EVAMINTOX Project

EVALUATION OF MOLECULAR AND INTERFACIAL BIOPHYSICAL ENDPOINTS FOR NANOPARTICLES TOXICITY TOWARD BACTERIA

A methodology is developed for evaluating in situ □at the nanometric and micronic scales□ the mechanisms underlying the toxic action over time of TiO2 nanoparticles (NPs, P25 Evonik□Degussa, ∅ 25nm) on bacterial surfaces. For that purpose, we shall first determine the electrophoretic mobility of bacteria exhibiting or not LPS structures (mutants KEIO E. coli JW3601 (ΔrfaJ), JW3596 (ΔrfaC) and JW3605 (ΔrfaP)) and exposed to NPs (0 to 50 mg/L) over periods from 0 to 20 hrs. The use of formalisms developed in our group on soft biosurface electrokinetics, will enable the identification of exposure and NP□concentration conditions leading to modification of bacterial surfaces as a result of abrasion, exulceration and/or perforation. We shall subsequently quantify these cell surface damages by Atomic Force Microscopy (AFM) via:

i) refined analysis of cell morphometry in liquid media, with few nms resolution, and

ii) spatial mapping □at the scale of a single cell□ of the mechanical, hydrophobic and osmotic bacterial properties.

On the basis of theory we established for interpreting AFM force curves, we shall quantify at every probed pixel of the cell surface, the dependence on time□ and NP□concentration of its membrane elasticity, Turgor pressure and hydrophobicity. This strategy will allow identification of preferential zones of the cell surface where nanoparticles operate. These spatial and kinetic signatures of NPs actions on cell surface will further define indicators reflecting precursory stages of toxicity. They will be discussed in connection to the cell surface nature prior to exposition, thus making possible the evaluation of barrier effects. All results obtained according to the aforementioned methodology will be compared to toxicity data collected from standard viability tests at the population level using flow cytometry (evaluation of membrane potential (DiBAC4(3)), membrane integrity (propidium iodide/Syto9 labeling or Live/Dead BacLight test), respiration (CTC labeling) and esterase activity (FDA / cFDA)).

PARTNERS

UMR 7360 - Laboratoire Interdisciplinaire des Environnements Continentaux

Leader: **Jérôme F. L. Duval** is a physical-chemist, currently Directeur de Recherche CNRS at the Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC, Vandeuvre-lès-Nancy). His current research interests include physical-chemistry of complex biotic and abiotic interphases with an emphasis on their electrokinetic and nanomechanical properties, bioadhesion issues, reactive transfer of metallic cations/nanoparticles to biological surfaces, and dynamics of metal speciation in colloidal suspensions.

Partners: Audrey Beaussart, Angelina Razafitianamaharavo, Céline Caillet, Bénédicte Sohm, Isabelle Bihannic, Pascale Bauda, Christophe Pagnout

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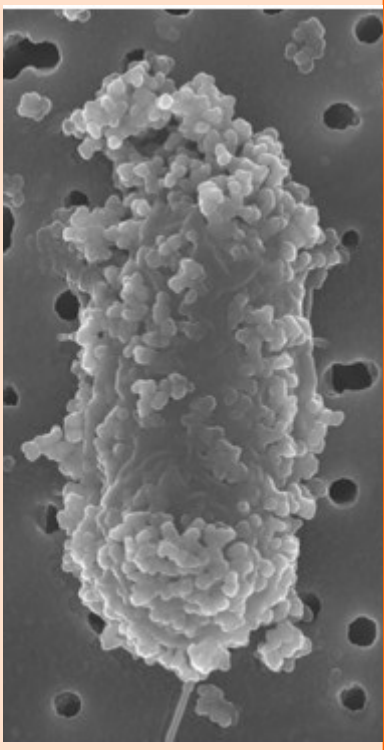

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