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In-situ formation of holmium oxide in pores of Mesoporous Carbon Nanoparticles as substrates for neutron-activatable radiotherapeutics

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ABSTRACT

Radionuclide therapy with nano-sized carriers is a very promising approach to treat various types of cancer. The preparation of radioactive nanocarriers can be achieved with minimum handling using a neutron-activation approach. However, the nanocarrier material must possess certain characteristics such as low density, heat-resistance, high metal adsorption, easy surface modification and low toxicity in order to be useful. Mesoporous Carbon Nanoparticles (MCNs) in which holmium oxide is formed in their pores by a wet-impregnation process are investigated as a suitable material for this application. Holmium (^{165}Ho) has a natural abundance of 100% and possesses a large cross-section for capturing thermal neutrons. After irradiation of Ho-containing MCNs in a neutron flux, ^{166}Ho, which emits therapeutic high energy beta particles as well as diagnostic low energy gamma photons that can be imaged externally, is produced. The wet impregnation process (16 w/w% Ho loading) is shown to completely prevent the leaching of radioactive holmium from the MCNs without compromising their structural integrity. *In vitro* studies showed that the MCNs containing non-radioactive holmium do not exhibit toxicity and the same formulation with radioactive holmium (^{166}Ho) demonstrated a tumoricidal effect. Post-irradiation PEGylation of the MCN surfaces endows dispersibility and biocompatibility.

1. Introduction

A variety of materials has been used in the design of nanocarriers for delivery of therapeutic agents to target organs and tissues. These agents include drugs, vaccines and radionuclides that emit particulate (α or β^-) radiation. For the latter application, creating nanocarrier materials containing large amounts of therapeutic radionuclides can be hazardous to the personnel involved in this process. This can be avoided by incorporating a carefully selected stable nucleus into the nanocarriers and subsequently exposing them to a high flux density of thermal neutrons such as is found in a nuclear reactor. If the stable nuclei have a sufficiently large thermal neutron capture cross-section, they can be activated to therapeutic (i.e., tumor cell killing) radioactive nuclei. This neutron-activation approach allows the stable isotope-containing nanocarriers to be produced without constraints related to handling hazardous radioactive materials or short isotopic half-lives. The nanocarriers can be subjected to neutron irradiation just prior to the time of administration to patients, thus limiting the handling of radioactive materials by personnel. For such an application, the nanocarrier materials must be able to entrap significant amounts of the stable isotope, withstand the harsh conditions in the core of a nuclear reactor, retain the radioactive isotope produced following neutron irradiation to prevent accumulation of the radionuclide in non-target tissues due to leaching, be of relatively low density for facile administration by the intravenous route, and be non-toxic.

Mesoporous Carbon Nanoparticles (MCNs) have been widely studied for use in a variety of applications including as fuel cells, supercapacitors, gas storage devices and as catalysts, but only rarely for drug delivery applications. This is in contrast to other carbon-based materials such as carbon nanotubes, fullerenes, graphene oxide nanoplatelets and carbon nanodiamonds, all of which have been used as drug delivery vehicles [1–4]. Zhu et al. explored the possibility of using MCNs for chemo-therapeutic drug delivery by exploiting the hydrophobic nature, large surface area, easy surface modification and low toxicity profile of MCNs [5]. A product known as Carbon Nanoparticles Suspension

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Injection (CNSI®), which contain particles 150 nm in diameter, has been tested in human clinical trials and was approved as a lymph node tracer by the China Food and Drug Administration [6]. Interestingly, these carbon nanoparticles did not induce major toxicities or allergic reactions [7]. Here we report on the investigation of MCNs as a carrier of stable isotopes for subsequent conversion to radioactive isotopes following neutron irradiation. This neutron-activation approach allows the preparation of therapeutic radionuclides in carriers that can be targeted to tumors (requiring large amounts of radioactivity) with minimum handling by the operator. Among the potential candidates of stable neutron-activatable elements, holmium (^{165}Ho) was chosen due to its high natural abundance (100%) and its large thermal neutron capture cross-section (64 b where 1 b = 10^{-24} cm^2) for activation to ^{166}Ho. This radioactive isotope of holmium decays by the emission of high energy β^- particles (E_{max} = 1.84 MeV) and γ-photons (81 keV, 6.7% photon yield) with a relatively short half-life (26.9 h). These properties make ^{166}Ho suitable as a 'theranostic' agent whereby the β^- particles provide their tumor-killing radiotherapeutic function while the emitted γ-photons allows the biodistribution of ^{166}Ho-labeled MCNs to be assessed non-invasively by SPECT/CT imaging (Fig. 1) [8,9]. Indeed, a ^{166}Ho/chitosan complex was formulated as a local tumor ablative agent in which their safety and efficacy were confirmed in a Phase IIb clinical study [10]. This formulation was approved under the brand name of Milican® in 2001 by the Korea Food and Drug Administration [11]. While this formulation demonstrated potential as a radiotherapeutic agent, it was produced by the cumbersome process of incorporating ^{166}Ho into the formulation and not by neutron-activation of a ^{165}Ho/chitosan complex.

When considering materials as suitable carriers of neutron-activatable elements, one must take into account the loading capacity of the carriers for these elements as well as their stability following irradiation in a nuclear reactor where the neutron flux density is great enough to yield therapeutically-sufficient amounts of the activated radionuclide. Holmium encapsulated polymeric microspheres comprised of poly (L-lactic acid) have been used as carriers of neutron-activatable elements, and one such composition is currently in clinical trials for treating liver cancer [8,12]. In spite of successful translation to the clinic, the biggest drawback of this formulation is degradation of the polymer by heat generated during the neutron-activation process. This can lead to premature leaching of the activated radionuclide which can result in radioactivity accumulating in non-target tissues after administration to a patient. In order to reduce this degradation, shorter neutron irradiation times must be used; however, this limits the total amount of radioactivity that can be produced to potentially sub-therapeutic levels.

Di Pasqua et al. explored the use of mesoporous silica nanoparticles (MSNs) as a carrier for neutron-activatable isotopes for intraperitoneal administration [9]. Because of our interest in using neutron-activated nanocarriers as a means to treat various types of cancers by systemic administration, high specific activities (mCi of ^{166}Ho mg^{-1} of nanocarrier) were required. This meant that long neutron irradiation times were needed to produce these high specific activities. Therefore, the nanocarriers required for this application need to have relatively high adsorption capacities for stable holmium and also need to be resistant to degradation in the relatively harsh nuclear reactor environment. In addition, it was desirable to avoid high-density nanocarriers to prevent settling of the particles during administration through an indwelling catheter. MCNs appeared to be an excellence choice as a nanocarrier for this theranostic application due to their desirable characteristics of lower toxicity than MSNs, heat resistance and high metal adsorption capacity [13–15]. Nanocarriers composed of mesoporous carbon were evaluated for their ability to serve as a suitable delivery vehicle for therapeutic radionuclides produced by neutron activation. Here we report that sufficient quantities of the stable isotope holmium (^{165}Ho) could be incorporated into MCNs using a wet impregnation technique, and that minimal leaching of the radionuclide produced by neutron activation (^{166}Ho) following irradiation in a nuclear reactor was observed. A novel NMR method was used to characterize the pore size distribution of the prepared MCNs. In addition, an *in vitro* cell-based assay was used to confirm

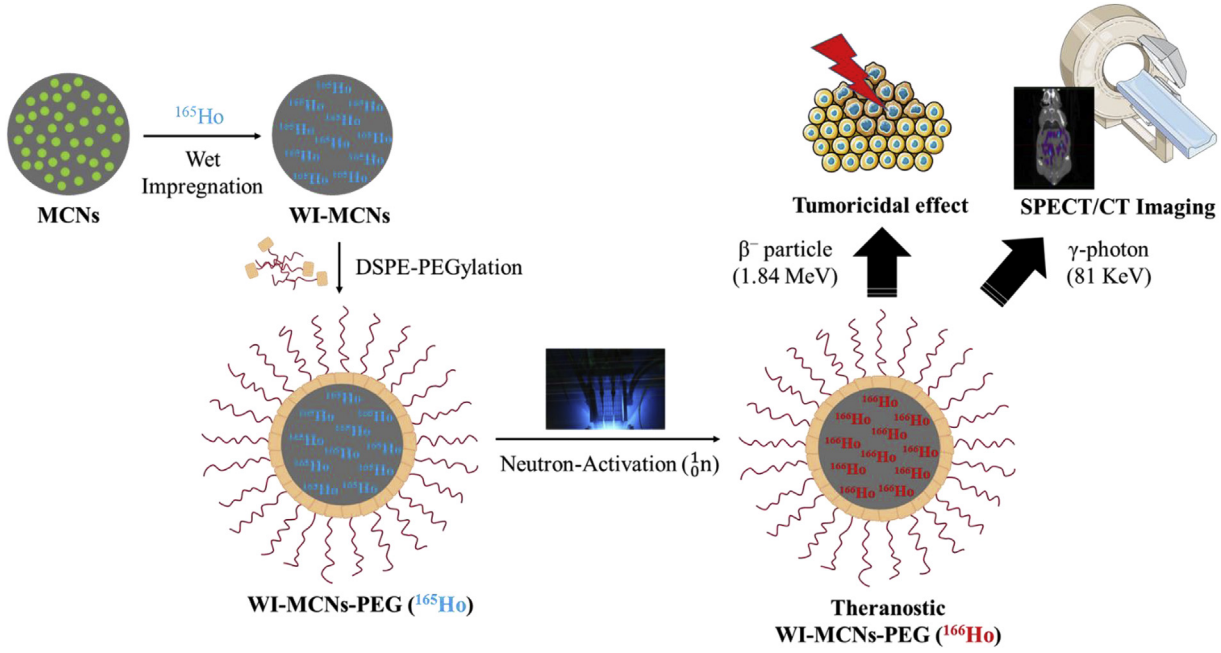

Fig. 1. Neutron-Activation and Theranostic Application of Mesoporous Carbon Nanoparticles (scheme produced using Servier Medical Art (www.servier.com)). (A colour version of this figure can be viewed online.)