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Synthesis and neuro-cytocompatibility of magnetic Zn-ferrite nanorods via peptide-assisted process

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ABSTRACT

In order to obtain magnetic nanorods (MNRs) with the neuro-cytocompatibility, silk-fibroin (SF)-coated Zn-ferrite NRs are successfully prepared via a mineralization process, and their saturation magnetization is 32 emu g^{-1}. After the mineralization of 2 d and 4 d in the mixed solution of the concentrations of 15 w/w% SF and 0.01 M HCl, the lengths of NRs are ~220 nm and ~2 μm, respectively. Cell tests of NRs with 220 nm length showed that the as-prepared Zn-ferrite NRs hardly produced toxicity on *Escherichia coli*, *Staphylococcus aureus*, L929, and PC12 cells. The results of the outgrown neurites from PC12 cells indicated that the neurite length and the number of neurites were not significantly decreased at the low concentrations of SF-coated NRs (less than 0.25 mg mL^{-1}) in 1–5 d culture time. TEM images of cell ultrathin sections indicated that, although Zn-ferrite NRs were split in the cytosol for 5 d at the NR concentrations of 0.125 mg mL^{-1}, some integrated mitochondria in a neurite suggested that SF-coated NRs inside cells did not influence the extension activity of neurites. Based on the good neuro-cytocompatibility and magnetic property of Zn-ferrite NRs, their potential applications in safe cell manipulation and axon guidance can be envisioned.

1. Introduction

In recent ten years, the applications of magnetic nanoparticles (MNPs) in the field of biomedicine have been studied based on their interesting and promising bio-magnetic property [1–3]. For example, iron oxide MNPs are believed to be nontoxic, biosafe, and biocompatible and have successfully applied in labeling of cell [4], cell separation [5,6], targeted drug carriers [7,8], high resolution magnetic resonance image (MRI) [9,10], and cancer therapy [11,12]. However, the iron oxide MNPs are also believed to have toxicology on target organisms based on the tests of bacteria and vertebrates [13–15]. For instance, γ-Fe_2O_3 MNPs are capable of diminishing the viability of neuron (PC12 pheochromocytoma) cells and the extension of neurites in response to their putative bio-cue [13].

In general, MNPs are the spherical shape, usually including a magnetic core and a functionalized sheath that allows the conjugation of bioactive ligands to realize desired biomedical applications [16,17]. If the magnetic nanomaterial is used as an externally guided platform, 1 D nanostructure is more advantageous than MNPs [18]. Magnetic nanorods (MNRs) and nanowires (MNWs) have better magnetic property than MNPs of comparable volume [19], and the precise architecture of MNWs along their axis can control their magnetic property for special biomedical applications [20].

Various approaches, such as the hard template method [21–23] and soft-template process [24,25], have been developed to prepare 1 D magnetic materials of metals, alloys, and metal oxides. Due to their stronger magnetic moment than MNPs, MNRs could induce hyperthermia in cells under lower frequency electromagnetic fields [26]. However, their preparation processes tend to yield multi-crystal MNRs without bio-compatibility, leading to the fact that they are difficult to be applied in the tests *in vitro* and *in vivo* due to their cellular toxicology [27]. Furthermore, MNRs still had the transverse and longitudinal plasmons [28–30], and they were easily localized in vesicles or in the cytoplasm [28]. Some cytotoxicity of MNRs might result from cetyltrimethyl-ammonium bromide (CTAB) coated on their surfaces [29] or from their stiffness and larger aggregation size [30]. According to Tanase's report, [31,32] MNWs with high aspect ratio could be bound to mammalian cells and had a potential for controlling cell populations in specific geometries. Naturally, it is thereby necessary to prepare the MNRs with cytocompatibility. Recently, several reports have shown that the hydrophilic-molecule-coated MNRs were biocompatible, such as polyhistidine/oleyamine [33,34], polyacrylic acid [35,36],

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poly(vinylpyrrolidone) [37], chitosan [38] and SiO_2 [39], and these MNRs could be applied in protein separation/adsorption, cell manipulation, MRI, pathogen detection, handling cell/subcell and cell-guidance [40], respectively.

More recently, dimercaptosuccinic acid (DMSA)- or antibody-coated Zn-ferrite MNPs have been applied in cell marker [41] and cell/biomolecular separations [42]. Our previous studies have shown that Fe_3O_4, Co/Ni-doped ZnO MNPs be mineralized and prepared in the solution of hydrolyzed silk-fibroin (SF) peptides, and they had good cellular compatibility [43–45]. However, to the best of our knowledge, uniform Zn-ferrite MNRs with good neuro-cyto-compatibility have not been reported in literature.

PC12 cell, a widely used cell line as the paradigm for neurobiological and neurochemical studies, could readily and rapidly respond to nerve growth factor (NGF), leading to the neurite-like outgrowth [46]. In this paper, we report a one-step bio-mineralization method to prepare Zn-ferrite NRs in the acidified SF peptide solution, and the aspect ratio of the as-prepared NRs can be adjusted via HCl concentration. Since MNRs can be used to control the grow direction of cell/axon [36,40], the neuro-cytocompatibility of these MNRs is investigated via their co-culture with PC 12 cells.

2. Experimental section

2.1. Materials

Bombyx mori silk fibers were purchased commercially and were hydrolyzed into SF peptides according to our previous work [47]. Luria–Bertani (LB) medium and Dulbecco's Modified Eagle Medium (DMEM) used for bacterial or cell cultures were purchased from Sigma–Aldrich and GIBCO (UK), respectively. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were kindly provided by Cell biology Laboratory of Southwest University for Nationalities, and L929/PC12 cells were purchased from Shanghai Institute of Biochemical and Cell Biology. NGF and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Peprotech (USA) and Beyotime Institute of Biotechnology (China), respectively. Paraformaldehyde was obtained from Merck Serono Co., Ltd. (GE). Other used chemicals are analytical grade, purchased from Chengdu Kelong Chemical Co., Ltd. (China), and they are listed in Supplementary data.

2.2. Bio-mineralization of Zn-ferrite NRs

In a typical experiment, SF peptides (1.6 g) were dissolved in 250 mL of deoxygenated water with mild stirring. Then, 20 mL of a stock solution 1 (including 0.4 mmol $FeCl_2{\cdot}4H_2O$ and 0.4 mmol $Zn(CH_3COO)_2{\cdot}2H_2O$ dissolved in 20 mL deoxygenated water) and 10 mL stock solution 2 (including 2.5 g hexamethylenetetramine dissolved in 10 mL deoxygenated water) were quickly added into this SF solution. The pH value of mixed solution was adjusted to 7 with 0.5 M HCl (~0.01 M of HCl concentration in the mixture), and then, the mixed solution was kept at room temperature (about 25 °C) for 2–4 days with mild stirring. The products were precipitated by adding ethanol, separated by centrifugation, washed several times with deoxygenated water, and then dried in a vacuum dry oven.

2.3. Characterization

The morphology and crystalline structures of Zn-ferrite were analyzed with high-resolution transmission electron microscopy (HRTEM, JEM-2100, Japan) and accompanying selected area electron diffraction (SAED), scanning electron microscopy (SEM, JSM-5900LV, Japan), Transmission electron microscopy (TEM, JEOL-200, Japan), and an X-ray diffractometer (XRD, X'Pert, Holland) with Cu Kα radiation, respectively. The ferromagnetic behaviors of the samples at room temperature were investigated by vibrating sample magnetometer (VSM, Lake shore-7400, USA). Their bacteria compatibility was analyzed through co-culture of *E. coli* (or *S. aureus*) and NR samples (See the Supplementary Material for details).

2.4. In vitro PC12 cell culture

The MTT assay was carried out to investigate the toxicity of Zn-ferrite NRs on PC12 cells, which were cultured with high glucose Dulbecco's DMEM containing 5% (v/v) fetal bovine serum (FBS), 10% (v/v) heat-inactivated horse serum, 1% (v/v) penicillin–streptomycin, and 2.5×10^{-4} mmol L^{-1} of $Fe(NO_3)_3$ [48] at 37 °C in 5% CO_2. Prior to being seeded, the cells were pre-cultured with the different medium (including 50 ng mL^{-1} NGF) for 1 day.

Zn-ferrite NRs should be sterilized and completely dispersed in culture medium with different concentrations (from 0.063 to 0.5 mg mL^{-1}). PC12 cells (2×10^4 cells mL^{-1}) in culture medium were added into 96 wells plate (BD Biosciences), and 30 ng mL^{-1} NGF in cell-culture medium was used to induce the differentiation of PC12 cells into nerve-like cells. After the cells were adherent to the culture plate, the medium with sterilized NRs were added into the above 96 wells plate. Finally, the cells were cultured in the incubator with 5% CO_2 at 37 °C for 2 d or 5 d. When cell tests were carried out, the same concentrations of NRs and NPs in culture medium (mg mL^{-1}) are used in order to avoid the effect of their size difference.

2.5. Ultrathin section observation of PC12 cells

First, cells were cultured as described above culture conditions. Then, cultured cells were washed three times with phosphate buffer saline (PBS). Later, they were centrifuged at 1500 rpm for 10 min and their supernates were removed. Subsequently, paraformaldehyde solution (0.5%) at 4 °C was slowly added into the centrifuge tube along the tube wall to fix cells for 30 min. Then, cells were centrifuged at 10,000 rpm for 10 min, and their supernates were removed. Subsequently, these cells were further fixed by 3% paraformaldehyde solution. Fixed cells were washed with PBS and dehydrated sequentially in a graded series of ethanol solutions (30, 50, 70, 90, and 100 v/v%, respectively). Then, fixed cells were embedded with resin. Ultrathin sections (ca. 70 nm of thickness) were obtained using a diamond cutting knife in an ultramicrotome (MT-X; RMC Inc., Tucson, AZ). Later, the ultrathin sections were mounted on copper grids and stained with 2% uranyl acetate and Reynolds' lead citrate (Shanghai Bioleaf Biotech Co., Ltd.), each for 7 min. Finally, thin sections were observed with TEM (JEOL-200, 160 kV).

2.6. Statistics

Results were expressed as means ± SD, and the data were analyzed using analysis of variance (ANOVA) of the Statistical Package for the Social Sciences (SPSS) version 13.0 software. Games-Howell tests were used for post hoc evaluations of differences among groups. In all cases, $p < 0.05$ was considered as statistically significance.

3. Results and discussion

Fig. 1 shows TEM images of the nanomaterial mineralized in different SF solutions, clearly revealing the effect of SF on the