Journal of
Nanomedicine & Nanotechnology

Marcon et al., J Nanomed Nanotechnol 2017, 8:5
DOI: 10.4172/2157-7439.1000460

Research Article Open Access

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

Marcon L[1*#], Gehan H[2*#], Khoshnevis M[3], Marmuse L[2], Carozzo C[3], Louis C[2], Ponce F[3] and Tillement O[1]

[1]*Institut Lumière Matière, UMR CNRS 5306, UCBL, Campus LyonTech - La Doua, Bâtiment Raulin, 10 rue Ada Byron, 69622 Villeurbanne, France*
[2]*Nano-H SAS, 2 place de l'Europe, 38070 Saint-Quentin Fallavier, France*
[3]*ICE (Interactions Cellules Environnement), UPSP 2016.A104, VetAgro Sup, Campus Vétérinaire de Lyon, 1 avenue Bourgelat, 69280, Marcy l'Etoile, France*
[#]*Authors contributed equally*

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, ^{166}Ho 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 Ho_2O_3 as a nanostructured precursor. The objective was to design particles with a high ^{165}Ho content combined with an enhanced stability suitable for brachytherapy of brain cancer following neutron activation. A sol-gel process was chosen to make Ho_2O_3 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 ^{165}Ho 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, ^{165}Ho polysiloxane nanoparticles have been developed for applications in brachytherapy of liver malignancies [8]. Holmium shows several advantages over other elements such as ^{90}Y used in brachytherapy because: 1) it is a highly paramagnetic element suitable for MRI-guided selective administration, 2) after neutron activation, ^{166}Ho 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 (E_{max}=1.84 MeV) exhibits radiotherapeutic properties appropriate for therapy. Additionally, the penetration range of ^{166}Ho into soft tissues is 1.23

***Corresponding authors:** Lionel Marcon, Institut Lumière Matière, UMR CNRS 5306, UCBL, Campus LyonTech - La Doua, Bâtiment Raulin, 10 rue Ada Byron, 69622 Villeurbanne, France, Tel: 0034665105506; Fax: 33472431233; E-mail: lionel.marcon@univ-lyon1.fr

Helene Gehan, Nano-H SAS, 2 place de l'Europe, 38070 Saint-Quentin Fallavier, France, Tel: 33663404380; E-mail: h.gehan@nano-h.com

Received: August 16, 2017; **Accepted:** August 24, 2017; **Published:** September 01, 2017

Citation: Marcon L, Gehan H, Khoshnevis M, Marmuse L, Carozzo C, et al. (2017) Synthesis of Highly-loaded Holmium-165 Siloxane Particles for Brachytherapy of Brain Cancer and Injectability Evaluation in Healthy Pig. J Nanomed Nanotechnol 8: 460. doi: 10.4172/2157-7439.1000460

mm on average with a maximum of 8.6 mm, sufficient for micro-brachytherapy of glioblastoma.

Similarly, holmium-166 poly(L-lactic acid) (^{166}Ho-PLLA) microspheres have been developed at University Medical Center Utrecht (Utrecht, Netherlands) for quantitative multimodal *in vivo* imaging [10]. Other types of microparticles composed of lower-density polymers, including alginate [11] and chitosan [12] containing ^{166}Ho, as well as nanosized carrier materials containing stable ^{165}Ho for subsequent neutron activation [13], have also been reported. However, most of these polymeric carrier materials cannot withstand long neutron irradiation times without degrading or aggregating. In this regard, Di Pasqua et al. designed mesoporous silica-based nanoparticles incorporating ^{165}Ho showing physicochemical stability before and after long reactor irradiation times [14]. Commercially available mesostructured silica nanoparticles were used to encapsulate a lipophilic acetylacetonate complex of ^{165}Ho in the pores. In addition, the use of "cold" isotope ^{165}Ho enables to overcome limitations of handling radioactivity (mostly characterization difficulties and necessity of radioprotection) during the synthesis.

On this basis, our strategy was to design particles with a high ^{165}Ho content combined with an enhanced stability suitable for brachytherapy of brain cancer. We synthesized 400 nm particles *via* a sol-gel process in acidic conditions using Holmium (III) oxide Ho_2O_3 as a nanostructured precursor. Such process was chosen to make Ho_2O_3 soluble in aqueous media and to improve particle's colloidal stability. In addition, this process is cost effective, scalable and allows for a fine control over thickness [15]. The resulting particles showed good 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 *in vivo* tolerability of the resulting ^{165}Ho siloxane suspension were assessed after stereotactic injection in the brain of healthy minipig. Pre- and post-operative computerized tomography (CT) imaging was performed to visualize the injection sites and to determine holmium distribution. This procedure allowed identifying suitable experimental conditions for future intratumoral injections of radioactive suspension.

Materials and Methods

Chemicals

All chemicals were reagent grade or higher and were used as received unless otherwise specified. Nanostructured holmium oxide Ho_2O_3 precursor was supplied by Nano-H SAS (Saint-Quentin Fallavier, France). N-[3-Trimethoxysilyl)propyl]ethylenediamine triacetic acid trisodium salt (Si-EDTA) 45% in water was obtained from Abcr. Acetic acid glacial was purchased from VWR Chemicals. Diethylene glycol ≥99% and polyethylene glycol 400 (PEG 400) were supplied by Sigma-Aldrich. Ethanol absolute anhydrous was obtained from Carlo Erba.

Characterization of the nanostructured Ho_2O_3 precursor

Nanostructured Ho_2O_3 precursor was characterized by X-ray diffractometry (XRD) using a Brüker D8 Advance diffractometer equipped with a Vantec high speed one dimensional detector. Analysis was performed by the Centre of diffractometry (CLEA - University Claude Bernard, Lyon, France). Holmium content was determined using Inductively Coupled Plasma – Mass (ICP-MS) spectrometry by analytical laboratory Antellis (Toulouse, France).

Holmium-165 (^{165}Ho) siloxane particles synthesis

Nanostructured Ho_2O_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}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}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 IR 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}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 SV-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 Dehag (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 abducted to clear the skull bone and the intersections. Six holes (diameter 4 mm) were drilled in the skull on both left and right hemispheres. Syringe pusher KDS Legato 130 (KD Scientific, USA) was mounted on the stereotactic system. In parallel, ^{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 Voxcan (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 pentobarbital (Dolethal) were administered intracardially, causing a painless and immediate death.

Results and Discussion

First, commercial nanostructured Ho_2O_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_2O_3 precursor in ethanol in presence of acetic acid and Si-EDTA silane (2% massic equivalent SiO_2) under reflux (Figure 1). Resulting particles were washed successively with ethanol and water, and then redispersed in mQ water.

Particle size was determined by Dynamic Light Scattering; suspension was diluted in a polyol solvent of moderate viscosity, *i.e.* diethylene glycol (DEG, 37.5 mPa.s at 25°C), to limit sedimentation following dilution. The particles exhibited a hydrodynamic diameter of 400 ± 150 nm (Figure 2).

The transmission FTIR spectrum of the particles is displayed in Figure 3. The broad band around 3400 cm^{-1} can be assigned to –OH and -COOH stretching vibrations. The peak at 1558 cm^{-1} is due to -COO$^-$ vibrations. Finally, the presence of a peak at 1412 cm^{-1} can be attributed to C-O bonds. Taken together, the presence of these peaks indicates the successful attachment of the Si-EDTA silane to the nanostructured Ho_2O_3 precursor.

The Ho content, as measured by ICP, was 28 ± 3% w: w, which is superior to the value of 17 ± 0.5% obtained by the Ho-loaded poly(L-lactic acid) microspheres produced by the University Medical Center (Utrecht, Netherlands) [10]. This value will potentially enable the delivery of a high radioactive dose to tumor cells. In addition, density (1.42 ± 0.04) as well as dry matter (550 ± 50 g.L^{-1}) high values confirmed the visual assessment that the suspension is highly concentrated. Viscosity is around 5-9 × 10^{-3} Pa.s which is comprised between the viscosity of blood (3-4 × 10^{-3} Pa.s) and ethylene glycol (16 × 10^{-3} Pa.s) [16]. The rheology of a particle suspension is a complex function of its physical properties since the key factors are particle volume fraction, particle shape, inter-particle forces and the spatial arrangement of particles [17]. Unlike parenteral suspensions, the formulation of stable and injectable suspensions for glioblastoma radiotherapy involves use of high solid content and/or increased viscosity to stabilize the formulation (*i.e.* to enhance colloidal stability).

To assess colloidal stability, we first measured the effect of pH on the zeta potential in a pH range between 7.0 and 12.5 (Figure 4). Under physiological conditions (pH 6-8), zeta potential is around 30 mV and decreases from 9.5 onward. It proves that inter-particle forces are greater than attractive forces among particles. Indeed, it is generally considered that particles with zeta potentials higher than 30 mV or lower 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_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 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

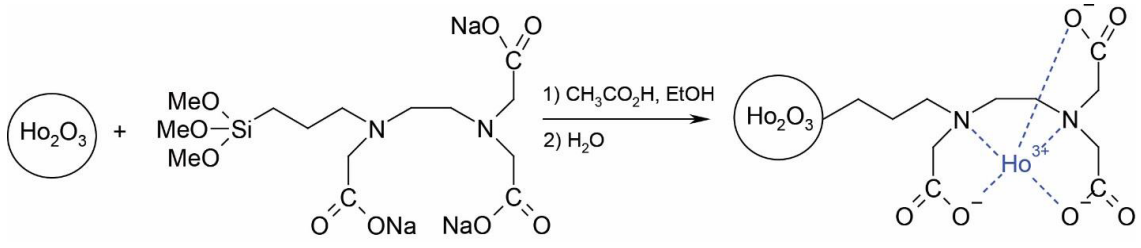

Figure 1: Synthesis scheme of ^{165}Ho polysiloxane particles.

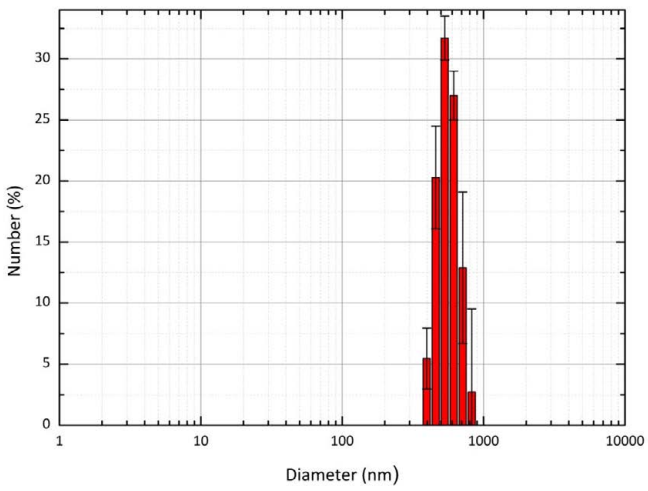

Figure 2: Averaged hydrodynamic diameter of ^{165}Ho polysiloxane particles. Each measurement consists of 6 runs.

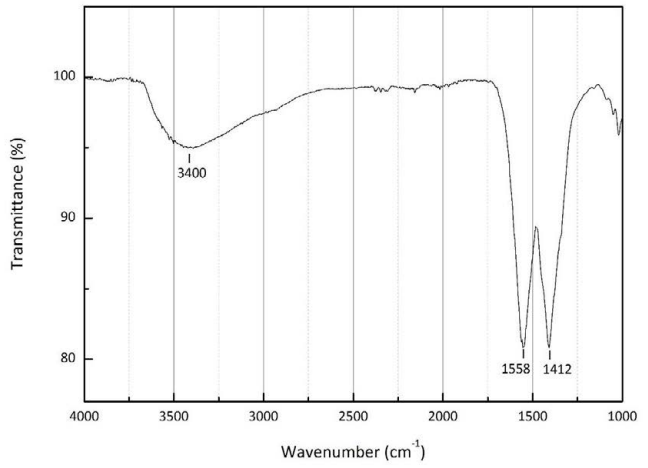

Figure 3: FTIR spectrum of the ^{165}Ho polysiloxane particles.

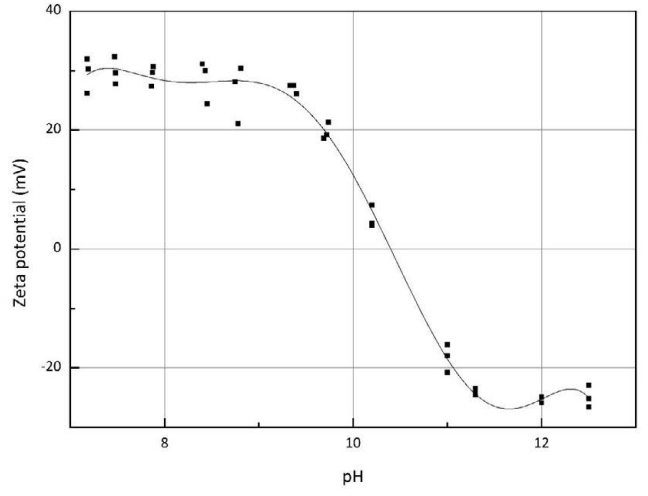

Figure 4: Variation in the zeta potential of the ^{165}Ho polysiloxane suspension in function of the pH.

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" ^{165}Ho 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.

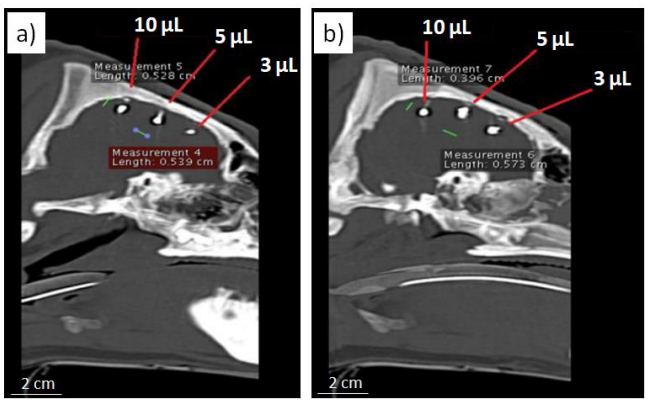

Figure 5: CT-Scan images after the injection of 3 µL, 5 µL and 10 µL of suspension from cranial to caudal respectively use a) a needle (left hemisphere) and b) a cannula (right hemisphere).

Tube characteristics (Injection site)	Injected suspension volume (µL)	Refluxed volume† (µL)	Distributed volume in brain tissue‡ (µL)
25G× 50 mm lumbar puncture needle (left hemisphere)	**3**	**/**	**6**
	5	1 µL	19
	10*	1 µL	16
25G × 40 mm cannula (right hemisphere)	3	/	16
	5	1µL	25
	10*	1 µL	14

*2 × 5 µL with 2 minutes idle time

†Volumes estimated visually

‡Estimated from the CT acquisitions

Table 1: Summary of injection parameters and experimental observations. Column 3: Refluxed volume as estimated visually. Column 4: Distributed volumes in brain tissues estimated from the CT acquisitions. Flow rate: 100 µL/min. Injection depth between 10.8 mm and 13.4 mm from the surface of the skull.

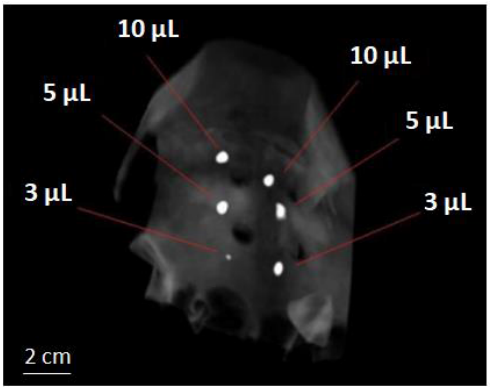

Figure 6: Three-dimensional (3D) CT-Scan images in caudo-ventral view after injection of the suspension. Left: Needle. Right: Cannula.

Three-dimensional volumes were then reconstructed using DICOM image viewers based on the post-operative CT acquisitions to track and estimate the volumes of distribution of the injected ^{165}Ho suspension in healthy brain tissue. The resulting image (Figure 6) enabled to estimate the reconstructed three-dimensional volumes (Table 1, last column). There is a difference in volume of 3 µL injections between needle and cannula (6 µL *vs.* 16 µL).

Interestingly, the distributed volumes after the injection of 10 µL suspension are smaller than in the case of the injection of 5 µL both in needle and cannula. This could be due to the additional idle time 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 ^{166}Ho 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 ^{165}Ho in ^{166}Ho. These studies will also help to define the potential *in vivo* acute toxicity of the suspension.

Acknowledgments

This work was financially supported by the project TheraneaM "Therapy by Neutron Activation using Microparticles" ISI (Innovation Stratégique Industrielle)..

References

1. Baba AI, Câtoi C, Baba AI (2007) Comparative oncology. The Publishing House of the Romanian Academ.
2. Buono S, Burgio N, Hamoudeh M, Fessi H, Hiltbr E, et al. (2007) Brachytherapy: State of the Art and Possible Improvements. Anticancer Agents Med Chem 7: 411-424.
3. Urbańska K, Sokołowska J, Szmidt M, Sysa P (2014) Glioblastoma multiforme-an overview. Współczesna Onkol 5: 307-312.
4. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, et al. (2005) Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. N Engl J Med 352: 987-996.
5. http://www.strategyr.com/MarketResearch/Radiation_Therapy_Equipment_Market_Trends.asp.
6. Gobitti C, Borsatti E, Arcicasa M, Roncadin M, Franchin G, et al. (2011) Treatment of recurrent high-grade gliomas with GliaSite brachytherapy: a prospective mono-institutional Italian experience. Tumori 97: 614-619.
7. Ruge MI, Kickingereder P, Grau S, Treuer S, Sturm V, et al. (2012) Stereotactic iodine-125 brachytherapy for brain tumors: temporary versus permanent implantation. Radiat Oncol 7: 94.
8. Nijsen JF, van het Schip AD, Hennink WE, Rook DW, van Rijk PP, et al. (2002) Advances in nuclear oncology: microspheres for internal radionuclide therapy of liver tumours. Curr Med Chem 9: 73-82.
9. Vente MAD, Nijsen JFW, de Wit TC, Seppenwoolde JH, Krijger GC, et al. (2008) Clinical effects of transcatheter hepatic arterial embolization with holmium-166 poly(l-lactic acid) microspheres in healthy pigs. Eur J Nucl. Med Mol Imaging 35: 1259-1271.
10. Zielhuis SW, Nijsen JF, de Roos R, Krijger GC, van Rijk PP, et al. (2006) Production of GMP-grade radioactive holmium loaded poly(l-lactic acid) microspheres for clinical application. Int J Pharm 311: 69-74.
11. Zielhuis SW, Seppenwoolde JH, Bakker CJ, Jahnz U, Zonnenberg BA, et al. (2007) Characterization of holmium loaded alginate microspheres for multimodality imaging and therapeutic applications. J Biomed Mater Res A 82A: 892-898.
12. Kim JK, Han KH, Lee JT, Paik YH, Ahn SH, et al. (2006) Long-term Clinical Outcome of Phase IIb Clinical Trial of Percutaneous Injection with Holmium-166/

Chitosan Complex (Milican) for the Treatment of Small Hepatocellular Carcinoma. Clin. Cancer Res 12: 543-548.

13. Bult W, Varkevisser R, Soulimani F, Seevinck PR, de Leeuw H, et al. (2010) Holmium Nanoparticles: Preparation and In Vitro Characterization of a New Device for Radioablation of Solid Malignancies. Pharm Res 27: 2205-2212.
14. Di Pasqua AJ, Yuan H, Chung Y, Kim JK, Huckle JE, et al. (2013) Neutron-Activatable Holmium-Containing Mesoporous Silica Nanoparticles as a Potential Radionuclide Therapeutic Agent for Ovarian Cancer. J Nucl Med 54: 111-116.
15. Claire Billotey (2009) Nouveaux agents de radiothérapie ciblée ou de curiethérapie à base d'oxydes et d'oxo-hydroxydes de terre rare.
16. Yaws CL (1995) Handbook of viscosity.
17. Mueller S, Llewellin EW, Mader HM (2010) The rheology of suspensions of solid particles. Proc R Soc Math Phys Eng Sci 466: 1201-1228.
18. van Driessche I, Hoste S (2006) In Functional Coatings.
19. Vallar S, Houivet D, El Fallah J, Kervadec D, Haussonne JM (1999) Oxide slurries stability and powders dispersion: optimization with zeta potential and rheological measurements. J Eur Ceram Soc 19: 1017-1021.
20. de Jong KP (2009) Synthesis of solid catalysts. Wiley Online Library.
21. Verhoeff JJC, Stalpers JLK, Coumou AW, Koedooder K, Lavini C, et al. (2007) Experimental iodine-125 seed irradiation of intracerebral brain tumors in nude mice. Radiat Oncol 2: 38.
22. Cilurzo F, Selmin F, Minghetti P, Adami M, Bertoni E, et al. (2011) Injectability Evaluation: An Open Issue. AAPS Pharm Sci Tech 12: 604-609.
23. Kararli TT (1995) Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dispos 16: 351-380.
24. Swindle MM, Smith AC, Hepburn BJ (1988) Swine as Models in Experimental Surgery. J Invest Surg 1: 65-79.
25. Gill HS, Prausnitz MR (2007) Coating Formulations for Microneedles. Pharm Res 24: 1369-1380.
26. Strickley RG (2004) Solubilizing Excipients in Oral and Injectable Formulations. Pharm Res 21: 201-230.
27. Pogoda K, Chin L, Georges PC, Byfield FJ, Bucki R, et al. (2014) Compression stiffening of brain and its effect on mechanosensing by glioma cells. New J Phys 16: 075002.
28. Khoshnevis M, Carozzo C, Bonnefont-Rebeix C, Belluco S, Leveneur O, et al. (2017) Development of induced glioblastoma by implantation of a human xenograft in Yucatan minipig as a large animal model. J Neurosci Methods 282: 61-68.