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Analysis of macrophage responses to titanium nanoparticles in hyperglycemic conditions

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Titanium biomaterials are widely used for implantation. Titanium implants release titanium nanoparticles (Ti NPs), known as implant debris. Ti NPs induce inflammation and are amongst the driving forces that contribute to the disruption of the mechanical stability of implant devices causing aseptic loosening and triggering adverse immune reactions that lead to chronic health complications. Macrophages are innate immune cells present in virtually all tissues. Macrophages are responsible for the recognition of foreign material and foreign body response. Pathological activation of macrophages can result in the failure of implants. Patients with Diabetes Mellitus (DM) have elevated blood sugar levels and can suffer from consequences triggered by compromised immune responses. Amongst those consequences are the complications produced by metal implants. Hyperglycemia is a major factor of diabetic pathology, which also promotes implant corrosion contributing to the release of metal wear-off particles. Macrophages are essential regulators of inflammation and play a critical role in DM and diabetic complications.

The aim of the study was to investigate the interacting effect of Ti NPs and hyperglycemia on innate immune responses, which can be responsible for the implant failure in diabetic patients. In the current study, the effects of titanium debris on primary human macrophages in hyperglycemic conditions were explored. Monocytes were isolated out of buffy coats by CD14+ positive selection and differentiated into the following subtypes: M0 (control or unstimulated), M1 (stimulated with IFNγ) and M2 (stimulated with IL-4). Macrophages were exposed to high concentrations of glucose (25mM) to mimic a diabetic environment. For the assessment of the effects of titanium debris, the cells were stimulated with 25 ppm and 100 ppm of Ti NPs. The results of the Alamar Blue assay revealed that Ti NPs at moderate concentrations do not affect macrophage viability. Using RT-PCR and ELISA, the expression and secretion of CHI3L1, CHIT1, CCL18 and TNF-α were quantified.

Ti NPs stimulated gene expression of CHI3L1 in M0 and M2 macrophages, however, the secretion of CHI3L1 was potentiated in all macrophage subtypes. Ti NPs stimulated gene expression of CHIT1 in all macrophage subtypes. Gene expression and secretion of CCL18 was suppressed by Ti NPs. In the presence of Ti NPs, the secretion of TNF-α was not changed. Hyperglycemia cooperated with Ti NPs-induced gene expression of CHI3L1 in M1 and M2 and Ti NPs-induced secretion of CHI3L1 in all macrophage subtypes. Hyperglycemia cooperated with Ti NPs-induced CHIT1 expression in all macrophage subtypes. Suppressed production of CCL18 by Ti NPs was enhanced by hyperglycemia. In the presence of a hyperglycemic environment, secretion of TNF-α was not changed. Dexamethasone suppressed Ti NPs-induced CHI3L1 expression in a high glucose environment but not in normal glucose. However, dexamethasone upregulated Ti NPs-induced CHIT1 expression in normal glucose and had no effect in high glucose.

Confocal microscopy demonstrated that Ti NPs promote intracellular accumulation of CHIT1 in the trans-Golgi network (TGN) in M1 macrophages, however, in M2 macrophages, Ti NPs trigger degradation of TGN. Flow cytometry demonstrated that Ti NPs induce ROS release in M0 macrophages and enhance ROS production in M1 macrophages. The results of Affymetrix gene expression analysis identified a group of differentially expressed genes induced by Ti NPs: DCSTAMP, OLR1, ORM1, MME, CSF1, CXCL8, CXCL9, CXCL10, MT1X and MT1G. RT-qPCR validation analysis of the differentially expressed genes revealed that hyperglycemia enhances Ti NPs-induced expression of CSF1, DCSTAMP, CSF1, MT1X, OLR1 and ORM1; and favors Ti NPs-induced suppression of CXCL9 and CXCL10.

In total, Ti NPs induced increased production of ROS, CHIT1, CHI3L1, DCSTAMP, OLR1, ORM1, MME, CSF1, MT1X and MT1G, and suppressed production of CCL18, CXCL9 and CXCL10 in primary human monocyte-derived macrophages. Hyperglycemia cooperated with the effects of Ti NPs in the production of CHIT1, CHI3L1, CSF1, DCSTAMP, MT1X, OLR1, ORM1, CXCL9 and CXCL10. In

summary, the results of the study clearly demonstrated that Ti NPs and hyperglycemia have synergistic effects, while the dominant effects are induced by Ti NPs. The synergistic effect of Ti NPs and hyperglycemia are characterized by the system of biomarkers including CHIT1, DCSTAMP, CHI3L1, OLR1, CSF1, ORM1 and MT1X that can potentially be used to predict the detrimental effect of Ti implants in diabetic patients.