

Thesis Title of Doctor: Study of Cytotoxicity in Granulocytes Functional Activity and Some Enzyme Release

ABSTRACT

Polymorphonuclear neutrophils (PMN) are the main inflammatory cells to act at implant interface where they encounter foreign materials and may produce reactive oxygen species (ROS). During the interaction between titanium and ROS, Ti-peroxy compounds may be formed. In this study Ti-peroxy particles made from TiO_2 and H_2O_2 were used to study the effects of Ti-peroxy particles on isolated PMN functional activity by means of chemiluminescence. The presence of Ti-peroxy particles decreased the oxidative response of PMN functional activity represented by percentage reduction of chemiluminescence of Luminol dependent functional activity of PMN stimulated by PMA (Phorbol-12-myristate-13-acetate)

The percentage reduction of functional activity of PMN were observed in both Luminol and Lucigenin dependent chemiluminescence. These effects could not be ascribed to the release of H_2O_2 from Ti-peroxy particles, because a steady state of H_2O_2 producing system failed to enhance Luminol CL in presence of Ti-peroxy particles where no PMN cells were present.

The anti-inflammatory properties of Ti-particles through a combined peroxide and physical contact effect support the idea that interactions between Ti and inflammatory cells are responsible for the scavenging effect of Ti-particles to ROS generated by the stimulated PMN with PMA.

The ultrafine TiO_2 is an important material used in commerce today; It generates reactive oxygen species (ROS) efficiently. Under UV light; The study of the characteristic cytotoxicity of this fine or ultrafine TiO_2 particles under different conditions of illumination with UV at different

concentrations in PMN produced ROS generated, using Luminol or Lucigenin dependent CL. The observed cytotoxicity and anti-inflammatory effects, the response of PMN exhibited dose-response behavior and the effects increased with time of exposure to UV illumination. The extent to which ultrafine and fine Ti-particles affected cellular PMN behavior was not dependent on simple physical properties only but also on cytotoxic generation of ROS under UV illumination and possible release of LDH (Lactate dehydrogenase) or depleted SH (glutathione) in PMN cells. The data suggested that ultrafine TiO₂ samples optimized for ROS production in photocatalysis are more likely to generate damaging ROS in PMN isolated cells. This can highlight the important role that in vitro measure of ROS production can be used to screen for cytotoxicity.

Determination of level of malondialdehyde (MDA), as an end product of lipid peroxidation; glutathione (GSH) depletion estimated in intact PMN cells were used as a key for antioxidant activity of neutrophils during formation of ROS. Lactate dehydrogenase was also assayed in serum samples as indication of cytotoxic result of cell lysis that causes the release of LDH as indication of cytotoxic death. These results suggest that Ti-particles are capable of enhancing the breakdown of the inflammatory mediators and may account for biocompatible properties of the material as antioxidant that degrade the ROS which mediate the inflammatory response. The annealing of Ti-particles caused reduction in ROS generation indicated by percentage reduction of CL functional activity of PMN due to oxygen vacancy defect in the crystal lattice.