| | |
|---|---|
| Title | 細胞小器官の高選択的磁気分離技術構築に向けた磁性－プラズモンハイブリッドナノ粒子の創製とオートファゴソームの単離への応用に関する研究 |
| Author(s) | 高橋，麻里 |
| Citation | |
| Issue Date | 2018-03 |
| Type | Thesis or Dissertation |
| Text version | ETD |
| URL | http://hdl.handle.net/10119/15332 |
| Rights | |
| Description | Supervisor:前之園　信也，マテリアルサイエンス研究科，博士 |

# Abstract

The present research demonstrates the capability of magnetic separation of intracellular organelles using magnetic-plasmonic heterostructured nanoparticles (NPs). Magnetic separation of cellular organelles has been considered to be a powerful analytical technique for cellular organelles which are difficult to be purified by conventional separation techniques. In order to separate these organelles, three aspects are required for the magnetic probe: small size, imaging ability and high magnetic property. In the present research the NP which is composed of Ag@FeCo@Ag core@shell@shell structure (Fig. 1a) is fabricated by a chemical synthesis. The size of the NP is about 15 nm. The formation mechanism and magnetic properties of the NPs are carefully analyzed, then the NPs are made to be water dispersible using hydrophilic polymer. Previously it was reported that a latex bead was transferred to an autophagosome after transfection. In this study we selected the autophagosome as a target organelle with magnetic separation using the current NPs. Incorporation of the NPs into autophagosomes was analyzed by a confocal laser scanning microscope by detecting plasmon scattering from the NPs (Fig. 1b). The results showed the localization of the NPs changed as incubation time increased after transfection of the NP according to the degradation pathway. Finally the autophagosomes which contain the NPs were magnetically separated by an autoMACS Pro Separator. The magnetically separated fraction (MSF) was investigated by western blot analysis. The results indicated the presence of LC3-II which is an autophagosomal membrane protein in the MSF, suggesting the success of magnetic separation of autophagosomes. The advantages of using magnetic-plasmonic heterostructured NPs for magnetic separation include easy organelle tracing due to localization of the probe, and straightforward separation timing. The technique described in this study will provide beneficial knowledge in both fundamental and practical aspects of the medical biology field.


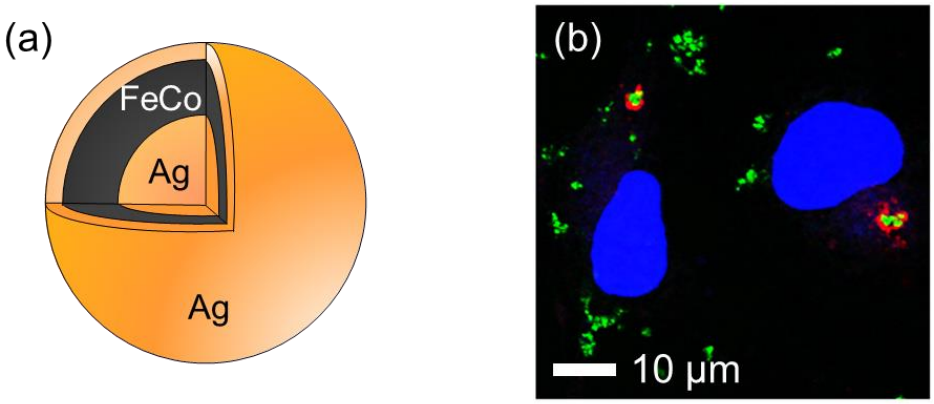


**Figure 1.** (a) Structure of the Ag@FeCo@Ag core@shell@shell NP. (b) Confocal laser scanning microscope image of COS-1 cells in which the NPs were introduced by transfection. Blue, red and green color represents nucleus, LC3 (autophagosomal marker protein) and plasmon scattering from the NPs respectively.

The framework of this thesis is as follows. Chapter 1 contains the introduction of magnetic separation technique showing some examples and importance of separation of cellular organelles. Then Ag@FeCo@Ag NPs are introduced as a magnetic-plasmonic probe for separation of cellular organelles. Chapter 2 focuses on the formation mechanism of Ag@FeCo@Ag NPs. The NPs are synthesized by combination of a hot injection and a polyol method. The synthesis of the NPs is not just a seed-mediated growth. It contains many important concepts such as size-dependent reducing ability of Ag core, size focusing and surface segregation. In Chapter 3 the magnetic properties of the NPs are discussed. The reduction of saturation magnetization is observed due to surface oxidation. As a result exchange bias at the interface between ferromagnetic FeCo and antiferromagnetic cobalt wüstite is clearly observed. Based on the investigation of the relationship between exchange bias field and structural parameter of the NPs, an analytical tool for estimation of the oxide layer in the NPs from its exchange bias field is produced. Chapter 4 describes the synthesis of hydrophilic polymer and ligand exchange method for water dispersible NPs. Colloidal stability of the NPs is investigated. In addition, the surface of the NPs is modified by specific proteins using biotin-avidin interaction. This demonstrates the potential of surface modification of the NPs by certain proteins. Chapter 5 includes several examples of nanoparticle introduction by endocytosis and/or transfection method. Then the Ag@FeCo@Ag NPs are introduced into cells using a transfection reagent in order to separate autophagosomes. The microscope observation and magnetic separation results are then summarized. Chapter 6 summarizes the conclusion of the present study and significant achievements in each chapter. Finally the ideal magnetic probe for more versatile magnetic separation of cellular organelles is discussed.

## Key words

Nanoparticle; Magnetic separation; Plasmon scattering; Cellular organelle; Surface modification