MULTIFUNCTIONAL MICROSPHERES WITH AN ULTRAHIGH HOLMIUM LOAD FOR IMAGING AND THERAPY

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Introduction Local administration of radioactive microspheres for microbrachytherapy is a promising therapy for irresectable liver malignancies. Currently, two FDA approved products use yttrium-90, a pure beta emitter, for internal radiation of the tumor(s). Beta irradiation does not allow for direct imaging. For this purpose, holmium-166 poly-(L-lactic acid) microspheres(^{166}Ho-PLLA-MS) were proposed for internal radiation therapy in the early 1990's and were further explored in the last decade[1]. The microspheres contained approximately 17% w/w holmium to be neutron activated in a nuclear reactor. After neutron activation, holmium emits both a gamma and a beta, which allows for imaging, and therapy respectively. Since holmium is highly paramagnetic it can be visualized by MR imaging, even without neutron activation. In higher concentrations, holmium can be visualized by CT imaging, since holmium has a high attenuation coefficient[2]. Direct intratumoral injection requires small deposits of radioactive microspheres in compact tumor tissue, limiting the overall injection volume to 0.1–0.3 ml[3]. Consequently, the amount of activity per injection should be very high. In this work we present the production of microspheres with a substantially increased holmium content to optimize internal radiation treatment of patients with liver malignancies by intratumoral injections. Furthermore, we investigated the sensitivity on both MRI and CT and determined the detection limit in liver tissue for both MRI and CT.

Materials and Methods The microspheres were produced by solvent evaporation. In short, holmium as its acetylacetonate complex was dissolved in chloroform and subsequently added to an aqueous solution of polyvinyl alcohol (2%). Under continuous stirring the chloroform was evaporated, and after 40 hours the holmium acetylacetonate microspheres (HoAcAc-MS) were collected, washed, sieved and dried under a continuous nitrogen flow.

The microspheres were characterized by determining holmium content and size distribution and visualized using light microscopy (LM), scanning electron microscopy (SEM) and were subjected to differential scanning calorimetry (DSC). Furthermore the microspheres were neutron irradiated. Multimodality imaging characteristics were studied using MRI and CT. First, sensitivity of both modalities was determined by a phantom experiment. Second, a gel with depots containing 1 µg to 1 mg of HoAcAc microspheres was constructed for qualitative imaging on both CT and MRI. Lastly, HoAcAc microspheres were injected into an excised rabbit liver, to assess the detection limit in an *ex vivo* experiment.

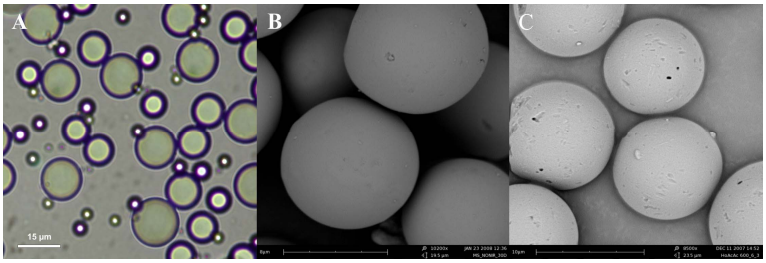

Figure 1 A) Light micrograph of HoAcAc microspheres before sieving (mean size 15 µm), B) scanning electron micrograph of non activated HoAcAc microspheres and C) scanning electron micrograph of HoAcAc microspheres after 6 hours of neutron activation.

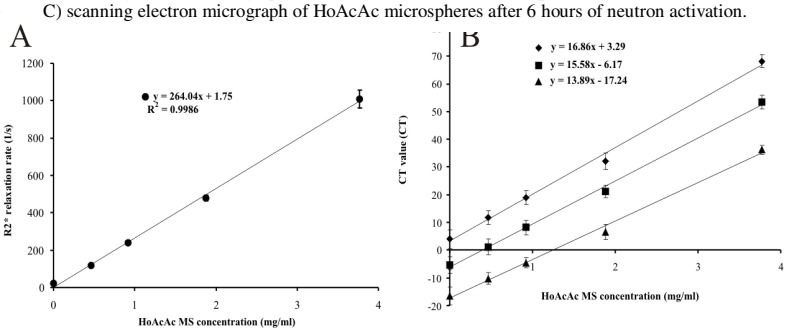

Figure 2 Regression curves, A) showing the R2* relaxation rate versus the concentration of HoAcAc microspheres suspended in agarose gel B) showing linear attenuation coefficient versus the concentration of HoAcAc microspheres suspended in agarose

Results It was demonstrated that microspheres could be prepared using HoAcAc crystals as the sole starting material. Interestingly the holmium content of the HoAcAc microspheres (45%) was higher than the holmium content of the HoAcAc crystals (31%). The particle size ranged between 5 and 25 micrometers, which could be adapted for different applications by altering the stirring speed during the solvent evaporation process. The size distribution remained the same both before and after neutron irradiation. LM revealed spherical particles, and when studied more in depth, the surface of the particles was smooth. Neither surface damage, nor agglomeration of particles was observed after neutron irradiation. MR and CT imaging experiments demonstrate the potential use of the particles as contrast agents, since the increased holmium content resulted in an increase in r2* relaxivity (264 ± 5.7 s^{-1} mg^{-1} ml) and an increase in the linear attenuation coefficient 15.6 HU mg^{-1} ml when compared to the HoPLLA microspheres (92 and 6.7, respectively). Moreover, MR imaging allowed for the detection of a 1µg depot of HoAcAc microspheres in a gel, and 5 µg in an excised liver.

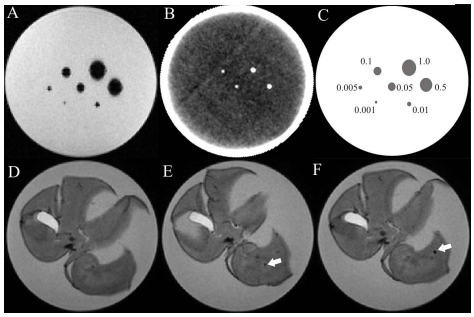

Figure 3 A) Gradient echo MRI image and B) CT image of agarose phantom with different depots of HoAcAc microspheres, C) schematic representation of agarose phantom with different depots of HoAcAc microspheres, including the amount of microspheres in milligram, D) MRI image of rabbit liver before administration of HoAcAc microspheres, E) MRI image of rabbit liver after administration of 5 µg and F) after administration of 10 µg

Discussion and conclusion Microspheres with high holmium load were produced following a straight forward methodology. The microspheres proved to be stable, both before and after irradiation. The holmium content was higher than the expected 33 %, which suggests that one acetylacetone molecule has left the complex, and that there was a rearrangement of the HoAcAc complex. This is supported by the DSC data where a higher melting temperature was found for the HoAcAc microspheres than for the HoAcAc crystals. We conclude that we have produced a stable particle that has a higher holmium loading, which is suitable for internal radiation therapy and as an imaging agent for nuclear, MR and CT imaging. The high stability, high holmium load and straightforward production methods allows for tailoring the size of the particles for different applications. For instance, small particles (ranging from 100 to 4000 nm) can be made by increasing the stirrer speed during solvent evaporation or by sonication which may be used for tumor detection as a safe and non-toxic alternative to iron-loaded agents. Larger particles (6 to 200 micrometer) can be used for embolising or lodging in and around the tumor vasculature to deposit radiation the tumor. Moreover, lanthanide chemistry is the same for all lanthanides, and therefore holmium can easily be replaced by other lanthanides, such as europium or dysprosium, to meet the required characteristics for different applications.

Literature

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