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The shape effect of magnetic mesoporous silica nanoparticles on endocytosis, biocompatibility and biodistribution

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ABSTRACT

Although the aspect ratio (AR) play a crucial role in determining biological effects of homogeneous nanomaterials, studies available concerning how the shape contributes to biological effect of heterogeneous nanomaterials is limited. To systematically clarify the shape influence on the endocytosis, biocompatibility and biodistribution of magnetic mesoporous silica nanoparticles (M-MSNPs), three FITC-labeled M-MSNPs with different aspect ratio (AR = 1, 2, and 4) were specifically designed and constructed through altering the ratios of CTAB/TEOS in a modified so-gel method. We have demonstrated that long-rod M-MSNP2 possessed higher intracellular internalization amount than the short-rod M-MSNP1 and the sphere-like M-MSNP0 in both cancer cells and normal cells due to the difference in the endocytosis pathways. However, there are no significant shape effects on biocompatibility including cytotoxicity and hemolytic rate. Moreover, biodistribution in HepG2 tumor-bearing mice showed that M-MSNPs administrated intravenously were mainly presented in reticuloendothelial system (RES) organs including liver, spleen and kidney. In particular, sphere-like M-MSNP0 were easily trapped in the liver, while long-rod M-MSP2 exhibited more retention in the spleen. It is worth noting that rod-like M-MSNPs are preferentially accumulated in tumor sites than sphere-like M-MSNPs, indicating an improved drug delivery efficacy in cancer therapy. Our findings may provide useful data for deeply understanding the interaction between the different shapes and biological behavior of M-MSNPs, which is expected to give rise to a new generation of heterogeneous M-MSNPs with significantly enhanced efficacy and safety for the cancer theranostics.

Statement of Significance

In this work, we systematically clarified the shape influence on the endocytosis, biocompatibility and biodistribution of homogeneous nanomaterials. We have demonstrated that rod-like magnetic mesoporous silica nanoparticles (M-MSNPs) were capable of higher intracellular internalization and tumor accumulation than sphere-like M-MSNPs, which was expected to give rise to a new generation of heterogeneous M-MSNPs with significantly enhanced efficacy and safety for the cancer theranostics.

1. Introduction

With the rapid development in the nanotechnology which has led to a huge boom in the diagnosis and therapy of disease, it is imperative to investigate how engineered nanomaterials affect human health [1,2]. A better understanding of the interactions between nanomaterials and biological systems is crucial to establish optimal standards for nanotechnology design, which in turn has facilitated the safe use of nanomaterials for biological applications [3]. In light of this, continuous efforts have demonstrated that the relationships between nanoscale structure and biological activity are strongly influenced by nanomaterial properties such as size,

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shape, surface chemistry, roughness and surface coatings [4–7]. In natural environments, bacterial pathogens are first and foremost recognized by their form or shape, which could help them to invade host cells through the nano-bio interaction [8]. Indeed, recent advance suggest that shape plays an instrumental role in both cellular interactions and the systemic distribution of nanomaterials, including uptake efficiency, endocytosis mechanism, intercellular transportation, biodistribution, and biocompatibility [9–11]. Therefore, the development of practical and effective carriers for optimized drug delivery may be realized by orchestrating their nanostructural shape for maximizing their biological effects.

The ability to manipulate the cellular uptake and tumor accumulation of nanomaterials makes it possible to improve sensitivity and efficiency in cancer diagnosis and therapy [12,13]. Although spherical nanomaterials has been traditionally employed for biological application due to their relative ease of fabrication, nonspherical nanomaterials (e.g., rods, disks, hemispheres, ellipsoids, and cylinders) possessed different impact on their biological effects [14]. Particularly, rod-like nanomaterials exhibited more rapidly and efficiently endocytotic behavior than sphere-like nanomaterials [15–18]. It was found that nonspherical mesoporous silica nanoparticles (MSNs) with a high aspect ratio (AR) were taken up in larger amounts and had faster internalization rates, as well as greater impacts on different aspects of cellular function including cell proliferation, apoptosis, cytoskeleton formation, adhesion and migration, as well as biodistribution, biodegradation, and biocompatibility [17–21]. Although MSNs with higher ARs exhibited slower degradable, lower systematic absorption and excretion manner in vivo, they possessed good biocompatibility [19–21]. Furthermore, the rod-shaped polymeric micellar system could accumulate distinctly in tumor tissue through the enhanced permeability and retention (EPR) effect, and DOX-loaded rod-like NPs exhibited great potential in the development of advanced drug-delivery systems for enhanced cancer therapy in comparison to spherical NPs [16].

In order to fulfill the future requirements of the ranostic nanomedicine, heterogeneous MSNs are currently employed as a promising class of multifunctional platforms owning to their synergic properties [22–26]. Among them, magnetic mesoporous silica nanoparticles (M-MSNPs) have been considered as intriguing drug delivery carriers due to their unique magnetic-mediated functions, convenience in drug-loading, appreciable adaptability in subsequent biomedical engineering and intrinsic safety profile [27–35]. However, researches still have limited information regarding the shape effects on biological behavior of these heterogeneous NPs. Therefore, an improved understanding of how shape affects on biological processes of M-MSNPs is desirable to give rise to a new generation of innovative drug carriers with significantly improved drug delivery outcomes for the cancer theranostics.

In this study, we constructed an M-MSNP library composed of sphere- and rod-shaped NPs to explore the impact of aspect ratio variation on their cellular uptake, biodistribution and biocompatibility. Firstly, different shaped M-MSNPs (AR = 1, 2, 4) are well fabricated and labeled with fluorescein isothiocyanate (FITC) for monitoring and quantification of cellular uptake. Then, we systematically investigate the endocytosis mechanism through utilizing specific inhibitors to cell uptake pathways by fluorescence-activated cell sorting (FACS). Subsequently, the biocompatibility of different shaped M-MSNPs is evaluated by the analysis of cell viability and hemolytic characteristics. Additionally, the biodistribution and tumor retention of different shaped M-MSNPs in HepG2-tumor bearing mice are analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-OES) and confocal laser scanning microscopy (CLSM). To the best of our knowledge, this is the first study showing shape critically affects endocytosis and biodistribution of M-MSNPs, which will provide a foundation for exploring the next generation of heterogeneous M-MSNPs in cancer theranostics.

2. Materials and methods

2.1. Cell culture and cytotoxicity assessment

The human hepatocelluar carcinoma cell line (HepG2), human breast cancer cell line (MCF-7), human cervical cancer cell line (Hela), and human hepatic embryo cell line (HL-7702) were maintained at 37 °C under 5% CO2 in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml). In vitro cytotoxicity of different shaped M-MSNPs against HepG2, HL-7702, Hela and MCF-7 cells was assessed at various time points using an SRB cell viability assay. The sulforhodamine B (SRB) assay is routinely used for cytotoxicity determination, based on the measurement of live cell protein content [36]. In brief, cells were seeded in 96-well plates at a density of 5×10^4 cells per well overnight, and treated with the indicated different concentrations (0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 μg /mL) of M-MSNPs. Relative cell viability was calculated as a percentage from the viability of the control (untreated) cells based on three independent experiments. The viability of the control cells was considered as 100%.

2.2. Identification of the cellular uptake and their pathways of M-MSNPs

To measure the intracellular localization of different shaped M-MSNPs firstly, HepG2 or HL-7702 cells were seeded on lean coverslips in a 24-well plate at a density of 2×10^4 cells per well. After 24 h incubation, the medium was discarded and fresh culture without serum was added. FITC-labeled M-MSNPs (12.5 μg/mL) were co-cultured with cells for 3 h. Then, endosome and lysosome of cells were labeled with 50 nM LysoTracker Red DND-99 fluoroprobe for another 0.5 h, the cells were washed twice with chilled PBS, and the nuclei were stained using Hoechst 33258 dye (5 mg/ml) for 5 min. The location of the NPs was observed using an Olympus FV1000 CLSM equipped with multi-line argon LASER (405, 488 nm) and a 30-mW Laserclass 3D laser.

We next confirmed the cellular uptake behavior of different shaped M-MSNPs via Prussian blue staining assay. HepG2 or HL-7702 cells were seeded in 96-well plate at a density of 5×10^3 cells per well. After 24 h incubation, the medium was discarded and fresh culture without serum was added. M-MSNPs (12.5 μg/mL) were co-cultured with cells for 3 or 24 h, then the cells were washed with PBS and fixed with 4% paraformaldehyde. Prussian Blue staining was performed by adding freshly prepared working solution consisting of 5% potassium ferrocyanide and 5% hydrochloric acid [33]. Finally, cells were washed with PBS and the photos of cells were taken by biological microscope (Olympus CX21FSEC Japan).

We further quantitated the uptake dynamics of different shaped M-MSNPs by fluorescence-activated cell sorting. HepG2 or HL-7702 cells were seeded in 6-well plates at the density of 5×10^4 -cells/well. After 24 h, FITC-labeled M-MSNPs (12.5 μg/mL) were co-cultured with cells for 3 or 24 h. Afterwards, the supernatant solution was removed, and cells were washed twice with chilled PBS to remove any extracellular NPs. Then, the cells were trypsinized and resuspended in chilled PBS to yield a concentration of 1×10^6 cells/mL and analyzed by FACS (Becton–Dickinson Biosciences, Drive Franklin Lakes, U.S.).

Finally, the endocytosis mechanisms of different shaped M-MSNPs were performed by blocking uptake pathway with different treatment under both CLSM and FACS. The cellular uptake