EXTRACELLULAR POLYMERIC SUBSTANCES AS ARMOR AGAINST CYTOTOXIC MINERALS: SURVIVAL OF PSEUDOMONAS AERUGINOSA CELLS IN OXIDE PARTICLE SUSPENSIONS

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Abstract: Our study explored the effects of extracellular polymeric substances (EPS) on the susceptibility to lysis of *Pseudomonas aeruginosa* in suspensions of submicron-sized oxide particles. We determined whether the effects were oxide-dependent and identified the mechanisms of oxide-induced cytotoxicity. Two strains of *Pseudomonas aeruginosa* were examined, a wild type (PAO1), and an isogenic mutant of the wild type (δ⁻*psl)*. The PAO1 cells produced EPS extensively and were capable of forming biofilms, while the δ⁻*psl* cells only produced minimum EPS and were incapable of forming biofilms. The oxides studied were amorphous silica, anatase (β-TiO_2), and g-alumina (γ-Al_2O_3), which have primary particle sizes in the range of 100 - 250 nm, and represent a spectrum of points of zero charge.

The presence and relative amounts of EPS in the PAO1 and δ⁻*psl* cultures were characterized using fluoro-conjugated lectins of wheat germ agglutinin and concanavalin-A. To remove the EPS completely from the δ⁻*psl* and PAO1 cell cultures, a gentle washing procedure was adopted, and the washed cells are referred to as EPS-devoid cell cultures. For EPS-devoid δ⁻*psl* cells, all three oxides investigated were cytotoxic and reduced cell viability by 20-60% compared with their oxide-free blanks within 6 h. The cytotoxic effect increased as amorphous silica (- 20%) < anatase (- 50%) ≤ γ-alumina (- 50-60%). The EPS-devoid PAO1 cells were initially impacted by the oxide suspensions, but recovered after 3 - 4 h of incubation. By comparison, the EPS-abundant and biofilm-forming cell cultures of PAO1 were unaffected by the presence of oxide particles. Estimates of cell viability by epifluorescence microscopy using LIVE/DEAD stains provided a consistent trend of oxide-induced cytotoxicity.

The mechanisms for the oxide cytotoxicity were investigated through a series of studies. Of the three oxides, γ-Al_2O_3 has a positive surface charge at the culture medium pH ~ 6 - 6.5 and held most strongly at the cell surface, which is negatively charged. Transmission electron microscopy study coupled by high-pressure freezing and substitution techniques indicated that a fraction of the γ-Al_2O_3 particles had penetrated through the outer and inner membranes of *δ⁻psl* cells. In contrast, negatively charged amorphous silica and anatase of comparable particle sizes were not observed inside the cells. Quantification of the highly reactive oxygen species (hROS) at oxide surfaces revealed that anatase had an hROS concentration two orders of magnitude higher than that of amorphous silica or γ-alumina. The high concentration of hROS on anatase likely resulted in lipid membrane peroxidation and cell lysis. The effects of "soluble" Si, Ti, and Al versus "nanoparticulate" oxides were separated by filtration through 0.2-mm filters and subsequent solution analysis by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES).

In summary, the study provided direct proof for the hypotheses that certain mineral particles can disturb the stability of bacterial cell surfaces and cause lysis, and EPS behaves as a shield for the cells in their interaction with such unfavourable minerals. Several roles have been proposed for the evolution of EPS including protection against ultraviolet radiation, desiccation, and soluble metal toxicity, as well as providing a dense medium for concentrating quorum-sensing molecules. We propose here that EPS may have evolved early in the history of prokaryotes as a protective barrier against cytotoxic mineral surfaces. Whatever the original role, EPS would likely have quickly provided redundant functions in protecting the cell surface.