INTRODUCTION: PEEK (polyetheretherketone) has found use in a broad range of medical applications. Amongst these, PEEK 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 as well as PEEK being demonstrated to be of lower wear, the particulate debris generated will be of equal or lower cell reactivity when compared with conventional polymer materials.

To test our hypothesis that PEEK elicits a reduced inflammatory response, human THP-1 macrophages were exposed to sterile sub-micron 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 and cytokine analysis.

METHODS: Bulk PEEK OPTIMA™ (Invibio Ltd., UK) and a commonly used, implantable grade 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 aspect ratio, roundness and form factor. The particles were designed to match particles generated previously from total disk arthroplasty test simulations. PEEK and UHMWPE particles were EtO sterilized, endotoxin cleaned (using serial incubation with PyroNClean™ and ethanol) and verified to be endotoxin free (using Kinetic QCL assay) prior to challenge of macrophages.

Differentiated human THP-1 macrophages (ATCC, Rockville, MD) cultured in Dulbecco’s Modified Eagle Medium, DMEM (Sigma, St Louis, MO) at 37°C and 0.5% CO₂, containing 10% fetal bovine serum (Hyclone Laboratories Inc., Logan, UT) were exposed to varying ratios of particles to cells for 24 or 48 hours (n=3 for each material, dose and time). Cytotoxicity was determined by lactate dehydrogenase (LDH) release (LDH Cytotoxicity Assay, Cayman Chemical Company, Ann Arbor, MI), according to the manufacturer’s instructions and fluorescence (560 nm excitation, 590 nm emission) was measured using a Wallac Microbeta 1450 fluorescence plate reader. Supernatants from particle-challenged cells were collected and analyzed for particle-induced IL-1β, IL-6, IL-8, MCP-1 and TNF-α expression by a pro-inflammatory Luminex suspension multiplex array (Invitrogen), according to the manufacturer’s instructions. By convention, to calculate group means, cytokine concentrations below the detection limit were assigned a value of one-half the method detection limit.

RESULTS: Following cryo-milling, pulverisation and filtering, PEEK OPTIMA and UHMWPE sterile particles were generated. The mean number-based particle sizes (equivalent circle diameter) of PEEK and UHMWPE were 0.7µm and 0.5µm respectively, as determined by LALLS. Particles had the same granular to flake-like shape with average aspect ratios from 1.1 to 1.5 (i.e. round to oval). 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) (Figure 1). 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 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 macrophages with PEEK and UHMWPE materials in this study resulted in a limited inflammatory response, with IL-6 levels in the supernatants of particle-challenged cells remaining below the level of detection for the duration of the experiment. After 24 hours, TNF-α levels were largely equivalent for both materials, although were slightly elevated for UHMWPE at the highest particle load (20:1). Similarly, MCP-1 levels remained below the level of detection, with the exception of UHMWPE at 20:1. Expression of cytokines IL-1β (Figure 2) and IL-8 was greatest for UHMWPE 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.

After 48 hours challenge of macrophages with particles, expression levels of cytokines were largely equivalent for the two materials at the low dose, with the exception of MCP-1, which was increased for UHMWPE. Despite the high degree of variability seen for PEEK, expression levels of IL-1β (Figure 2), TNF-α, MCP-1 and IL-8 in UHMWPE-challenged cells were raised compared with PEEK at doses of 10:1 and 20:1, and generally showed a greater sensitivity to the increase in particle load.

DISCUSSION: Both materials elicited a relatively low inflammatory response from macrophages following particle challenge. Cytotoxic effects and inflammatory cytokine response were generally more evident following exposure to UHMWPE, and displayed a greater sensitivity to increasing dose of UHMWPE compared with PEEK. This in vitro study is in 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 offers greater biocompatibility than UHMWPE with a reduced inflammatory response, thereby providing a viable alternative to UHMWPE in total disc arthroplasty, where similar amounts and sizes of debris are generated.