# Primary Human Macrophage Responses to PEEK-OPTIMA® and UHMWPE Particulate Implant Debris

[1]Hallab, N. J., [2]Kinbrum, A., [2]Brady, M.
[1]Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL, USA, [2]Invibio Biomaterial Solutions, Thornton-Cleveleys, UK.

**INTRODUCTION:** PEEK (polyetheretherketone) has found use in a broad range of medical applications. Amongst these, PEEK-OPTIMA® has been demonstrated to be a suitable, low wearing alternative to UHMWPE in spine arthroplasty [1]. Debris derived from implant wear commonly results in a pro-inflammatory response elicited predominantly by macrophages, with the release of the cytokines TNF-α, IL-1β, IL-6 and other mediators [2, 3]. We hypothesise that PEEK particles will induce equal or lower innate reactivity responses when compared with conventional polymeric implant materials, *in vitro*. To test our hypothesis that PEEK does not elicit an increased inflammatory response, human macrophages from n=3 subjects were exposed to sterile 1-2micron sized particles of PEEK and UHMWPE, representative of those generated through wear of articulating surfaces in total disk arthroplasty test simulations. The effect of particles on cell function was then assessed by cytotoxicity assay (on THP-1 macrophages) and cytokine analysis (on primary human macrophages).

**METHODS:** Bulk PEEK-OPTIMA (Invibio Biomaterial Solutions, UK) and a commonly used, implantable grade UHMWPE and X-UHMWPE underwent sterile cryo-milling, pulverisation and filtering to create predetermined particle sizes. These particles then underwent analysis by Low Angle Laser Light Spectroscopy (LALLS), to provide measurements of equivalent circle diameter, aspect ratio, roundness and form factor. The particles were designed to match particles generated previously from total disk arthroplasty test simulations. PEEK and PE particles (2μm diameter) were EtO sterilized, endotoxin cleaned (using serial incubation with PyronCleanTM and ethanol) and verified to be endotoxin free (<0.01uE using Kinetic QCL assay). Differentiated human THP-1 macrophages and primary human monocytes (n=3 subjects) negatively isolated from Ficol separated PBMC cell fractions via antibody binding (AutoMacs) cultured in Dulbecco's Modified Eagle Medium, DMEM (Sigma) at 37º C and 0.5% CO2, containing 10% fetal bovine serum (FBS; Hyclone Laboratories, Inc) were exposed to varying ratios of particles to cells for 24 or 48 hours. Cytotoxicity was determined by lactate dehydrogenase (LDH) release (LDH Cytotoxicity Assay, Cayman Chemical Company, Ann Arbor, MI), and fluorescence (560 nm excitation, 590 nm emission) was measured in triplicate using a Wallac Microbeta 1450 fluorescence plate reader. Supernatants from particle-challenged cells were collected and analyzed for IL-1β, IL-6, IL-8, MCP-1 and TNF-α expression in triplicate by Luminex suspension multiplex array (Invitrogen). Comparative analysis with controls and PEEK data was conducted using non-paired t-testing, for all data predeterimed to be normally distributed.

**RESULTS:** Following challenge of the cells with particles, the LDH release assay revealed that UHMWPE particles elicited a statistically significant increase in cytotoxicity in macrophages after 24 hours compared with PEEK-OPTIMA ($p<0.05$), (data not shown). This difference was observed for each ratio of particles to cells. No dose-related increase in cytotoxicity was evident for the PEEK material. However, an increase in cytotoxicity was observed in THP-1 macrophages with increasing dose of UHMWPE particles. After 48 hours, no difference in cytotoxic effect of the two materials was evident. Of note, cytotoxicity attributed to the high dose (20:1) of UHMWPE particles remained significantly elevated after 48 hours.

In general, challenge of THP-1 macrophages with PEEK and UHMWPE materials in this study resulted in a limited inflammatory response. Expression of cytokines IL-1β and IL-8 was greatest for UHMPE compared with PEEK at the low (1:1) and high (20:1) dose of particles, but was equivalent or greater for PEEK at a dose of 10:1 (data not shown).

All three subjects demonstrated roughly similar ranges of IL-1β, IL-6, IL-8, and TNFa cytokine responses to PEEK and PE particles. However the IL-1β response to X-UHMWPE was greater than to PEEK in 2 of 3 subjects (Figure 9). Additionally, endotoxin free particles of PEEK and PE particles induced significantly ($p<0.05$) greater for IL-1β than LPS challenge. The chemokine MCP-1 demonstrated the greatest variability in response to particle challenge in subjects 1 and 3. There was a significantly greater IL-1β cytokine response to X-UHMWPE compared to PEEK particles in Subject 1, and a significantly greater response to MCP-1 (chemokine) X-UHMWPE compared to PEEK particles in Subject 3 (Figure 1).

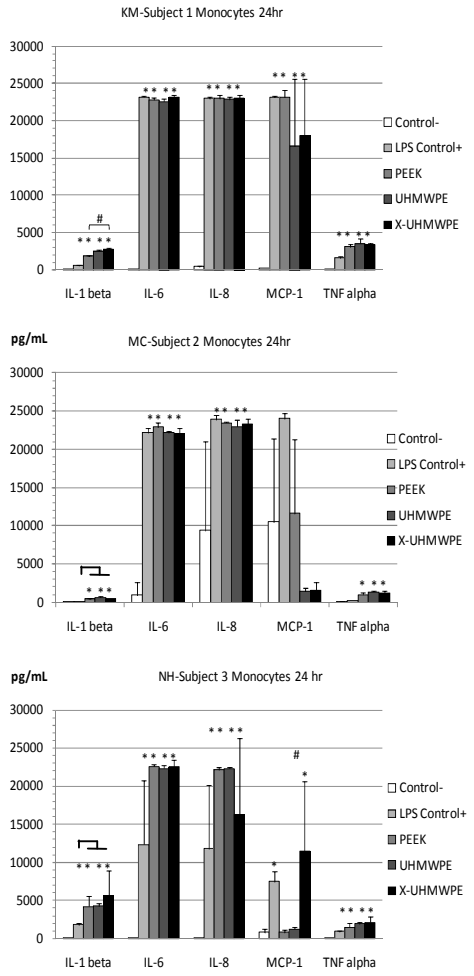


**Figure 1:** *PEEK and UHMWPE particles induced significant increases in IL-1β, IL-6, IL-8, and TNFα at 24 hours. All particles induced significantly ($p<0.05$) greater IL-1β compared to LPS (bars). Note: *=$p<0.05$, compared to controls (medium alone) and #=$p<0.05$ compared to PEEK at an equal particle size.*

**DISCUSSION:** THP-1 macrophage cytotoxic effects and primary macrophage inflammatory cytokine responses were generally more evident following exposure to UHMWPE, when compared with PEEK. This *in vitro* study is accordance with a previous *in vivo* study in which PEEK particles showed no adverse response in the spine [4]. These findings therefore support our hypothesis that PEEK-OPTIMA particles are as biocompatible as UHMWPE particles and demonstrate reduced inflammatory responses, providing a viable alternative to UHMWPE as bearing surface, where similar amounts and sizes of debris are generated.

**REFERENCES:** [1] Brown T., *et al*. 6th Annual SAS Global Symposium on Motion Preservation Technology. 2006, Montreal, Canada. [2] Punt I.M., *et al*. Biomaterials 2009; 30: 2079-84. [3] Jacobs J.J., *et al*. J Bone Joint Surg Am 2006; 88(Suppl 2): 99-102. [4] Rivard C.H., *et al*. J Biomed Mater Res 2002; 62: 488-98.