Local IL-4 Delivery Increases Systemic Macrophage Trafficking and Prevents Bone-loss in a Mouse Model of Wear Particle Induced Osteolysis

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Statement of Purpose Aseptic loosening remains one of the main long-term complications of total joint replacement surgery. Peri-implant osteolysis and total joint replacement loosening are driven by macrophage-mediated inflammation to implant-derived ultra-high molecular weight polyethylene (UHMWPE) and other biomaterial wear particles. Wear particle activated macrophages secrete chemokines and pro-inflammatory cytokines that lead to further macrophage recruitment, increased osteoclastogenesis, and suppression of osteoblast formation and function ultimately leading to peri-implant osteolysis. In vitro and in vivo studies have shown that induction of M2 macrophage polarization by IL-4 treatment mitigates this biomaterial particle-induced inflammation and osteolysis (**1-3**). In this study, the effect of local, continuous, IL-4 delivery on the UHMWPE particle-induced osteolysis and on the systemic macrophage trafficking to the peri-implant tissue were investigated in a murine continuous femoral intramedullary particle infusion model (**4**).

Methods The animal protocol was approved by the Stanford University Animal Care Committee. Alzet miniature osmotic pumps were loaded either with carrier solution; carrier solution with UHMWPE particles; or carrier solution with UHMWPE particles and IL-4. Pumps were then connected to 1 mm x 6 mm hollow titanium rods via vinyl tubing and implanted in the subcutaneous tissues in the dorsal sides of 30, adult, male BALB/cByJ mice under isoflurane anesthesia and buprenorphine analgesia. A subcutaneous tunnel reaching the right knee was made for the tubing and a lateral parapatellar arthrotomy was performed via second skin incision made to the lateral side of the right knee. A series of needles was used to drill through the intercondylar notch of the distal femur to gain access to the medullary cavity. The titanium rod, connected from another end to the pump via tubing, was then press fit into the drill hole resulting in a continuous delivery of UHMWPE particles with or without IL-4 to the medullary cavity (**4, 5**). Green fluorescent protein (GFP) and firefly luciferase (FLUC) expressing mouse primary macrophages were produced by infecting BALB/cByJ-derived bone marrow macrophages with pFU-Luc2-eGFP containing lentivirus vector. After one week of pump implantation these reporter macrophages were injected to the tail vein of the mice implanted with PE and PE+IL-4 containing pumps. Trafficking of the reporter cells to the distal femur was observed by obtaining whole body BLI images of the mice in 2 day intervals up to 20 days post injection. Uniformly sized region of interest was drawn over the right distal femur and the total flux (photons/second) was determined. The bone volume fraction (BVF) at the distal femur was quantified from µCT images obtained prior to rod implantation and 28 days later when mice were sacrificed and titanium rods removed prior to imaging.

Results The continuous infusion of UHMWPE particles lead to the systemic recruitment of macrophages, as evidenced by strong BLI signal originating from the right distal femur. Local delivery of IL-4 together with UHMWPE particles increased reporter macrophage recruitment to the peri-implant tissue, the difference between the groups reaching statistical significance at day 12 with day 14 showing a clear trend (**Figure 1a**). Using µCT, the continuous infusion of UHMWPE particles lead to decreased BVF compared to the controls, while continuous IL-4 delivery with UHMWPE particles lead to increased BVF **(Figure 1b**).

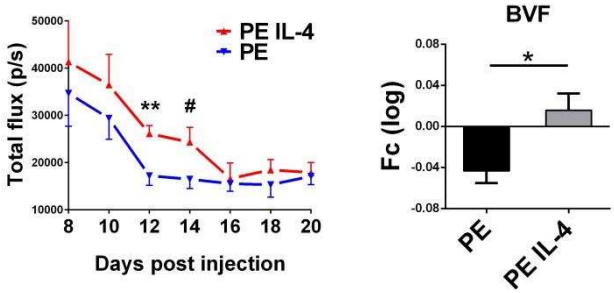

Figure 1. UHMWPE particles were infused into the mouse right distal femur with or without IL-4. Systemic trafficking of GFP and FLUC expressing reporter macrophages to the distal femur was determined by bioluminescence imaging (BLI) and the amount of bone at the distal femur was quantified using µCT imaging. **a)** The BLI signal originating from the distal femur at days 8 to 20 after the systemic injection of reporter cells. **b)** Bone volume fraction (BVF) at distal femur expressed as logarithm-transformed fold change (fc) to control femurs. **) $p<0.01$, *) $p<0.05$, #) $p=0.075$ as determined by Student's t-test.

Conclusions In agreement with previous reports, continuous infusion of UHMWPE particles led to local bone loss and systemic trafficking of reporter macrophages to the peri-implant tissue (**4**). IL-4 delivery prevented particle induced osteolysis but somewhat surprisingly also led to increased macrophage trafficking and/or survival. We are currently investigating the emerging hypotheses that continuous IL-4 delivery will lead to local M2 macrophage polarization with decreased inflammatory response to UHMWPE particles, increased macrophage survival and increased ability of macrophages to support local bone formation.

References (**1**) Rao AJ, et al. Acta Biomater. 2012;8: 2815-23. (**2**) Pajarinen J, et al. Acta Biomater. 2013;9: 9229-40. (**3**) Rao AJ, et al. J Biomed Mater Res A. 2013;101: 1926-34. (**4**) Ren PG, et al Clin Orthop Relat Res. 2011;469(1): 113-22. (**5**) Pajarinen J, et al. J Biomed Mater Res A. 2014 In press (doi: 10.1002/jbm.a.35278.).