OSTEOLYTIC POTENTIAL OF CLINICALLY RELEVANT ALUMINA CERAMIC WEAR DEBRIS GENERATED UNDER MICROSEPARATION CONDITIONS IN A HIP JOINT SIMULATOR

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Introduction
Aseptic loosening is the most common cause of long term failure of UHMWPE-on-metal total hip replacements. It is currently believed that this is caused by activation of macrophages by UHMWPE particles in the 0.1-1.0 µm size range [1]. This has lead to an increased interest in alternative bearing materials, such as alumina ceramic-on-ceramic for total hip prostheses. There are conflicting reports regarding the incidence of osteolysis around loose ceramic-on-ceramic hips. There have been few studies investigating the osteolytic potential of alumina ceramic wear debris and these have used artificial particles that are not clinically relevant. The recent introduction of microseparation during the swing phase of the gait cycle in ceramic-on-ceramic hip joint simulations has produced clinically relevant wear rates, wear mechanisms and wear particles. This provided an opportunity to test the osteolytic potential of the wear particles in vitro.

Methods
Alumina ceramic particles were generated using non-HIPed alumina ceramic (BIOLOX; CeramTecAG) components on the Leeds Mk II hip simulator using microseparation conditions [2]. Tests were run in pyrogen free water for 600,000 cycles at a frequency of 1 Hz. The lubricant containing the debris was centrifuged, and a portion sequentially filtered through a series of filters with descending pore size (5.0, 1.0, 0.1, 0.05 and 0.015µm) to determine the mass of the debris in the different size ranges. The remainder of the debris was treated to remove endotoxin (soaked in E-Toxaclean™ and heated to 180°C for 5h), tested for endotoxin (Limulus Amaebocyte Lysate Turbidimetric Assay;™ Associates of Cape Cod) and analysed by SEM, TEM and image analysis (Image Pro-Plus™).

Blood (50ml) was collected from six normal healthy donors and the mononuclear cell fractions isolated on Lymphoprep™ gradients (Nycomed, UK). The mononuclear cells were resuspended in RPMI-1640 culture medium plus supplements to a final density of 4 x 10⁸ monocytes/ml³ and seeded into 96-well plates. The cells were incubated at 37°C in 5% (v/v) CO₂ in air., after which the culture supernatants were harvested and assayed for TNF-α (ELISA). The viability of the cells was determined by MTT conversion. Results are expressed as the mean specific activity of TNF-α (ng.ml⁻¹/absorbance at 560nm; MTT).

Results
SEM analysis of the microseparation wear particles revealed polygonal shaped particles ranging from less than 1µm to over 10µm, with the mode of the particle length of 0.3 to 0.4µm. The smaller particles were analysed by TEM. This revealed particles in the nanometre size range with the majority being 5 to 20nm in length. The mass distribution of the alumina wear particles as a function of size is shown in Fig (1). The majority of the volumetric concentration of the particles was greater than 1 µm. Despite the small proportion of the volumetric concentration in the less than 50µm size range, this debris represented the approximately 98% of the total number of the particles. The particles were endotoxin free (less than 0.002 endotoxin units per 945µg).

The particles had no effect on the viability of the mononuclear cells from any of the six donors over the 24 h period of incubation. The results for TNF-α production by the mononuclear cells in response to different concentrations of the particles are shown in Fig (2). For all six donor mononuclear cells, the levels of TNF-α produced in the control cultures were low. The levels of TNF-α produced in response to LPS varied between donor mononuclear cells and ranged from 2.49 (Donor 5) to 28.8 (Donor 6) units of specific activity. Mononuclear cells from Donors (1), (2) and (6) produced significantly elevated levels of TNF-α (p<0.05) when stimulated with particles at 100 and 500µm³ per cell. Donors (3), (4) and (5) only produced significantly elevated levels of TNF-α when cultured with the highest volume of particles tested (500µm³ per cell).

Discussion
In this study, TNF-α production was used as a marker for osteolytic potential since it has been shown to be a major cytokine involved in particle induced osteolysis in vitro [3] and in vivo [4]. This study demonstrated that the alumina ceramic wear particles could stimulate human peripheral blood mononuclear cells to produce TNF-α in vitro, but that the volumetric concentration of the particles and the individual mononuclear cell donor were critical factors in the response. Previous studies using UHMWPE particles showed that UHMWPE particles of 0.1 to 1.0 µm were the most biologically active and stimulated significantly enhanced cytokine production at 10 and 100 µm³ per cell[1]. Thus it can be concluded that a greater volume of the microseparation debris was needed to expose the cells to an equivalent volume of the critically sized particles. Given a sufficiently high local volumetric concentration of critical size alumina ceramic wear particles, the potential for osteolysis around ceramic-on-ceramic implants does exist, however, whether the volume of alumina debris will reach this threshold in vivo is questionable, given the extremely low wear rates of ceramic-on-ceramic prostheses even under microseparation conditions, in the region of 4µm³ per million cycles [2], which is approximately ten times lower than UHMWPE.

References
Ingham et al. (1999), Proc 45th ORS, 899.

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