Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

Analysis of macrophage responses to zinc-modified calcium phosphate coatings on Ti6Al4V alloy

Autor: Guanhao Li
Institut / Klinik: Institut für Transfusionsmedizin und Immunologie
Doktormutter: Prof. Dr. J. Kzhyshkowska

Ti6Al4V (Ti64) is widely used in biomedical applications, including orthopedic and dental implants, due to its excellent mechanical properties, biocompatibility, and corrosion resistance. However, its inert surface lacks bioactivity and antibacterial properties, leading to delayed osseointegration and implant-related complications. Additionally, wear and corrosion release nanoparticles and ions like aluminum and vanadium, causing inflammation, oxidative stress, and cytotoxic effects. To address these issues, calcium phosphate coatings have been developed to enhance bioactivity and promote bone regeneration. While CaP coatings mimic bone's mineral composition and improve osteoinductive properties, they lack antibacterial effects and may worsen inflammation-related complications. Zinc (Zn), a trace element known for its antibacterial, antioxidative, and immune-modulating properties, has shown promise in improving CaP coatings, though its effects on macrophage biology remain unclear. This study explored the biological effects and immunocompatibility of zinc-modified CaP coatings on Ti64 alloys, focusing on macrophage responses. Six types of Ti64 samples were evaluated: uncoated Ti64, CaP coated Ti64 (CaP), and Ti64 coated with CaP incorporating four different concentrations of zinc acetate during preparation (Zn25, Zn50, Zn75, and Zn100). This study used primary human monocyte-derived macrophages differentiated into M0, M1, and M2 phenotypes, which were subsequently cultured on six distinct Ti64 alloy surfaces. The aims of the study included: 1) evaluation of the expression of titanium debris induced biomarkers in macrophages interacting with modified Ti64 samples; 2) identification of differential gene expression associated with various Zn-modified CaP coatings in macrophages; 3) to assess the impact of CaP and Zn-doped CaP coatings on the scavenging function of macrophages, essential for the resolution of inflammation. The experimental approach included evaluating the gene expression profiles of TiNPs-induced biomarkers using RT-PCR and comprehensive transcriptomic analyses through RNA sequencing. Macrophage scavenging function was assessed by the endocytosis assays using fluorescently labeled acLDL. RNA-seq analyses revealed extensive transcriptional alterations induced by CaP and Zn-modified coatings. Differential gene expression analysis revealed substantial transcriptional changes, particularly pronounced in macrophages cultured on CaP and Zn50. Transcriptomic profiling via clustering method revealed CaP and Zn50 coatings significantly altered gene expression in elevating inflammatory and stress-related pathways including IL-17, MAPK, and HIF-1 signaling pathways. Additionally, CaP inhibited oxidative phosphorylation in M2 macrophages and Zn50 induced endoplasmic reticulum stress in M0 macrophages. In contrast, macrophages cultured on Zn25, Zn75, and Zn100 coatings exhibited substantial suppression of inflammatory gene signatures and enhanced expression of genes associated with tissue repair, antioxidant responses, and anti-inflammatory functions. Functional assays assessing macrophage scavenging capacity further confirmed the transcriptomic findings. Endocytosis assays using fluorescently labeled acLDL revealed significant impairment of phagocytic function in M2 macrophages cultured on Zn50-CaP coated surfaces, documented quantitatively via flow cytometry and qualitatively by confocal microscopy. In contrast, M2 macrophages cultured on Ti64 alloys coated by CaP modified with Zn25, Zn75 and Zn100 exhibited restored clearance capacity. In conclusion, Zn modifications of CaP coatings on Ti64 alloys elicited profound and various effects on macrophages. The intermediate Zn concentration (Zn50) in coating preparation induced pro-inflammatory and stress responses, adversely affecting macrophage function. Conversely, the lower and higher Zn concentrations in coating preparation (Zn25, Zn75, Zn100) showed significant immunomodulatory advantages, reducing inflammation, supporting macrophage scavenging functions, and fostering conditions favorable for tissue repair and implant integration. The result of this study contributes to implant surface engineering by demonstrating that optimal Zn concentrations can enhance macrophage responses, mitigate inflammation, and promote effective implant integration.