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Analysis of reactions of macrophages to titanium and biodegradable coating materials

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Nowadays implants and medical devices are seen as efficient and practical solutions for a multitude of health associated problems. Titanium and titanium alloys have been successfully used in orthopedics, dentistry, cardiology and otorhinolaryngology. Superior mechanical properties, excellent corrosion resistance, low magnetic susceptibility and high biocompatibility have been the reason for choosing titanium for implantation. Still, up to 9% of implants fail due to the development of various complications. A major cause of implant failure is aseptic loosening, characterized by a progressive destruction of the periprosthetic tissue as a result of foreign body response. Macrophages are essential cells involved in the recognition of the foreign body and in the initiation and coordination of the foreign body response. In order to investigate the extent of macrophage reaction to titanium, human peripheral blood monocyte-derived macrophages were cultured with polished and porous titanium discs. To simulate different macrophage activation states and to discriminate among their reactions to titanium, macrophages were brought to 3 distinct activation states: M(Control), M(IFNγ) and M(IL-4). ELISA analysis revealed that both polished and porous titanium discs induce similar inflammatory cytokine profiles in macrophages. Affymetrix microarray analysis revealed a total of 1690 genes were differentially regulated by polished titanium and 4648 genes were differentially regulated by porous titanium. In both experiments, the highest number of differences between titanium and control settings were found in M(IL-4). Microarray analysis showed that both polished and porous titanium affected several genes involved in inflammation and matrix remodeling. RT-qPCR analysis confirmed that polished titanium up-regulates CSF1, TNFSF14, CHI3L1 and MMP9 and down-regulates CCL8, CCL13, CCL18, CCL23 and IL17RB in M(IL-4) macrophages. Likewise, porous titanium up-regulates the expression of CHIT1, CHI3L1, MMP8 and MMP9 and suppresses CCL8, CCL13, CCL18, CCL23 and IL17RB in M(IL-4) macrophages. Additionally, in order to model wear debris, titanium microbeads and nanoparticles were used. RT-qPCR analysis demonstrated that nanoparticles induce stronger up-regulation of CSF1, CHIT1 and CHI3L1 and stronger down-regulation of CCL8, CCL13, CCL18, and CCL23 when compared to polished titanium discs or to titanium microbeads. In summary, it was demonstrated that titanium induces pro-inflammatory and tissue-remodeling responses first of all in M(IL-4) macrophages, that model healing macrophages. This can explain failure of implants on later stages of implant integration.
In order to simulate a bacterial infection of titanium implants, heat killed Staphylococcus Aureus were added to mature macrophages and naive monocytes cultured with polished titanium discs. Cytokine release analysis revealed that when Staphylococcus Aureus is added to naive monocytes higher levels of TNFα, IL-1β, IL-6, IL-8, CCL18, MMP-7 and MMP-9 are secreted. However, the reaction to Staphylococcus Aureus is not affected by titanium.
In order to examine the effect of peripheral blood mononuclear cells and fibroblasts on the secretion of MMP-7 and MMP-9 by macrophages in response to titanium, co-culture models were established. MMP-7 and MMP-9 secretion levels were increased when the whole fraction of peripheral blood mononuclear cells was exposed to titanium. However, depletion of CD14+ monocytes abrogated this effect. Similarly, fibroblasts stimulated the secretion of MMP-7 in both juxtacrine and paracrine macrophage-fibroblast interaction models. In contrast, MMP-9 secretion was inhibited by fibroblasts in the juxtacrine interaction model. Still, neither peripheral blood mononuclear cells nor fibroblasts were able to substantially increase the concentration of active MMP-7 and MMP-9 in response to titanium.
To increase implant biocompatibility implant coatings can be applied. In this study two types of coatings have been used: polyarginine/hyaluronic acid-based coatings and polylactic acid-based coatings. To test macrophage reaction to these coatings the secretion of TNFα and CCL18 was analyzed. Polyarginine/hyaluronic acid-based coatings inhibited the secretion of TNFα and CCL18 in all donors

analyzed. In contrast, the reaction of macrophages to polylactic acid-based coatings was donor-dependent.

Collectively the results of this study indicate that macrophage reaction to titanium implants is determined by several factors: 1) macrophage activation state; 2) titanium surface; 3) titanium size; 4) other cellular components of the periprosthetic tissue; 5) donor-specific factors. However, macrophage reaction to Staphylococcus Aureus is independent of titanium. Furthermore, analysis of macrophage responses to the main implant material as well as to coating materials in a donor-specific way represents a promising approach for the selection of optimal personalized implant materials.