DIFFERENT CELLULAR AND APOPTOTIC RESPONSES TO VARIANT SHAPES OF UHMWPE PARTICLES IN A MURINE MODEL OF ASEPTIC LOOSENING

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Introduction: Cellular response to particulate debris present in periprosthetic tissue surrounding the failed joint prosthesis is critical in the pathogenesis of aseptic loosening. It is generally accepted that particulate debris generated by mechanical wearing of orthopaedic components attract and activate macrophages to provoke local inflammation, produce proinflammatory cytokines such as IL-1 and TNFα, and subsequently cause osteolysis around implant and arthroplasty failure. The role that debris-induced-apoptotic-change plays in pathogenesis of aseptic loosening has also become an active area of interest. Periprosthetic tissue associated with loosened implants contains particulate debris that varies in chemical composition, shape, and size, and cells present in this tissue exhibit considerable heterogeneity among different individuals. We therefore hypothesized that different wear particle shape and size may have different activity in provoking cellular and cytokine responses. Using a well-developed murine air pouch model, this study examined how different shapes of UHMWPE particles act in provoking cellular and apoptotic responses.

Materials & Methods: A pin-on-flat wear tester with two metal surfaces, one with cross-hatched and the other with uni-hatched grooves, was employed to generate two distinct populations of UHMWPE wear particles from UHMWPE pins (GUR415, extruded, gamma irradiated with 25 kGy in air). One population of particles was predominantly globular or blunt in shape, while the other population was predominantly sharp elongated rods ranging in the average size from 5 to 25 µm. The aspect ratio of these particles were about 1.5 and 3 respectively, while the volume was similar. These particles were produced under sterile environment and no further sterilization steps were taken before injecting into air pouches.

Air pouches were established by injecting 3 ml of filtered air subcutaneously on the mid dorsal area of BALB/c mice. After six days, the two populations of UHMWPE particles suspended in 10%FCS/saline were introduced respectively into the air pouches. Vehicle (10%FCS in saline) was given to a particle-free control group. Pouch membranes and fluid were harvested 7 days later and divided for histological/apoptotic evaluation and molecular/biochemical analyses. Pouch membrane sections were cut with a microtome and stained with H&E or analyzed with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL). An image analysis system was used to evaluate the thickness of the pouch membranes, the magnitude and diversity of the cellular infiltrate, and to calculate cellular apoptotic status (Apoptotic Index) within the membranes. RT-PCR and ELISA were performed to assess expression and secretion of inflammatory cytokines in pouch lavage fluid and pouch membrane homogenates.

Results: The results revealed that sharp elongated particles generated more active inflammatory air pouches, with increased erythematous and edematous changes compared with blunt particles. Figure 1 showed the typical macroscopic appearance of air pouches in each group at termination. Microscopically, the pouch membranes containing elongated-particles averaged 0.223±0.021 mm in thickness, significantly thicker than pouches with globular-particles (0.14±0.005 mm, p=0.03) and non-particle control pouches (0.071±0.01 mm, p=0.001). Cellular infiltration in membranes with elongated particles (8495±502 per mm²) were also significantly increased compared with the other two groups (6815±513 /mm², p=0.05 and 4344±648 /mm², p=0.01, respectively). The cellular diversity analyses showed that 62% of cellular composition in elongated-particles containing pouches was mononuclear inflammatory cells, significantly higher than the blunt particle-stimulated pouches where fibrocytes are predominant (p<0.05). Significantly elevated level of inflammatory cytokines IL-1ß and TNFα was detected in the lavage fluid (p<0.01) and homogenate (p<0.05 for IL-1ß) of the pouches with sharp particles compared with blunt particles (Figure 2). Apoptotic evaluation concluded that both particles clearly induced apoptotic changes within the inflammatory membrane compared to non-particle controls. However, the Apoptosis Index for sharp elongated particle-containing pouches (33.1%) was significantly higher than that for pouches with blunt globular particles (18.7%). Figure 3 demonstrates positive stained apoptotic cells within the pouch membranes.

Conclusions: This study suggests that the cellular responses to UHMWPE wear debris are dependent on the shape of the particles. Particles with blunt globular shape appear to have less effect on provoking local inflammation in terms of proinflammatory cytokine production and inflammatory cellular infiltration compared to sharp elongated-particles. Further, the finding of different apoptotic changes within inflammatory membranes to various shapes of particles provides evidence that apoptosis play a role in the process of aseptic loosening of the prosthetic implants.

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![Figure 1: Air pouches with UHMWPE](image1.png)

![Figure 2: Air Pouches with UHMWPE particles](image2.png)

![Figure 3: TUNEL stain for Apoptotic cells](image3.png)

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