Study on Rare Earth Doped Nano - hydroxyapatite Biosignal Tracer

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Abstract. In recent years, bioluminescent probes with labeled traces have been one of the research hotspots for doping rare earth ions into hydroxyapatite (HA) nanomaterials. Because the 20~40nm HA nanomaterials can easily enter the interior of the cells, the role of the rare earth doped, well - dispersed, 20 ~ 40nm spherical or short rod-like nano HA bioluminescent probe materials for the role of Mechanisms and other studies provide good experimental basis and theoretical support.

1. Nano Bioluminescent Probes Overview

1.1. Introduction of nano-bioluminescent probe materials
With the development of modern life sciences and the enhancement of people’s health awareness, the clinical requirements for disease detection are getting higher and higher. Especially in recent decades, due to changes in the environment and people’s own various pressures, the number of people in the world with worrying physical conditions is increasing, and cancer patients are also increasing. Under such circumstances, how to better integrate life science and medical care and explore the process and mechanism of drug treatment in the human body is becoming more and more important to reduce suffering and cure patients. Many biomolecules have low sensitivity for detection, and often fail to meet the detection requirements. To obtain higher sensitivity, the markers are needed to obtain the required information. The nano-bioluminescent probe material is a luminescent marker material developed under this background. A good bioluminescent probe material should have the following characteristics: (1) Excellent biocompatibility, including non-toxicity, no teratogenicity, and no carcinogenicity. (2) Under certain conditions, such as ultraviolet light or longer wavelength light irradiation, can produce relatively stable fluorescence. (3) The morphology conforms to certain requirements, the size is within a certain range, and has certain functions.

1.2. The research basis, purpose and research content of this study
It has become one of the research hotspots to prepare bioluminescent probes with labeled traces and so on by doping rare earth ions into hydroxyapatite (HA) nanomaterials. Although studies on the doping of rare earth-doped nano HA, especially Tb3+ and Eu3+, have been reported in recent years, there are still some tackling problems. For example, the problem of product agglomeration, the change in morphology after doping, the fluorescence quenching of the doping amount, etc., have not been well resolved, so the research in this area remains to be further explored.
Other studies have shown that 20 to 40 nm HA nanomaterials can easily enter the interior of cells, so preparation of rare earth doped, well dispersed, 20 to 40 nm spherical or short rod shaped nano HA bioluminescent probe materials, for better To solve the above-mentioned problems to provide a good experimental basis and theoretical support, has become the main purpose of this paper.

2. The path of experimental research
The basic research idea is to prepare HA nano-materials that meet the requirements of certain morphologies and sizes, and then select rare-earth-doped HA nano-materials with good luminescence properties and Tb3+. Hydrothermal method was used to select suitable amino acid additives. The effects of different hydrothermal temperature, hydrothermal time and amino acid addition amount on the structure, morphology and size of synthetic HA nanomaterials were discussed. Then, Tb3+ with good luminescence intensity is selected as the doping ion. Based on the preparation of HA nanomaterials, Tb3+-doped Tb-HA nanoparticles are prepared, and the effects of different Tb3+ doping amounts on the structure, morphology size and fluorescence properties of the product Tb-HA nanomaterials were analyzed and studied. Finally, different doses of Tb-HA nanomaterials were co-cultured with L929 mouse fibroblasts for a certain period of time to evaluate their toxicity to normal cells; they were co-cultured with colon cancer cells Caco-2 to determine whether they passed the cells. The endocytosis enters the cell, and if it enters the cell it also has fluorescent properties.

2.1. Screening for L-amino acids
Using hydrothermal method, using Ca(NO3)2•4H2O, (NH4)2HPO4 as raw material, according to a certain ratio of Ca/P/amino acid, use NH3•H2O to adjust the pH value of the solution to control the hydrothermal temperature and hydrothermal temperature Reaction under time conditions. Twenty HAnp samples were prepared by the addition of 20 L-amino acid preparations. The sample with a very thin concentration was dropped on an ultra-thin carbon film copper mesh, and its morphology and size were observed with a transmission electron microscope (TEM). An amino acid was determined as an additive for subsequent experiments, and the addition of this amino acid was able to assist in preparation of a small-particle-size HA nanomaterial having good dispersibility, particle-like spherical shape or short rod shape.

(2) Preparation of Spherical HA Nanomaterials
The HA nanomaterial was prepared by using the amino acid selected in the content (1) as an additive, hydrothermal method, and controlling the conditions of different hydrothermal temperature, hydrothermal time, and the amount of added amino acid under certain pH conditions. X-ray diffraction (XRD), infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) were used to characterize the effects of different conditions on the structure, morphology, and size control of HA nanomaterials.

(3) Rare earth doping of HA nanomaterials
Tb3+ with good luminescence intensity is selected as the doping ion. On the basis of HAnp preparation, Tb3+-doped HA nanoparticles are prepared. XRD, FTIR, TEM, DES, PL and other characterizations were used to analyze and study the effects of different Tb3+ doping on the structure, morphology size and fluorescence properties of the Tb-HA nanomaterials.

(4) Cytotoxicity test of Tb-HA nanomaterials
Different doses of Tb-HA nanomaterials were co-cultured with L929 mouse fibroblasts for a certain period of time. CCK-8 cytotoxicity assay was performed. The absorbance values were measured by enzyme-linked immunosorbent assay (ELISA), and cell viability and cell inhibition rate were calculated according to the formula. To evaluate the toxicity of Tb-HA nanomaterials to normal cells.

(5) Cellular endocytosis of Tb-HA nanomaterials
The Tb-HA nanomaterials were co-cultured with colon cancer cells Caco-2 to see if they could enter the cells via phagocytosis of cells and whether they also had fluorescent properties if they entered the cells.
HA nanomaterials are used as drug carriers due to their biological activity, biocompatibility, and the unique surface effects of nanomaterials. In order to carry out direct monitoring to facilitate the exploration of its activities and mechanism of action, it is necessary to prepare HA into nanoluminescent materials. In recent years, the preparation of bioluminescent probes with labeled traces by doping rare earth ions into HA nanomaterials has gradually become one of the research hotspots. Since 20 to 40 nm HA nanomaterials can easily enter the interior of the cell, this paper prepared rare earth-doped, well-dispersed, 20 to 40 nm spherical or short rod-like nano HA bioluminescent probes. Provide a good experimental basis and theoretical support for the study of its mechanism of action.

![Figure 1. XRD patterns of Tb-HA synthesized with different amounts of Tb3+, (a) 0mol%. (b) 1mol%. (c) 2mol%. (d) 3mol%. (e) 4mol%.

3. The experimental results are as follows:

(1) Using hydrothermal method, under conditions of hydrothermal temperature of 150°C, hydrothermal time of 20h, and pH value of 11, different L-amino acids were added according to the ratio of Ca/P/amino acid = 10/6/1 to synthesize HA nanomaterials. Among the 20 kinds of L-amino acids, the addition of polar amino acids tends to make HA nanomaterials tend to grow to short rod-like particles with smaller size, and the effect on the morphology and size of HA nanomaterials is greater than that of non-polar amino acids; Among the amino acids, L-lysine can help regulate the synthesis of spherical HA nanomaterials.

(2) Using hydrothermal method and using L-lysine as additive, the effects of hydrothermal temperature, hydrothermal time, and L-lysine addition amount on the morphology and size of HA nanomaterials were investigated. With the increase of the hydrothermal temperature and the prolongation of time, the crystallinity of the HA nano-particles tends to increase, and the morphology of the HA nano-particles changes from a long rod-like tendency to a short rod-like or spherical shape. When the hydrothermal temperature is 185°C and the hydrothermal time is 25h, the HA nanomaterial produced has a good crystallinity, a short rod shape, and a small amount of spherical particles. Compared with hydrothermal temperature and hydrothermal time, the addition amount of L-lysine has more obvious effect on the crystallinity, morphology and particle size of HA nanomaterials. When the amount of L-lysine was 7.4144g, the HA nano-materials were basically spherical, with high...
crystallinity, uniform particle size, and an average particle size of about 30 nm, which was in line with the particle size requirements for entering biological cells.

(3) To investigate the effect of Tb$^{3+}$ doping amount of 0-4 mol% on the composition, structure, morphology, size and fluorescence properties of the synthesized Tb-HA nanomaterials. The undoped HA nanomaterials are mainly spherical, and the four doped samples are mainly short rods with spherical particles and the crystallinity is reduced, it shows that the doping of Tb$^{3+}$ affects the growth process of HA Nan crystals and thus affects the composition and structure of nanomaterials. Fluorescence analysis showed that the undoped HA nanomaterials were not fluorescent, and the four doped samples emitted green fluorescence, and all had strong emission peaks at 491nm and 545nm. When the doping amount was 2mol%, Tb-HA nanomaterial has the highest fluorescence.

(4) Tb$^{3+}$ doped Tb-HA nanomaterials in concentrations of 250μg/mL, 500μg/mL, and 1000μg/mL suspensions with L929 mouse fibroblasts at 0, 2 and 4 mol% after co-cultured for 12h, the cell viability was good and the cell inhibition rate was small, which accorded with the relevant national medical device biological standards. At a concentration of 250μg/mL, 500μg/mL, 1000μg/mL, 1500μg/mL and 2000μg/mL suspensions were co-cultured with L929 cells for 24h. The cell viability is good, the cell inhibition rate is small, and the cells are basically non-toxic.

(5) Different amounts of 2mol% Tb-HA nanomaterials were co-cultured with Caco2 colon cancer cells for 48h. Cell micrographs showed that Caco2 cells were normal and elongated in both blank and experimental groups. Fluorescence microscopy showed that the blank group had no fluorescence, while the experimental group showed fluorescence. The Tb-HA nanomaterial is fluorescent after being endocytosis by Caco2 cells, and is expected to become a bioluminescent probe material.

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