Synthesis of Highly-loaded Holmium-165 Siloxane Particles for Brachytherapy of Brain Cancer and Injectability Evaluation in Healthy Pig

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Abstract

Glioblastoma is the most invasive type of glial tumors, rapidly growing and commonly spreading into nearby brain tissue. Internal radiotherapy, also called brachytherapy, is an advanced cancer treatment where radioactive seeds are placed in or near the tumor itself, giving a high radiation dose to the tumor while reducing the radiation exposure in the surrounding healthy tissues. Among the available radioactive materials, 166Ho emits high-energy β radiation required for tumor destruction and low-energy γ radiation which can be used for quantitative SPECT imaging. In addition, Ho is a highly paramagnetic element and can therefore be visualized by MRI. In this context, we synthesized 400 nm particles via a sol-gel process in acidic conditions using holmium (III) oxide Ho2O3 as a nanostructured precursor. The objective was to design particles with a high 165Ho content combined with an enhanced stability suitable for brachytherapy of brain cancer following neutron activation. A sol-gel process was chosen to make Ho2O3 soluble in aqueous media and to improve particle’s colloidal stability. The resulting suspension showed enhanced colloidal stability in water, as evidenced by zeta potential and sedimentation rate measurements, combined with a high Ho content (28% w/w).

Injectability and preliminary acute toxicity of the suspension were assessed after stereotactic injection in the brain of healthy minipig. Computerized tomography (CT) imaging was performed to visualize the injection sites and to determine the holmium distribution. It turns out that our suspension provided high 165Ho concentration to the site of action by way of stereotactic injection. This procedure allowed identifying suitable experimental conditions for future intratumoral injections of activated suspension.

Keywords: Holmium-165; Brachytherapy; Suspension; Minipig; Injectability; Glioblastoma

Introduction

Most common types of tumor treatments include surgery, chemotherapy and/or external radiotherapy. Radiation therapy or radiotherapy is a treatment using ionizing radiations (photons or charged particles) to damage DNA of malignant cells. Internal radiotherapy (also called brachytherapy) is an advanced cancer treatment. Radioactive seeds or sources are placed in or near the tumor using tube-like catheters, giving a high radiation dose to the tumor while reducing the radiation exposure in the surrounding healthy tissues [1]. Radioactive seeds may be produced under the shape of microspheres doped with β emitting radionuclides; this technique is known as micro-brachytherapy [2].

Glioblastoma is the most invasive type of primary brain tumors, rapidly growing and commonly spreading into nearby brain tissue. Its incidence is 2-3 per 100,000 adults per year in the US and Europe and approximately 13,000 cases are diagnosed each year in Europe. It shows the worst prognosis of all brain cancers because surgical resection of entire tumors is almost impossible [3]. Due to the high recurrence rate of glioblastoma, it is considered as incurable despite new medications such as Temozolomide and improved surgical techniques [4]. Consequently, many patients are eligible for micro-brachytherapy. The market for radiation therapy equipment is expected to reach US$8 billion by 2020 due to rising incidence of cancer and increasing preference for non-surgical treatment options [5].

Recently, Gliasite® (IsoRay Medical) has given a new impulse to brain tumors brachytherapy. This system is a form of intracavitary brachytherapy where a balloon catheter is introduced into the brain and inflated with a radioactive Iodine-125 solution [6]. Radiation is delivered locally to the surrounding brain tissues to treat tumors with greater accuracy, ensuring a high dose delivery. Unfortunately, this technique is controversial since it is not applicable for inoperable tumors, it shows a limited efficacy and induces higher costs for healthcare facilities [7].

In contrast, 165Ho polysiloxane nanoparticles have been developed for applications in brachytherapy of liver malignancies [8]. Holmium shows several advantages over other elements such as 90Y used in brachytherapy because: 1) it is a highly paramagnetic element suitable for MRI-guided selective administration, 2) after neutron activation, 165Ho is a combined β- and γ-emitter making the particles visible by Single Photon Emission Computed Tomography (SPECT) enabling nuclear imaging (useful for dosimetric calculations) relevant for personalized patient treatments [9]. Beta particles (Eβmax=1.84 MeV) exhibits radiotherapeutic properties appropriate for therapy. Additionally, the penetration range of 165Ho into soft tissues is 1.23
Holmium-165 (\(^{165}\text{Ho}\)) siloxane particles synthesis

Nanostructured \(\text{Ho}_2\text{O}_3\) precursor (0.397 mol) was first slurried and refluxed in 1.5 L ethanol together with acetic acid (0.262 mol) and Si-EDTA (0.05 mol) for 20 hours. Mixture was then allowed to cool down to room temperature and transferred into 200 mL plastic centrifuge bottles (Fisher Scientific). Particles were washed in ethanol twice and water by centrifugation cycles (10 minutes at 4100 rpm) and resuspended in 160 mL mQ water. Then, particle size was homogenized by stirring the suspension for 4 days at 30°C.

Characterization of the holmium-165 (\(^{165}\text{Ho}\)) siloxane particles

Particle size was determined via Dynamic Light Scattering using a zetasizer Nano-ZS (Red Badge) Zen 3600 (Malvern Instruments) equipped with a 633 nm laser. \(^{165}\text{Ho}\) siloxane suspension was diluted 200 times in diethylene glycol (DEG) to reach a mass concentration of approximately 2.5 g/L\(^{-1}\). A total of 6 measurements were performed after 5 minutes equilibration time. Zeta potential was determined using the same apparatus coupled with an automatic titrator MPT-2 (Malvern Instruments). pH of the suspension was automatically adjusted between 7.0 and 12.5 using NaOH 0.1 M and HCl 0.12 M. Measurements were performed at 25°C after a temperature homogenization time of 2 minutes.

Infra-red spectra were acquired using an IRAffinity 1 (Shimadzu Scientific Instruments) spectrophotometer controlled by LabSolutions software. One milliliter of suspension was dried in an oven at 80°C for 12 hours. Subsequent powder analysis was carried out by ATR technique using a single reflection diamond crystal on ZnSe plate. Density of the final \(^{165}\text{Ho}\) siloxane suspension was measured in triplicate at 25°C by weighing 1 mL of suspension.

Dry matter corresponds to the mass of particles once the suspension has been entirely dried. Three glass vials were filled with 300 µL suspension and left in an oven B170 (Nabertherm) at 300°C for 10 hours. Final value was given in g L\(^{-1}\). Viscosity was measured at 25°C using a 5V-10 vibro-viscometer (A&D, Japan) by detecting the electric current necessary to resonate two sensor plates in a sample fluid.

Sedimentation rate was determined using a method based on erythrocyte sedimentation rate (ESR). ESR is the rate at which red blood cells sediment in one hour. Graduated glass sedimentation tubes and frame with cross-lever bar were obtained from Degag (Germany). Tubes were filled with 700 µL suspension until the zero mark. Suspension was then allowed to stand in the tube placed vertically for one hour. Under gravity, particles tend to settle out from the solvent. The rate at which they settle is measured as the number of graduations (millimeter) of clear solvent present at the top of the tube after one hour (mm per hour or %). Each measurement was made in triplicate.

Animal and surgical procedure

A four months Yucatan minipig (19.3 kgs, INRA Saint-Gilles, France) was left 10 days for acclimatization. Minipig was premedicated with an intramuscular injection of atropine sulfate and azaperone. After 20 minutes, anesthesia was administered intramuscularly with Zoletil\(^{100}\) (Tiletamine + Zolazepam). Sedation was achieved by a continuous inhalation of Isoflurane 2%. Pre-operative analgesia consisting of morphine hydrochloride (Aguettant\(^{+}\)) was injected. Head of the pig was subsequently fixed in a Large Animal Stereotactic Frame (Stereotaxic Instruments, RWD 68901). Incision site was disinfected with a povidone-iodine solution. A 7 cm longitudinal skin incision was
made on the midline of skull, 2 cm in cranial part of occipital crest. Periosteum was abraded to clear the skull bone and the intersections. Six holes (diameter 4 mm) were drilled in the skull on both left and right hemispheres. Syringing pusher KDS Legato 130 (KD Scientific, USA) was mounted on the stereotactic system. In parallel, \textsuperscript{165}Ho siloxane suspension was vortexed for 1 minute and then loaded into a 1 mL syringe (BD Plastipak) connected to either a 25G × 40 mm cannula (Magic Needle) or a 25G × 50 mm lumbar puncture needle (BD Neonatal) pre-wetted with PEG 400 surfactant. The syringe-needle combination was mounted on the syringe pusher. A total of six injections were performed.

Three injections were made in the right hemisphere using 25G cannula as follows: 1) 3 µL of suspension at an injection rate of 100 µL/min (injection depth: 10.8 mm from the surface of the skull), 2) 5 µL at 100 µL/min (injection depth: 11.3 mm), 3) 10 µL (2 × 5 µL with 2 minutes idle time in between) at 100 µL/min (injection depth: 13.4 mm). Then, three injections were made in the left hemisphere using 25G needle as follows: 1) 3 µL at 100 µL/min (injection depth: 10.8 mm), 2) 5 µL at 100 µL/min (injection depth: 11.3 mm), 3) 10 µL (2 × 5 µL with 2 minutes idle time in between) at 100 µL/min (injection depth: 13.4 mm). Cannula and needle were gently withdrawn in about 45 seconds from the brain two minutes after the injections.

Post-operative CT scans of the brain were performed by Voxscan (Marcy l’Etoile, France) using a CT-Scan GE BrightSpeed 16 (GE Healthcare) to determine the location of injection sites and observe the distribution of cold holmium. Three-dimensional volumes were reconstructed using OsiriX and RadiAnt softwares. Then, 30 ml of a solution made on the midline of skull, 2 cm in cranial part of occipital crest. Periosteum was abraded to clear the skull bone and the intersections. Six holes (diameter 4 mm) were drilled in the skull on both left and right hemispheres. Syringing pusher KDS Legato 130 (KD Scientific, USA) was mounted on the stereotactic system. In parallel, \textsuperscript{165}Ho siloxane suspension was vortexed for 1 minute and then loaded into a 1 mL syringe (BD Plastipak) connected to either a 25G × 40 mm cannula (Magic Needle) or a 25G × 50 mm lumbar puncture needle (BD Neonatal) pre-wetted with PEG 400 surfactant. The syringe-needle combination was mounted on the syringe pusher. A total of six injections were performed.

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Results and Discussion

First, commercial nanostructured Ho\textsubscript{2}O\textsubscript{3} precursor was analyzed using X-Ray diffractometry (XRD), exhibiting a crystallite size of 60 ± 15 nm. Inductively Coupled Plasma – Mass (ICP-MS) measurements showed an high Holmium content of 85 ± 3% w:w. Submicronic particles were then obtained using a sol-gel process. Reaction consisted in mixing the Ho\textsubscript{2}O\textsubscript{3} precursor in ethanol in presence of acetic acid and Si-EDTA silane (2% massic equivalent SiO\textsubscript{2}) under reflux (Figure 1).

The transmission FTIR spectrum of the particles is displayed in Figure 1. The broad band around 3400 cm\textsuperscript{-1} can be assigned to –OH and –COOH stretching vibrations. The peak at 1558 cm\textsuperscript{-1} is due to –COO\textsuperscript{-} vibrations. Finally, the presence of a peak at 1412 cm\textsuperscript{-1} can be attributed to C=O bonds. Taken together, the presence of these peaks indicates that particles with zeta potentials higher than 30 mV or less than -30 mV are stable in solution [18,19]. The high positive value is mainly imparted from the predominant presence of holmium oxide slightly modified by the very negative zeta potential provided by the thin polysiloxane layer (2% massic equivalent SiO\textsubscript{2}). Indeed, the isoelectric point (IEP) of the suspension was found at pH 10.5 ± 1, which is consistent with values measured for trivalent rare earth oxides (around 11) [20].

Finally, the sedimentation rate was measured since it provides invaluable information about the suspension stability and injectability. When suspension is placed in a test tube, particles gradually settle to the bottom. The sedimentation rate test measures the distance particles fall in a test tube in one hour and provides valuable information about colloidal stability and future behavior during syringe injections. The obtained suspension exhibits a sedimentation rate around 1-2% after 1 hour and 10-15% after 24 hours. An ideal suspension is said to be in flocculation equilibrium, meaning that no clear supernatant is visible within the tubes. In our case, the values were obtained were extremely low from a clinical point of view, considering that intracerebral injections are typically performed within 10-20 minutes during high-dose rate treatments [21]. Also, sedimentation rate of the same suspension was measured after one year storage following redispersion under moderate
vortex during 30 seconds and was unchanged. These results indicate that the suspension is easy to redisperse into a uniform mixture upon moderate shaking and keeps its stability.

Taken together, these data indicate that the suspension exhibits a high solid content that enhances its physical stability in water, making it a priori suitable for brachytherapy of brain cancer.

**Stereotactic injection in the brain of a minipig**

Injectability, i.e. the performance of the formulation during injection [22], was then assessed in preclinical settings in the brain of a healthy pig. Yucatan minipig has been used as a relevant model to assess the injectability of the “cold” 165Ho siloxane suspension because of its similarities to humans in both structure and function [23,24]. For ethical concerns, only one animal was observed as a proof-of-concept. Various volumes (3, 5 and 10 µL) of the suspension were injected in the brain of a minipig by coupling a stereotactic frame to a syringe pusher (Table 1). Injections were performed using either a metallic flexible cannula or a rigid needle pre-wetted with polyethylene glycol 400 (PEG 400). PEG 400 is a low-molecular-weight grade of polyethylene glycol widely used as a pre-wetting agent in a variety of pharmaceutical formulations due to its viscosity and low toxicity [25,26]. Also, blunt cannula has been included in the surgical procedure since practitioners increasingly use it over sharp needles due to its less traumatic shape. Injectability was subsequently assessed macroscopically and monitored by performing post-operative computerized tomography (CT) acquisitions.

The ideal suspension should be delivered to the site of interest in a controlled manner, be retained for a prolonged period of time and minimize reflux. In our case, suspension could be delivered successfully in the brain and no needle clogs were observed. A waiting time of two minutes was implemented following injection and the needle/cannula was then slowly removed from the brain in about 45 sec to prevent reflux. However, a suspension volume of 1 µL refluxed in the holes after the injection of 5 and 10 µL, both with needle and cannula. In contrast, no backflow was observed following injection of 3 µL. It turns out that a lower injection volume decreases the risk of reflux through the penetration tract.

In parallel, CT-Scan observations showed that the suspension (whatever the volume) is retained at the site of injection (Figure 5) although the slight reflux observed macroscopically is distinguishable.
by adjusting needle’s gauge, flow rate and injection pressure [28].

Insight about the reflux issue and 2) to circumvent it inside the tumor pig xenograft model of brain cancer will be necessary 1) to get some backflow after intratumoral injections [27]. So, further tests using a than healthy tissue and can potentially increase the risk of suspension

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during the injection of 10 µL suspension as 2 × 5 µL with 2 minutes idle
time in between, meaning that 4 minutes in total for the injections of 10
µL. It suggests that a longer post-injection waiting time would enable

a better localization of the injected holmium suspension and would reduce the subsequent risk of reflux. However, this parameter will need to be adjusted in function of the clinical procedure (i.e. dosimetry, number of injections and idle time).

In addition to these results, it must be considered that permeability and elasticity of striatum tissue in healthy brain are different from those of tumoral tissue. In fact, the density of tumoral tissue is higher than healthy tissue and can potentially increase the risk of suspension backflow after intratumoral injections [27]. So, further tests using a pig xenograft model of brain cancer will be necessary 1) to get some insight about the reflux issue and 2) to circumvent it inside the tumor by adjusting needle’s gauge, flow rate and injection pressure [28].

Conclusions and Perspectives

The suspension described previously can potentially enable the delivery of high 166Ho concentration to the site of action via stereotactic injection. CT-Scan images following intracerebral injection of the suspension in a pig model showed that reflux issues may appear. However, this problem will be solved in future studies by decreasing the flow rate and the needle/cannula gauge. Next steps will consist in 1) evaluating the intratumoral injectability of cold holmium suspension and then 2) checking that the physicochemical properties of the suspension remain unchanged following the neutron activation of 165Ho in 166Ho. These studies will also help to define the potential in vivo acute toxicity of the suspension.

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References


