

Five million MDA-MB-231 cell (300 μ L of serum free DMEM) were injected subcutaneously in to the back of nude mice and allowed to establish tumors for 3 weeks (approx. 300–700 mm³) prior to intravenous particle injection. Mice were injected with silica beads (10^7 or 10^8 in 100 μ L saline) and non-spherical particles (10^8 in 100 μ L saline) via tail vein (4mice per group). Four animals were also injected with the identical volume of normal saline as a negative control. Two to six hours after the injection, the mice were sacrificed and the organs (liver, spleen, heart, lungs, kidneys, and brain) as well as tumors were excised and weighed. Retro-orbital bleeding was performed to collect peripheral blood using a plastic capillary. Each organ was divided into two parts: one for elemental analysis of Si and another for histological evaluation. End points of the experiments were chosen so that the concentration of beads left in circulation was negligibly small. It was of 2 h for the 1, 2 and 3 μ m beads, 4 h for the 0.7 μ m beads and of 6 h for the non-spherical particles. Consequently, the Si content measured in each organ normalized to the basal level can be directly related to the number of beads permanently accumulated. The portion of the organs intended for the Si content analysis were weighed, homogenized in 3ml of 20% EtOH in 1 N NaOH and left for 48 h at room temperature for extraction of Si. The extracts were centrifuged at 4200 rpm for 25 min and 0.5 mL of the supernatant was withdrawn, diluted with 2.5 mL of de-ionized 18- $\mu\Omega$ water and analyzed for Si content. Silicon content was measured using a Varian Vista-Pro Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) housed in the Rice University Geochemistry Laboratory. Si was detected at the following wavelengths 250.69, 251. 43, 251.61 and 288.158 nm. Six standards were prepared using 1 ppm sodium silicate as a stock solution and 18- $\mu\Omega$ water as a diluent. Yttrium (1 ppm) was added to both standards and samples in order to correct for instrumental drift during the run. A calibration run including the internal control was made before each group of 15 samples. In addition, samples were analyzed in random order to avoid any bias in data acquisition. The detection limit of Si was 15 ppb, corresponding to about 1 μ g ($\sim 10^{+6}$ spherical particles of 1 μ m). Therefore for the 700 nm particles, only the high dose can be considered, since at a low dose (10^7 beads) the total mass of Si injected was lower than 3.5 μ g. For measurement of the total silicon in each sample (100%), the original particle suspensions were diluted and dissolved in 1 N NaOH for 24 h at 37 °C. Further, all results were recalculated considering the dilutions performed and also normalized to percentage of total silicon resulting from the number of particles injected, or to the individual organ weight. Whole blood (200 μ L) was processed to determine the residual Si content in the peripheral blood. Serum fractions were first removed by low speed centrifugation at 1000 x g for 10 min and the red blood fraction was further lysed in red blood cell lysis buffer for 10 min at 37 °C. Supernatant was removed by high speed centrifugation at 12,000 x g. The precipitant was washed by distilled water 3 times for further Si element analysis.