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Top-down preparation and characterization of crystalline nanosilica for toxicological investigations

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Crystalline silica (CS) is a well-known human carcinogen and freshly fractured CS is strongly held to be more toxic than aged dust. Mechanical fracturing indeed generates on CS surface a specific family of Nearly-Free Silanols (NFS), a moiety that was proved to destabilize cell membranes and initiate inflammation *in vivo*. During milling, silica nanoparticles are also generated, however their contribution to the overall toxicity of quartz is still largely unexplored. Nanoquartz can be synthesized via bottom-up methods, but the surface chemistry of those crystals strongly differs from nanoparticles resulting from fracturing. We report here a top-down milling procedure to obtain a nanometric quartz that shares with fractured quartz the key surface properties relevant to toxicity. Fracturing procedure was optimized by coupling dry and wet milling steps, using water as a dispersing agent, and varying milling times and rotational speeds. We obtained a set of samples that would be classified as nanomaterials under EU CLP regulation (>50% of particles in number are < 100 nm) and exhibited a strong tendency to form submicrometric agglomerates. Deagglomeration with surfactants or simulated body fluids was negligible. Partial lattice amorphization and bimodal crystallite domain size were observed, as confirmed by the presence of two distinct domains of scattering with nanometric (< 50 nm) and submicrometric (0.8-1 μm) crystallite size. A moderate membranolytic activity, which nicely correlated with the amount of surface NFS, signalled that our nanoquartz may induce inflammation *in vivo*. Overall, a membranolytic nanoquartz for investigating the toxic activity of nanometric silica was obtained.