

Excellence in Electron Microscopy Award – Project Report

Particle Characterization of Non-Radioactive Preparations of a Beta-Emitting Liquid Brachytherapy Agent for Solid Tumor Treatment

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Overall Hypothesis and Goals

Brachytherapy is a type of cancer radiotherapy in which radioactive implants are inserted directly into diseased tissue. Conventional brachytherapy uses radioactive sources that emit low-energy gamma rays and are encapsulated in a metal shell. While low-energy gamma rays can penetrate the metal housing, beta particles are severely attenuated by metals such as titanium and stainless steel. The liquid brachytherapy approach overcomes this limitation via the incorporation of beta-emitting radionuclides into small, micrometer-scale particles. Our partners at IsoTherapeutics Group (ITG) have developed BetaBrach™, a novel beta-emitting form of liquid brachytherapy that employs unsealed yttrium-90 (Y-90) as the radioactive source co-precipitated with non-radioactive yttrium in hydroxycarbonate microparticles (putatively Y(OH)CO₃). BetaBrach particles are administered into the tumor to deliver a permanent implant that does not migrate and is retained for days within the target, thereby delivering high doses of radioactivity to the diseased tissue with minimal radiotoxicity to adjacent healthy tissues.

BetaBrach is injected directly into solid tumors as a suspension via a syringe, a unique feature that distinguishes BetaBrach from other clinically-approved ⁹⁰Y-microparticles, such as the radioembolization agents TheraSphere® or SIR-Spheres® (administration via catheterization of an artery). The aim of this work was to characterize non-radioactive BetaBrach preparations via Electron Microscopy imaging. By utilizing SEM techniques, we sought to investigate the size and morphology of the particle preparations, evaluate the reproducibility of the synthetic method, assess the particle stability in aqueous conditions, and test the feasibility of an alternative synthetic approach.

Approach and Methods

BetaBrach particles are produced by the co-precipitation method in aqueous environment; a radioactive ⁹⁰YCl₃ solution is mixed with non-radioactive ⁸⁹YCl₃ and urea, and the mixture is heated in a water bath to yield ⁹⁰Y/⁸⁹Y hydroxycarbonate particles. The synthetic mechanism involves the degradation of urea to CO₂ and NH₃ *in situ* and subsequent reaction with the Y⁺³, which leads to homogeneous precipitation of Y-hydroxycarbonate particles. The mean particle size and distribution is affected by the amount of yttrium, the concentration of urea, and the heating time. For most of the Electron Microscopy studies, the non-radioactive particles were prepared using the BetaBrach method (in the absence of ⁹⁰Y), to allow for characterization by conventional techniques (radioactive materials may not be analyzed at the EMC). An alternative enzymatic precipitation method utilizing urease at room temperature was also evaluated and compared with the original method. In particular, urease leads to enzymatic degradation of urea to CO₂ and NH₃ to cause subsequent precipitation of the yttrium cations. All microscopy studies were performed on the FEI Quanta 600F & Bruker EDS.

Results and Discussion

Three different batches of freshly-prepared particles were analyzed by SEM, Figure 1. Mean particle size, size distribution and morphology were reproducible across all three batches. More

specifically, non-radioactive BetaBrach consisted of clusters of spherical particles connected via thin bridges with a mean sphere size that ranged from 125 nm to 250 nm, while the average diameter was approximately 200 nm. Repeated analyses demonstrated that microparticles remained intact in the reaction mixture for over a month with no morphological changes. Stability in water was also assessed over the same period (one-month) and again no signs of decomposition or changes in the size and shape of the particles were observed, Figure 2. EDS elemental mapping confirmed the expected $Y(OH)CO_3$ composition, with reproducible elemental profiles among all batches analyzed, Figure 3. Interestingly, when the particles were suspended in phosphate-buffered saline (PBS) over a one-week period, the shape of the PBS-suspended particles became ill-defined; the outer particle surface was not as smooth and a thicker bridging between individual particles was observed compared to the original particles, Figure 4. Elemental mapping with EDS, Figure 5, demonstrated the absorption of phosphorus (P) into the particles, presumably due to an ion exchange process between the OH^- or CO_3^{2-} anions in the $Y(OH)CO_3$ particles with the phosphate anions. Therefore, the differences in the morphology observed in SEM imaging may be explained by the different elemental profile of the particles. Nevertheless, more studies are needed to clarify the mechanism of this ion exchange process. Finally, a feasibility study utilizing an alternative enzymatic method with urease at room temperature demonstrated that the resulting particles differed in size and morphology compared to particles synthesized via our conventional method, Figure 6. Additionally studies will be needed to optimize the enzymatic reaction parameters to achieve homogeneous precipitation as for the original formulation.

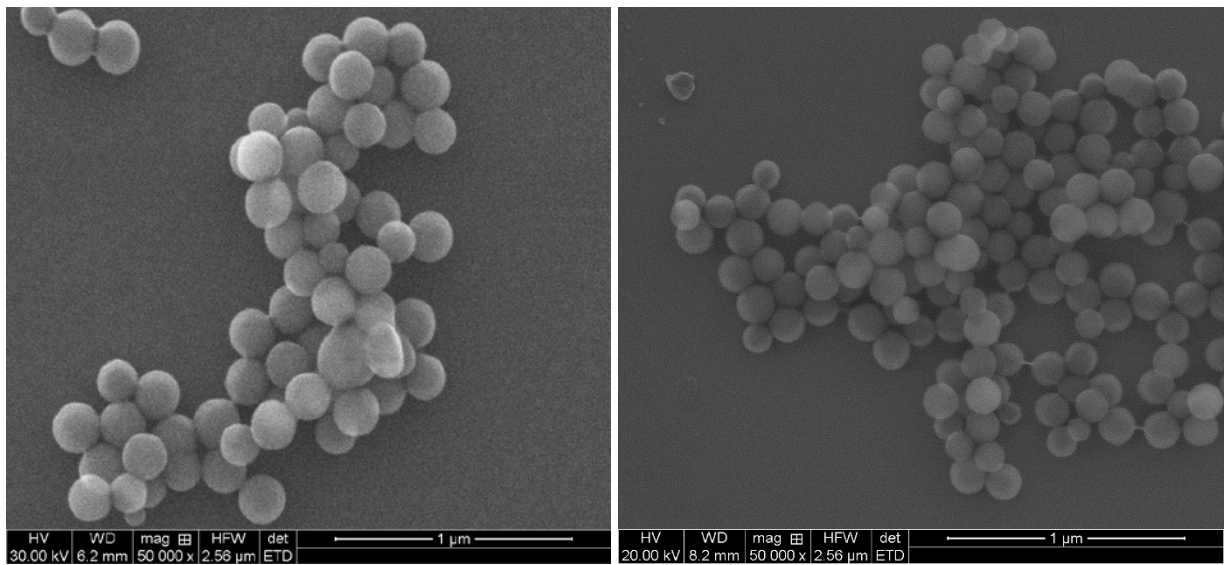


Figure 1: Representative SEM images of fresh, non-radioactive $Y(OH)CO_3$ particle clusters analyzed on the day of synthesis.

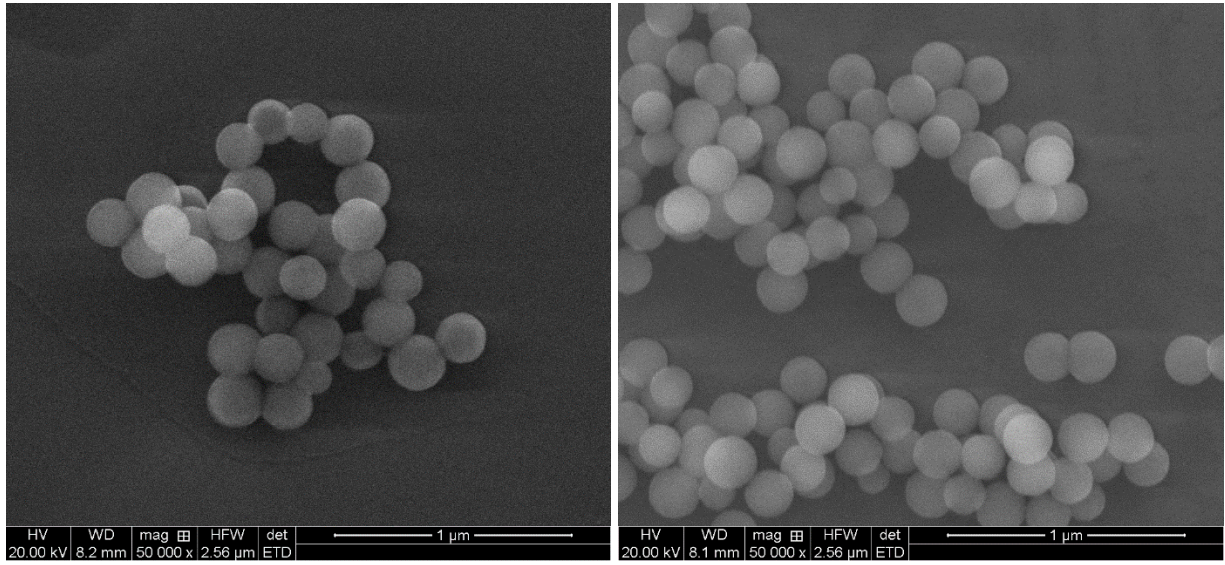


Figure 2: Stability analysis images of the $Y(OH)CO_3$ particles stored in water (left: one-week stability, right: two-week stability).

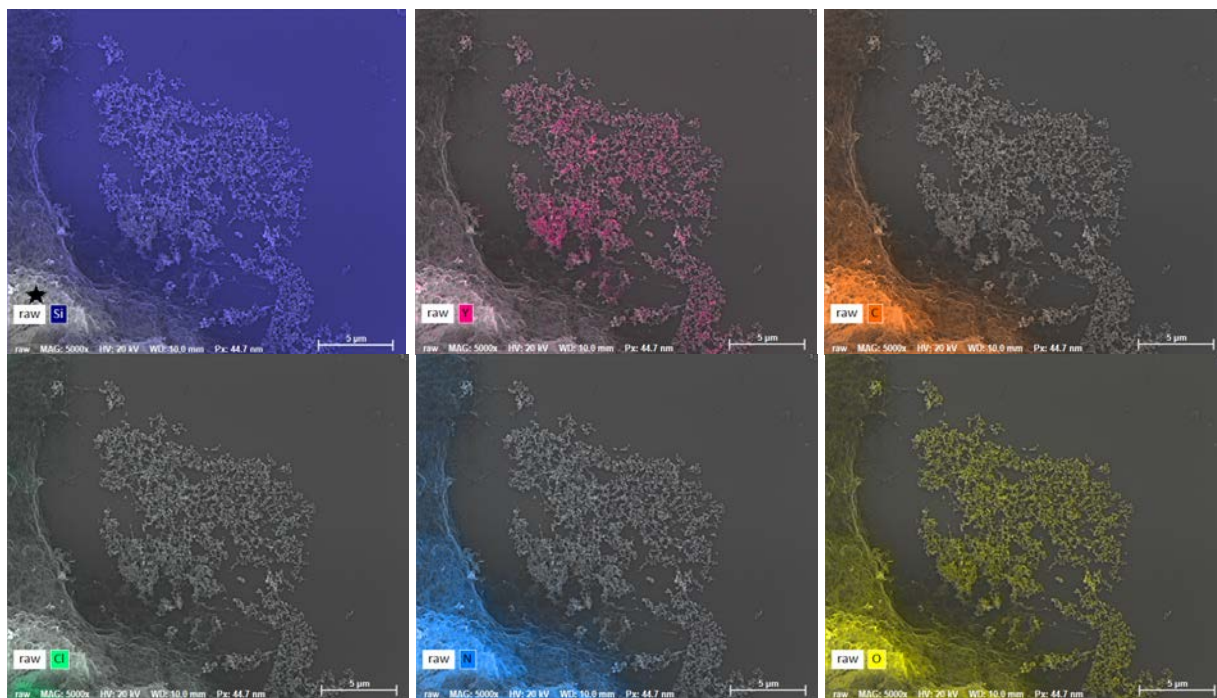


Figure 3: EDS mapping of the $Y(OH)CO_3$ particles mounted on a silicon wafer. The star in the upper left image denotes urea crystals from the mother solution.

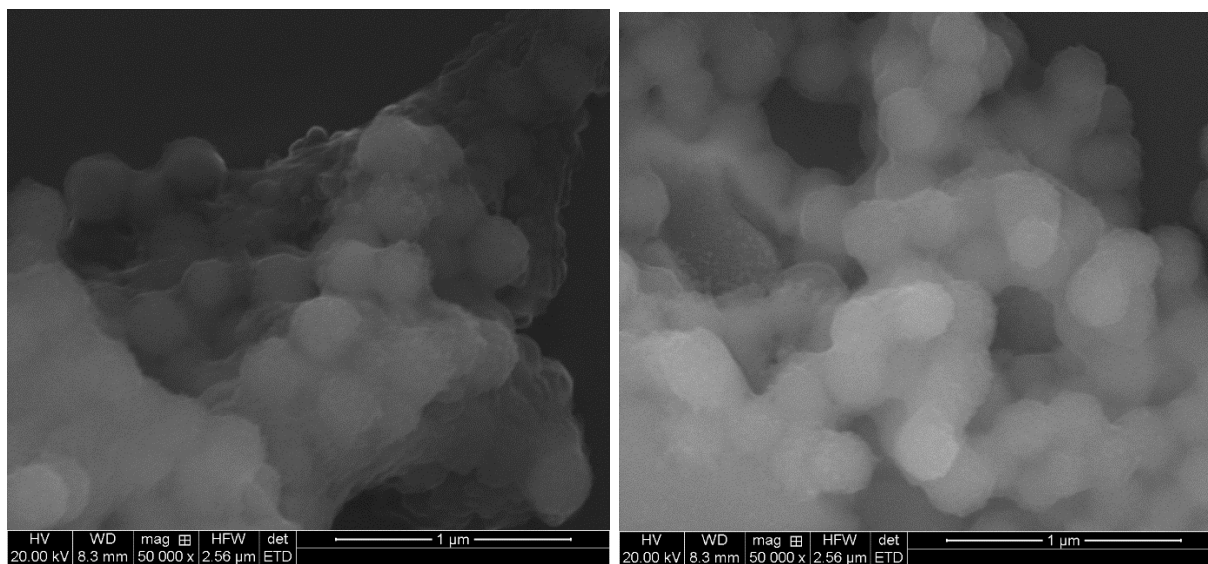


Figure 4: Stability analysis images of the $Y(OH)CO_3$ particles stored in PBS (one-week stability).

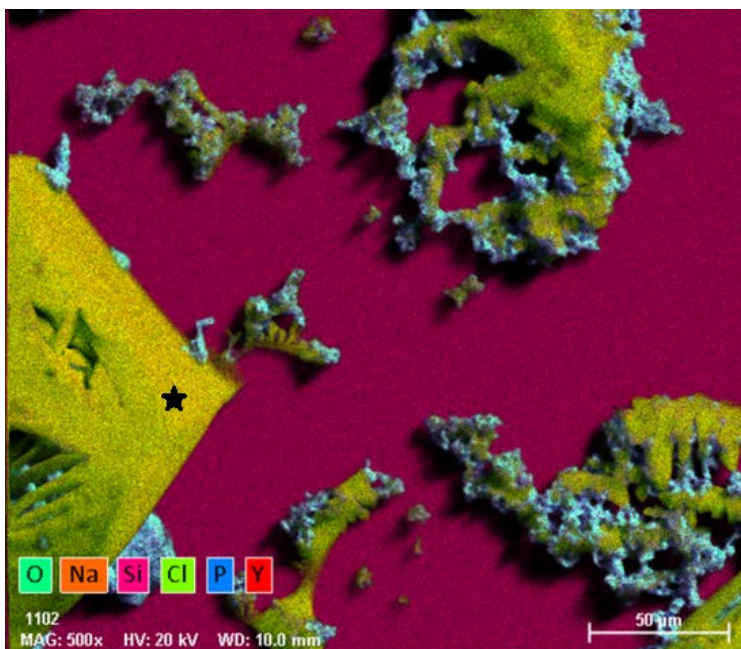


Figure 5: Layered EDS map of the PBS stability sample (one-week). The star denotes a large sodium chloride crystal from the mother solution.

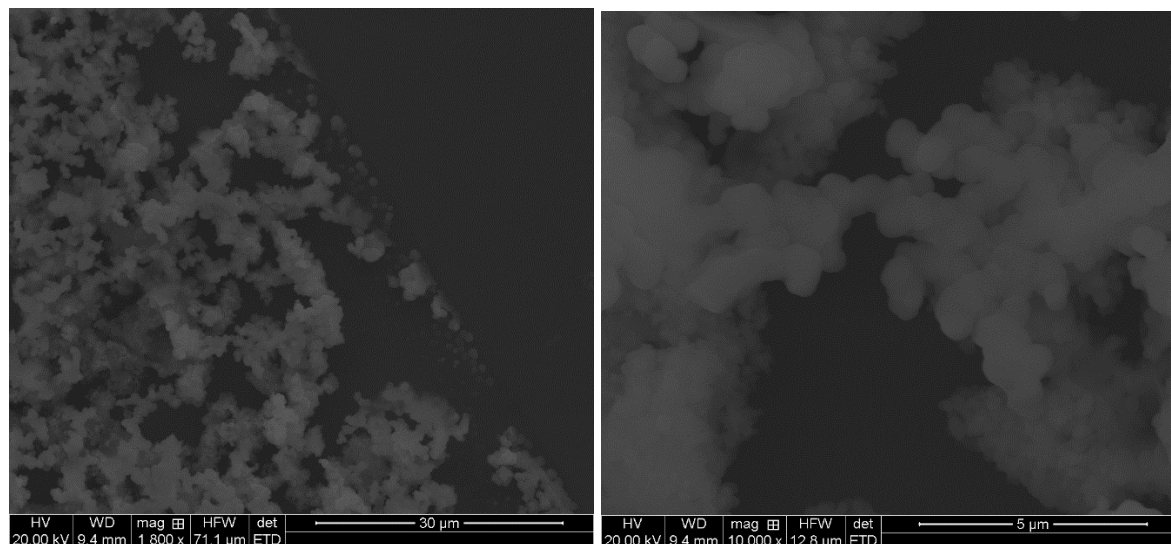


Figure 6: Particles synthesized with the urease enzymatic method at room temperature.