**Toxicological assessment of food-grade nanoparticles**

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Synthetic amorphous silica (SAS) is used in a wide variety of industrial applications including food products. According to the EU specifications, the forms of SAS used as food additive (E551) include pyrogenic or hydrated silica depending on the process (thermal or wet) used for their manufacture. These processes lead to the production of nanoparticles (NPs) of SAS that interact to form larger aggregates and agglomerates. In the last few years, there has been increased debate regarding the health and safety concerns related to the use of consumer products containing NPs.

During the first year of my PhD in Biotechnology and Life Sciences, I have evaluated the biological effects of SAS NPs produced by wet route (precipitated silica, NM-200) or thermal route (pyrogenic silica, NM-203) and I have observed that NM-203 exhibits greater cytotoxicity than the precipitated form in several human cell lines. To study the molecular interactions of NM-200 and NM-203 in a human monocytic cell line differentiated into macrophages (THP-1), I have isolated and identified the set of proteins from cell lysates which are adsorbed with a high level of affinity to the SAS NP surface. These proteins form a so-called “hard corona”, the structure of which defines the biological identity of NPs. SAS NPs and protein extracts were incubated for 24 h with gentle agitation, and multiple centrifugation steps and extensive washes with buffers of different ionic strengths were used to release almost all non-bound and soft corona proteins. Hard corona proteins were recovered by centrifugation and identified (after tryptic digestion) using liquid chromatography–high-resolution mass spectrometry. I have observed that NM-203 adsorbs on their surface more proteins than NM-200. SDS-PAGE analysis shows similar protein profiles, but a different abundance of specific proteins that form the corona of NM-200 and NM-203. The increased cytotoxicity and protein binding of pyrogenic SAS NPs compared to precipitated form could be attributed to the higher surface reactivity of NM-203.

The hard corona of these SAS NPs was composed of a number of distinct proteins involved in crucial metabolic pathways: pre- and post-transcriptional modifications, translation, cell motility, molecular chaperoning. These proteins show large unstructured regions that provide high flexibility that promotes their adsorption to SAS NPs. Furthermore, we have observed that many proteins found in the hard corona of SAS NPs are represented in specialized macrophage structures called podosomes. The physical interaction with these structures (that are crucial for macrophage functionality) would explain the high toxicity of SAS NPs in the THP-1 cells. In line with these results, Western blot and confocal microscope analyses using antibodies that recognize some proteins found in the hard corona [Heterogeneous nuclear ribonucleoprotein K (HnRNP-K) and Actin] show that SAS NP treatments dose-dependently decrease their abundance levels.

The identification of these structural determinants of SAS NP toxicity could be essential for a “safety-by-design” synthesis of these NPs used as food additives.