Ulupure Industrial Co., Ltd. Phosphate Buffered Saline (PBS) was supplied by GE Healthcare Life Sciences.

2.2. Preparation of drug-attached magnetic nanoparticles

8 mg of EDC and 1 mg of NHS were dissolved in 2 mL of deionized water, and 2 mL of Ferro ferric oxide nanoparticles were added and stirred at room temperature for 20 min. The magnets were used to help precipitate the magnetic nanoparticles in the beaker, then the supernatant is poured out, and deionized water is added for washing for 2-3 times. The nanoparticles were then added to 5 mL of insulin solution (in insulin solution preparation: 5 mg of insulin was dissolved in 1 mL of 0.01 mol/L hydrochloric acid, then diluted to 5 mL with PBS of ph7.4) and stirred for 2 hours at room temperature. The magnetic nanoparticles were then precipitated by a magnet and washed with PBS pH 7.4, and the washing liquid was subjected to ultraviolet spectroscopy until the absorption peak of the washing liquid had no insulin absorption at 276 nm. Finally, the obtained nanoparticles were dispersed in PBS and stored at 4°C for use.

2.3. Property characterization

Fourier-transform infrared spectra (FTIR) were obtained using Infrared Spectrometer 6700 from Thermo Nicolet Corporation (USA). Thermo gravimetric analysis (TGA) was measured on TGA 8000 from Perkin-Elmer (USA). Vibrating sample magnetometer (VSM) was measured using HH-10 system from Nanjing NanDa Insutrument Plant (China).

2.4. Establishment of in vitro model

In order to control the motion path of MNPs or MNPs-Insulin in the container through the magnetic field generated by the electromagnetic coil, we built a local motion control system, as shown in Figure 1. The control system mainly includes: electromagnetic coil, soft iron core (DT4), working platform Bracket (PLA), MNPs solution container (PDMS), control circuit board, DC current source, and PC.
2.5. *Histochemistry analysis*

To assess the biocompatibility of MNPs-Insulin, we performed histochemical analysis. Hematoxylin-eosin staining (HE) is a staining method used to observe and identify whether cells are necrotic. Using mice as animal model, they were injected with solutions, fed for 6 days and then killed. After the mice were killed, the peritoneal, liver, and spleen tissues of mice injected with 500 nm MNPs-Insulin were selected. They were fixed in the 10% formalin and performed by conventional dehydration embedding (Leica HistoCore ArcadiaH). Consecutive serial sections of 4 μm thickness were alternatively stained with hematoxylin and eosin (H&E). We stored and fixed the slices with neutral gum. Finally, we observed the tissue sections under the microscope (OLYMPUS BX51).

3. Results and Discussions

3.1. Properties of MNPs-Insulin

Successful coupling of insulin onto the surface of Fe₃O₄ @ PEG-COOH nanoparticles with different size was confirmed by FTIR spectra and TGA (Fig. 2). Comparing the FTIR spectra of insulin attached MNPs with MNPs without insulin, the peak intensity at 3300 cm⁻¹, 1630 cm⁻¹ and 1085 cm⁻¹ were enhanced compared with that before insulin attachment. And for the TGA results, the weight loss curves of 500 nm MNPs and 500 nm MNPs-Insulin were shown in Fig. 2b. The weight loss at temperature lower than 200 °C was corresponded to the removal of physically absorbed water or other solvents. The main weight loss at temperature 200-800 °C was attributed to the removal of functional organic moieties on the particle surface, including PEGylation and covalent bonded insulin. Combining the results of the FTIR spectra and TGA results, it can be concluded that insulin was successfully coupled onto the surface of Fe₃O₄ @ PEG-COOH nanoparticles.

![FTIR spectra comparison](image)

*Figure 2.* (a) FTIR spectra comparison of insulin, MNPs and MNPs-Insulin; (b) (c) Comparison of thermal weight loss of 500 nm MNPs and MNPs-Insulin by TGA.

The magnetic properties of the 500 nm MNPs-Insulin before and after surface modification were measured using a vibrating sample magnetometer. The hysteresis loop is shown in the Fig.3 (a-b). It can be seen that for the 500 nm nano-magnetic particles, the saturation magnetization before and after insulin labelling is unchanged, about 23.54 emu/g. The 500 nm nanoparticles ensure a fast-magnetic response. As the magnet approached the suspension of 500 nm microspheres, the microspheres rapidly accumulated toward the magnet. In about 30 s, the suspension became completely transparent, see Fig. 3(c).
Figure 3. (a-b) The hysteresis loop of 500 nm MNPs and 500 nm MNPs-Insulin. (c) The responses of 500 MNPs-Insulin to external magneto static field.

3.2. In vitro experiments of magnetic locomotion control

Figure 4 (a) exhibited the physical prototype of the locomotion control system and Fig. 4 (b) showed the successful controlled movement of nano-magnetic particle clusters to a certain position as expected. Because of the cluster of the MNPs-Insulin, we can see the locomotion directly.

Figure 4. (a) MNPs local motion control system. (b) The images showing the controlled motion path.

3.3. In vivo biocompatibility

The biocompatibility of magnetic nanoparticles has been widely investigated for biomedical application. In this study, the in vivo biocompatibility of MNPs-Insulin was evaluated via Hematoxylin-eosin staining (H&E) on days 6 after MNPs-Insulin treatment on mice. The mouse performed daily dietary iron ingestion per day. As shown in Fig.5, no neutrophil infiltration or fibrosis were observed at injected site of peritoneum as well as liver, spleen and kidney tissues. The cells were tightly arranged and intact, which showed the good biocompatibility of MNPs-Insulin.
Figure 5. H&E staining of tissues at the injection site of peritoneum, liver, spleen and kidney on day 6 after MNPs-Insulin treatment (16 mg/kg of 500 nm MNPs-Ins).

4. Conclusion
In summary, we proposed a drug-attached magnetic nanoparticle with drug effects and controllable locomotion. The successful conjugation of model drug insulin was confirmed by FTIR and TGA. VSM and in vitro magnetic-filed controlled locomotion of the MNPs-Insulin showed the successful response to the magnetic-field application. The in vivo experiments performed on mice demonstrated the good biocompatibility of the proposed MNPs-Insulin. We believe that this work will be very beneficial for the future application of MNPs-Insulin on blood glucose regulation.

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