Acta Biomaterialia 37 (2016) 165–173

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Hafnium-doped hydroxyapatite nanoparticles with ionizing radiation for lung cancer treatment

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ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form 29 March 2016
Accepted 6 April 2016
Available online 6 April 2016

Keywords:
Hafnium
Doping
Hydroxyapatite
ROS
Ionizing radiation
pH-dependent solubility

ABSTRACT

Recently, photodynamic therapy (PDT) is one of the new clinical options by generating cytotoxic reactive oxygen species (ROS) to kill cancer cells. However, the optical approach of PDT is limited by tissue penetration depth of visible light. In this study, we propose that a ROS-enhanced nanoparticle, hafnium-doped hydroxyapatite (Hf:HAp), which is a material to yield large quantities of ROS inside the cells when the nanoparticles are bombarded with high penetrating power of ionizing radiation. Hf:HAp nanoparticles are generated by wet chemical precipitation with total doping concentration of 15 mol% Hf^{4+} relative to Ca^{2+} in HAp host material. The results show that the HAp particles could be successfully doped with Hf ions, resulted in the formation of nano-sized rod-like shape and with pH-dependent solubility. The impact of ionizing radiation on Hf:HAp nanoparticles is assessed by using *in-vitro* and *in-vivo* model using A549 cell line. The 2′,7′-dichlorofluorescein diacetate (DCFH-DA) results reveal that after being exposed to gamma rays, Hf:HAp could significantly lead to the formation of ROS in cells. Both cell viability (WST-1) and cytotoxicity (LDH) assay show the consistent results that A549 lung cancer cell lines are damaged with changes in the cells' ROS level. The *in-vivo* studies further demonstrate that the tumor growth is inhibited owing to the cells apoptosis when Hf:HAp nanoparticles are bombarded with ionizing radiation. This finding offer a new therapeutic method of interacting with ionizing radiation and demonstrate the potential of Hf:HAp nanoparticles in tumor treatment, such as being used in a palliative treatment after lung surgical procedure.

Statement of Significance

Photodynamic therapy (PDT) is one of the new clinical options by generating cytotoxic reactive oxygen species (ROS) to kill cancer cells. Unfortunately, the approach of PDT is usually limited to the treatment of systemic disease and deeper tumor, due to the limited tissue penetration depth of visible light (620–690 nm). Here we report a ROS-enhanced nanoparticle, hafnium-doped hydroxyapatite (Hf:HAp), which can trigger ROS when particles are irradiated with high penetrating power of ionizing radiation. The present study provides quantitative data relating ROS generation and the therapeutic effect of Hf:HAp nanoparticles in lung cancer cells. As such, this material has opened an innovative window for deeper tumor and systemic disease treatment.

1. Introduction

Reactive oxygen species (ROS), in the form of superoxide anion (O^{2-}), singlet oxygen ($_1O^2$), hydroxyl radicals (OH), various peroxides (ROOR), hydroperoxides (ROOH) or the hydrogen peroxide (H_2O_2), are known to play a dual role in biological application,

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http://dx.doi.org/10.1016/j.actbio.2016.04.004
1742-7061/

for they can be either harmful or beneficial to living systems [1]. ROS could induce mitogenic response at low concentration. In contrast, excessive ROS may cause irreversible oxidative stress damage and interfere with cellular function, followed by the damage of several cellular components, including lipid membranes, protein, and DNA [2,3]. They have also been regarded as a common mediators to regulate mitochondrial respiratory chain and cause cells apoptosis [2].

Photodynamic therapy (PDT) is one of the new clinical options, based on the concept of producing cytotoxic ROS and free radicals, to treat cancer by exposing a photosensitizer to a specific light wavelength [4]. However, PDT is limited to the treatment of systemic diseases and deeper tumors, due to the limited tissue penetration depth of visible light (620–690 nm) [4,5]. For this reason, PDT is usually used to treat cancer on or just under the skin or located in areas, such as lining of internal organs or cavities that are easily reached with the light source during the treatment [6].

Recently, new pharmaceutical agents containing high atomic metallic ions have been regarded as an attractive material, based on the concept of interacting with ionizing radiation, to generate free radicals for deeper cancer treatment. By use of ionizing radiation, such as X-rays and gamma rays, the tissue penetration depth can easily reach to the range of 8–14 cm [5]. Exposed to ionizing radiation, the metallic nanoparticles of high electron density, such as gadolinium-based, titanium-based, silver-based, and gold nanoparticles, are regarded as the most appropriate ROS-ehanced radiosensitizers [7,8]. These metallic nanoparticles can interact with ionizing radiation to produce Auger electrons, Compton electrons, secondary electrons, and photoelectrons, which in turn interact with water molecules to produce free radicals and trigger the quantities of ROS in cells [9]. However, these metallic nanoparticles usually show reactive surface, which may easily interact with healthy tissues and damage them [10,11].

Nowadays, there is a new inert material, named NBTXR3, being developed to replace metallic nanoparticles with composition of hafnium oxide (HfO_2) [12]. HfO_2 nanoparticle has a high electron density and possesses photo-luminescent properties [13]. NBTXR3 has been demonstrated *in-vitro* and *in-vivo* that once HfO_2 nanoparticle accumulates in deeper tumor cells, particles will lead to generate large quantities of electrons when they are bombarded with ionizing radiation. In the preclinical studies, it has been shown that HfO_2 nanoparticles could enhance the effect of radiation therapy to kill the cancer cells while reducing the damage of side effects to healthy tissues. The application of NBTXR3 nanoparticles is now stepping toward in European Phase II/III clinical trial [14]. Nevertheless, despite these advances, HfO_2 nanoparticles with inert properties have been regarded to be easily excluded from cells through exocytosis, causing to reduce cellular uptake, and decreasing a curative effect in comparison of biodegradable materials [15,16]. Additionally, particles administered into the body are eventually taken up in the mononuclear phagocyte system, leading to the accumulation of particles in the liver or spleen organs; therefore, unbiodegradable materials will increase the likelihood of unintended acute or chronic toxicity [17].

Hydroxyapatite (HAp) is the major inorganic component in nature bones with biodegradability, and numerous studies have shown that HAp properties can be easily regulated by different metallic ions doping [18]. For instance, when Ca^{2+} in HAp is replaced by ions, such as iron, cobalt or nickel, this HAp could become a useful agent in magnetic resonance imaging contrast, cell separation, drug delivery and hyperthermia treatment of cancers [19–21]. HAp with lanthanide ions doping may endow the material with novel fluorescent properties [22]. Furthermore, C.S. Ciobanu et al. [18,23,24] showed that silver ions would be the best choice of dopants to create antimicrobial activity properties of HAp without causing cytotoxicity. These findings demonstrate that HAp is an ideal host material for metallic ions substitution, which convert HAp into a material with desired properties for biological applications.

Thus, in this study we use HAp as the host material with the doping of Hf ions into HAp structure (Hf:HAp), and ask whether this doped-particle could enhance ROS for anti-cancer treatment when it is bombarded with ionizing radiation. Hf:HAp nanoparticles were synthesized by wet chemical reaction with total doping concentration of 15 mol% Hf^{4+} relative to Ca^{2+} in HAp host material. The human lung epithelia cell line A549 was employed as the *in-vitro* and *in-vivo* model to assess the impact of ionizing radiation on Hf:HAp nanoparticles.

2. Materials and methods

2.1. Synthesis of Hf:HAp nanoparticles

Hf:HAp particles with nominal cell contents $Ca_{10-x}Hf_x(PO_4)_6(OH)_2$, x = 0, 0.5 and 1.5 (respectively, representative of HAp, Hf (5 mol%):HAp and Hf (15 mol%):HAp) were synthesized by wet chemical precipitation method previously described [25]. Briefly, 0.3 M of H_3PO_4 solution (Wako, Japan) contained with various concentration of hafnium chloride (Sigma-Aldrich) was slowly droped (1.3 ml/min) to an aqueous suspension of 0.5 M $Ca(OH)_2$ solution (Wako, Japan) with the atomic ratio (Ca + Hf)/P fixed at 1.67. The suspension was then vigorously stirred at 80 °C for 2 h with the solution maintained at pH 8.0. The ultimate suspension was aged at room temperature for 24 h. After aging, the suspension was centrifuged and washed with de-ionized water for three times and freeze-dried for further analysis. Particles re-suspended in culture media were used for *in-vitro* and *in-vivo* administration.

2.2. Materials characterization

X-ray powder diffractometer (XRD; Geiger Flex, Rigaka) was utilized to identify the crystalline phase composition using Cu Kα radiation (λ = 0.15406 nm) with the potential at 30 kV and the current at 20 mA. The specimens were scanned in a range from 20° to 60° at a speed of 0.05°/s. Patterns and unit-cell parameters were analyzed using a model automatched to the International centre for diffraction data (ICDD) database by using Jade 6.0 software.

The lattice parameters (a-axis and c-axis) were calculated from the major reflection peaks, (2 1 1), (3 0 0), (0 0 2), (2 2 2) and (2 1 3), with the following Eq. (1), where *hkl* are Miller's indices, d is the interplanar spacing. The unit cell volume (V) of pure and Hf:HAp particles were calculated using the Eq. (2) [26,27].

$$\frac{1}{d^2} = \frac{4}{3}\left[\frac{h^2 + hk + k^2}{a^2}\right] + \frac{l^2}{c^2} \quad (1)$$

$$V = \sin(60^\circ)a^2c \quad (2)$$

The reflection peak (0 0 2) from the XRD pattern was used to calculate the grain size (D) of HAp and Hf:HAp, using the equation of Debye-Scherrer [28]. Where λ is the wavelength of the monochromatic X-ray beam (0.15406 nm), B is the full width at half-maximum intensity (FWHM) of peak, and θ is the Bragg angle.

$$D = \frac{0.94\lambda}{B\cos\theta} \quad (3)$$

Energy-dispersive X-ray spectroscopy (EDX; Microscope TM3000, Hitachi) was used to analyze the composition of ions in particles by randomly detection of five areas. The composition of Ca/P and Hf/Ca molar ratio with varied concentration of Hf ions dopant was expressed.

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