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The biotoxicity of hydroxyapatite nanoparticles to the plant growth

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HIGHLIGHTS

- Mung bean sprouts were first used as the experimental model to research the cytotoxicity of the HAP nanomaterials.
- The biotoxicity depends on the concentration and particle size of HAP nanomaterials.
- The biotoxicity mechanism of HAP nanomaterials was discussed.

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ABSTRACT

In the present study, hydroxyapatite (HAP) nanoparticles of different particle sizes with high crystallinity and similiar structure were prepared by hydrothermal method. The crystal structure and particle size were characterized by X-ray diffraction pattern (XRD), transmission electron microscopy (TEM) and Fourier transform infrared (FT-IR) spectroscopy. Mung bean sprouts were first used as experimental models. Instead of by MTT assay, the cytoxicity of HAP nanoparticles were proved and evaluated by measuring the hypocotyle length of mung bean sprouts in the culture media. The result showed that the inhibition effect to the growth of mung bean sprouts enhanced when HAP nanoparticles existed. Culture media of HAP nanoparticles with different concentrations and particle sizes was prepared to investigate the level of inhibition effect to the growth of mung bean sprouts. The result found that hypocotyl length of mung bean sprouts were the shortest cultured in 5 mg/mL culture media in which the HAP nanoparticles were prepared by hydrothermal method for 24 h. It was concluded the inhibition effect depended on the amount of intracellular HAP nanoparticles. The nanostructure and Ca^{2+} concentration were considered as the main factors to cause cell apoptosis which was the reason of inhibition. The study provided a preliminary perspective about biotoxicity of HAP nanomaterials to the plant growth.

1. Introduction

The worldwide growing interest to biomaterials over the last years results from their irreplaceable role in medical clinic [1]. Hydroxyapatite is used in bone reconstruction because of its similar chemical structure compared to the inorganic composition of human bone and is a basic building component of many newly prepared biomaterials [2–5]. Because of its biocompatibility, biodegradability, ease of synthesis and amenability to achieve highly controlled morphologies [6], nano-sized hydroxyapatite could be used extensively as delivery vehicles for proteins [7], antibiotics [8], drugs [9], radioisotopes [10], genes [11], and even antigens for vaccines [12] and anticancer medicines for human hepatocellular carcinoma cell [13], ovarian carcinoma cell [14], cervical cancer Hela cell [15], anthropogenic urinary tumor cells[16], and leukemia cells [17].

The biocompatibility of HAP materials was undeniable which can be reflected by their widely applications in clinical medicine such as bone reconstruction. However, the cytotoxicity of nanoparticles generated in vivo has been brought to the forefront in recent years [18–20]. Casey A. [18] pointed out Arc discharge and HiPco, two kinds of single-walled carbon nanotube, could induce cytotoxicity by changing the environment of cell culture. Derfus A. M. [19] pointed out the dissociative cadmium ion generated from damaged lattice of cadmium selenide was related to the cytoxicity. Di Pasqua A. J. [20] points out the shape and surface adsorption of silica nanoparticles (MCM-41, MP-T, AP-T, SiO_2) had certain influence on the neuroblastoma cells.

As the nanocrystallization of HAP, the surface activity would be enhanced and some properties which the macro-sized HAP did not have would reveal. Hence, a problem arose that whether HAP nanoparticles had biotoxicity or not. Some researches on the

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biotoxicity of HAP materials to the cancer cells have been reported [13–17], but rare discussion about the mechanism of the interaction between HAP nanoparticles and living cells. In this paper, plant cells would be used to study the relationship between biocompability and biotoxicity of HAP nanoparticles. The problem that whether HAP nanomaterials would be beneficial to the plants since calcium ions would promote the growth of the plants or harmful since nanoparticles would enter into the cells, causing certain cytotoxicity to inhibite the growth of the plants would be answered. Further mechanism of the reaction between HAP nanoparticles and cells has been discussed. The work provided a preliminary perspective about biotoxicity of HAP nanomaterials to the plant growth.

The study used mung bean sprouts as experimental modes to research the effect of HAP nanoparticles on the growth of the plants. HAP nanoparticles were prepared by hydrothermal method [21,1,22–24]. Several culture media were prepared by with HAP nanoparticles of different concentrations and particle sizes. The experimental result showed HAP nanoparticles inhibited the growth of the mung bean sprouts which confirmed the cytotoxicity of HAP nanomaterials. Only when the size of HAP nanoparticles was small enough, these HAP could pass the cell membrane into the cytoplasm to inhibit the growth of the mung bean sprouts. The inhibition effect depended on the amount of the particles of intracellular HAP nanoparticles (HAP nanoparticles in the cell of the plants). It was discussed that the structure of HAP nanomaterials and Ca^{2+} concentration were the main factors of cell apoptosis which caused the inhibition.

2. Experimental

2.1. Preparation of HAP >nanoparticles

0.1 mol/L $CaCl_2$ (250 mL) was added dropwise to 0.06 mol/L KH_2PO_4 (250 mL) within 60 min in a magnetically stirred apparatus. The pH of the solutions was maintained at 8 by adding ethylenediamine ($C_2H_8N_2$ 85 wt%). The suspension was then transferred into the PTFE bottle settled in the hydrothermal kettle at 180 °C for 0, 2, 6, 12, 24, 48 and 72 h, respectively. The precipitates were centrifuged and washed by anhydrous ethanol and deionized water before dried for 12 h at 80 °C. The products were named as HAP (0), HAP (2), HAP (6), HAP (12), HAP (24), HAP (48) and HAP (72).

2.2. Preparation of the culture media of HAP nanoparticles

2.2.1. The preparation of the culture media with different concentration of HAP (2) nanoparticles

After being sterilized and washed by anhydrous ethanol, 1, 2, 3, 5, 7.5 and 10 mg HAP (2) nanoparticles were respectively added into 100 mL deionized water and stirred for 30 min. The turbid liquid as culture media was transferred into the culture dishes.

2.2.2. The preparation of the culture media with different particle sizes of HAP nanoparticles

After being sterilized and washed by anhydrous ethanol, 5 mg HAP (2), HAP (6), HAP (12), HAP (24), HAP (48) and HAP (72) were respectively added into 100 mL deionized water and stirred for 30 min. The turbid liquid as another kind of culture media was then transferred into the culture dishes.

2.3. Instrument and characterization

HAP nanoparticles were analyzed by Fourier transform infrared spectroscopy (FT-IR, Perkin-Elmer 580B, America, 500–4000 cm^{-1}) and X-ray diffraction (XRD) with Cu Kα radiation, $2\theta = 10°–80°$, at a scanning rate of 6°/min, a voltage of 40 kV, and a current of 40 mA.

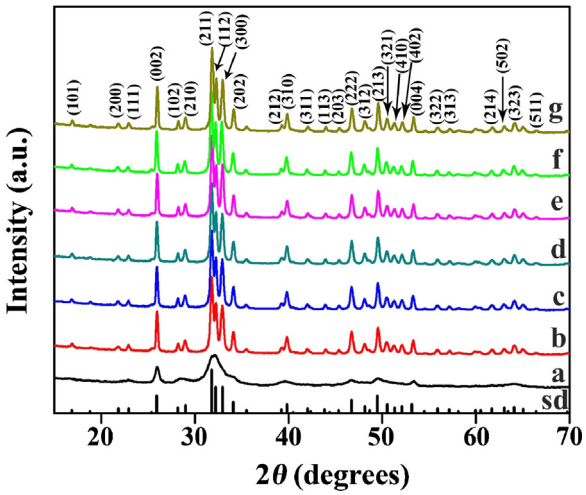

Fig. 1. (a–g) XRD patterns of HAP nanoparticles prepared by hydrothermal method (a, 0 h; b, 2 h; c, 6 h; d, 12 h; e, 24 h; f, 48 h; g, 72 h).

The morphology of HAP nanoparticles was observed by transmission electron microscopy (TEM, Hitachi-800, Japan) and electron diffraction spectrum (ED).

3. Results and discussions

3.1. Discussion of the synthesis conditions of HAP nanoparticles

Hydrothermal route was used to prepare HAP nanoparticles for the following reasons: First, compared with direct precipitation method [25], sol-gel method [26] and other preparation methods [27], hydrothermal route was proceeded under the high temperature and pressure, which would improve the reactivity due to the reaction process and mechanism were relatively different from that under the ordinary condition. The nanoparticles prepared by hydrothermal method had high purity and good dispersibility. Secondly, the product prepared by hydrothermal route without calcining process could form crystal and avoid agglomeration. The crystal shape of the nanoparticles was favourable and controllable. Hence, the research on the biotoxicity of HAP nanoparticles to the plant growth could be conducted under the similar crystallinity and morphology.

Ethylenediamine was used to modulate the pH value. Pure HAP nanoparticles could be easily prepared and calcium-phosphorus ratio was close to 1.67 when pH was adjusted to 8 [28]. Secondly, as a kind of organic molecule, ethylenediamine could control morphology features of crystal by oriented attachment and taking up different crystal space. Amino with positive electricity could take up the void positions of Ca^{2+}, it could be attached on the (0 0 0 1) and (1 0 $\bar{1}$ 0) facet of HAP. However, preferred growth on (1 0 $\bar{1}$ 0) facet would happen since amino had weaker inhibitional effect on crystal growth on (1 0 $\bar{1}$ 0) facet than on (0 0 0 1) facet. Therefore, HAP nanoparticles could be turned into the rod-shaped particles [29].

3.2. The crystallinity of HAP nanoparticles

The XRD pattern of synthesized HAP nanoparticles was presented in Fig. 1. The crystalline phase analysis of the HAP powder was carried out by X-ray diffraction studies. Identification of the phases was realized by comparing the experimental XRD pattern to standards complied by the International Centre for Diffraction Data (ICDD) using the cards (JCPDS, No: 09-0432) for hexagonal HAP crystal structure. All samples showed the characteristic peaks of the hydroxyapatite structure. No other crystalline phases of other