Inflammatory and Immunological Response to Orthopedic Implants

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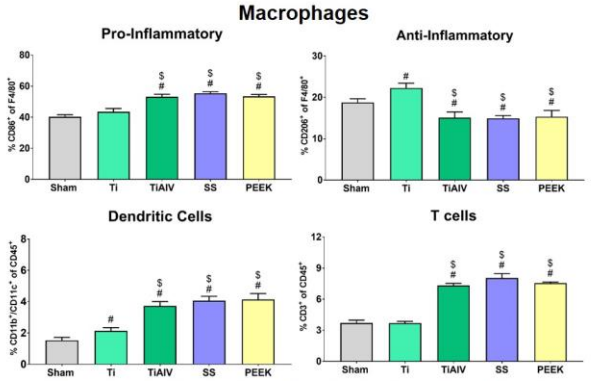
Background: Orthopedic injuries and associated pathologies are an important health issue globally. Musculoskeletal conditions affect an estimated 1.71 billion individuals globally. These conditions often require surgery and the use of permanent devices like orthopedic implants to restore function. Orthopedic implants are commonly selected based on their mechanical properties and high corrosion resistance. While the biocompatibility of orthopedic materials has been assessed, usually through in vitro cell lines studies, little is known about the inflammatory and immunological response to these materials. The aim of this study was to evaluate the inflammatory and immunological response to common orthopedic biomaterials *in vitro* and *in vivo*.

Methods: Primary bone-marrow-derived macrophages were isolated from 12-week-old C57BL/6 mice. Cells were seeded on polystyrene (TCPS) or disks fabricated from titanium (Ti), titanium-aluminum-vanadium alloy (TiAlV), stainless steel 316L (SS) or polyether ether ketone (PEEK). Samples were cultured for 12h for gene expression or 24h for cytokine analysis (n=6/variable). In a second experiment, phenotype of macrophages was examined on the same materials after 24h by flow cytometry. For *in vivo* studies, intrafemoral Ti, TiAlV, SS, or PEEK implants were placed in 12-week-old C57BL/6 mice (n=6/variable). Sham surgeries in which surgical procedures were performed without biomaterial implantation were used as control. Immune and stem cell phenotypes were analyzed by flow cytometry 1, 3, or 7 days after implantation.

Results: Macrophage gene expression of pro-inflammatory molecules (*Il1b, Il6, Il12, Il17, Tnf, Ccl2, Nos2*) were higher on TiAlV, SS, and PEEK when compared to Ti and TCPS. Furthermore, gene expression of anti-inflammatory molecules (*Il4, Il10, Il1rn, Arg1*) was higher on Ti when compared to TiAlV, SS, and PEEK. Pro-inflammatory cytokine levels in conditioned media were the lowest on Ti and the highest on SS. Similarly, immunophenotyping of macrophages by flow cytometry showed that macrophages cultured on Ti surfaces induced the highest anti-inflammatory macrophage polarization and the lowest pro-inflammatory activation. Interestingly, SS, PEEK and TiAlV activated the most macrophages to a pro-inflammatory phenotype, while PEEK polarize the least macrophages into an anti-inflammatory phenotype. In vivo, neutrophil infiltration was similar in all implant at day 1, and remained high after 7 days on TiAlV, SS, and PEEK implants. Neutrophil percentage on Ti implants reduced by day 3 and 7. Ti implants recruited the most macrophages at 1-day post-surgery decreasing macrophage percentage by day 7. Inflammatory macrophages were higher on TiAlV, SS, and PEEK by day 3 and continue high by day 7. Contrary, percentage of anti-inflammatory macrophages was similar in all implants at day 1 and increased by day 3 and 7 in sham and Ti groups, while remain low in TiAlV, SS, and PEEK. Dendritic cells were the highest on TiAlV and PEEK. Interestingly, we identified T cell presence at day 1 and were the highest on TiAlV on day 3. SS and PEEK implants recruited similar number of T cells than TiAlV by day 7. B cell percentage was similar between the groups at all times analyzed.

Conclusion: While biomaterials used for orthopedic applications have been shown to be biocompatible, our results showed that common orthopedic biomaterials differentially induce inflammatory response. Pure titanium produced the least inflammatory response *in vitro* and *in vivo*. Stainless steel produced the highest inflammatory response *in vitro*; however, *in vivo* studies showed similar immunological response for titanium-aluminum-vanadium alloy and PEEK implants. Taken together our results showed that TiAlV, SS, and PEEK implants induce a stronger inflammatory response with high infiltration of neutrophils and T cells.



Immunophenotyping of peri-implant tissue cells at day 7 post-implantation. Macrophage are differentially activated towards a pro- and anti-inflammatory phenotype in response to the biomaterial implanted with Ti activating the most anti-inflammatory phenotype. Dendritic cell and T cell percentage remain higher after 7 days of TiAlV, SS, and PEEK implantation. $p<0.05$: # vs. sham; $ vs. Ti.