UHMWPE Particles Stimulates Increased Release of MCP-1 in Traditional Cell Cultures without Coating Materials

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Statement of Purpose: Wear particles generated from implants are an important cause of loosening of orthopaedic prostheses. Due to difficulties in isolating and enriching clinically relevant UHMWPE particles and the lower density of UHMWPE particles (0.94 g/cm^3, compared to water, 1.0 g/cm^3), to date, a simple and effective in vitro model for testing the cell response to UHMWPE particles is still lacking.

Two different culture models have been attempted to improve the contact of macrophages to UHMWPE particles. One model fixed UHMWPE particles in a solid state, either on the bottom of culture dish, or using a solid culture medium [1,2]. The other model used an inverted culture system, in which cell culture dishes were placed upside down after initial cell adherence [3]. We isolated UHMWPE particles collected from joint simulation tests and compared the macrophage responses to UHMWPE particles using two different methods. In one method, the particles were coated directly on culture dishes and in a second method, particles were fixed on the bottom of culture dishes by collagen [1].

Methods:

Isolation of UHMWPE: Conventional UHMWPE particles, a generous gift from Dr. Tim Wright at the Hospital for Special Surgery in New York were obtained from knee joint simulator tests and isolated according to an established protocol [3]. The average diameter of the isolated particles was 2~3 um (**Fig. 1**). The particles were processed in a sterile manner and the absence of endotoxin was confirmed by LAL assay.

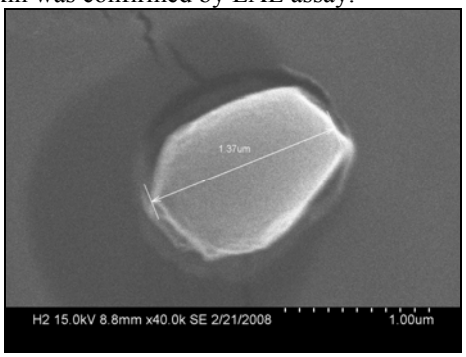

Fig. 1. UHMWPE particles under SEM (Hitachi S-3400N).

Direct coating of particles on culture dishes: The particles at various concentrations were suspended in 200 ul of sterile, distilled water and allowed to dry in a sterile culture hood for 2 days. Before cell seeding, these culture wells were gently washed with PBS.

Particle-collagen coating method: The particles at various concentrations were suspended in 200 ul of 0.01% type I collagen solution (Cat# C8919, SIGMA) and coated onthe cell culture plate. The culture plate was left dry under UV light in a sterile culture hood at room temperature for 2 days. Before cell seeding, these culture wells were gently washed with PBS.

Cells: RAW 264.7 cells (Cat#: TIB-71, ATCC), a mouse macrophage cell line was grown in 10% FBS in DMEM as recommended by the manufacturer. 0.5 million cells were seeded onto the culture wells of 24-well plates.

MCP-1 assay: The concentration of MCP-1 in the culture media was assayed by ELISA (R&D Systems) at 24 hour and 48 hour time points.

Results:

UHMWPE particles coated on the bottom of culture dishes together with collagen resulted in higher levels of MCP-1 release at the 48-hour time point when compared to collagen coating without particles. At the 24-hour time point, no significant difference was found (**Fig. 2**).

When particles were coated on the culture dishes directly, there was a significant release of MCP-1 at 48-hour time point than control with neither particles nor collagen, but not at 24-hour time point (**Fig. 2**).

Collagen coating by itself led to a significantly higher level of MCP-1 release than the control in which no exogenous coating materials were applied (**Fig. 2**).

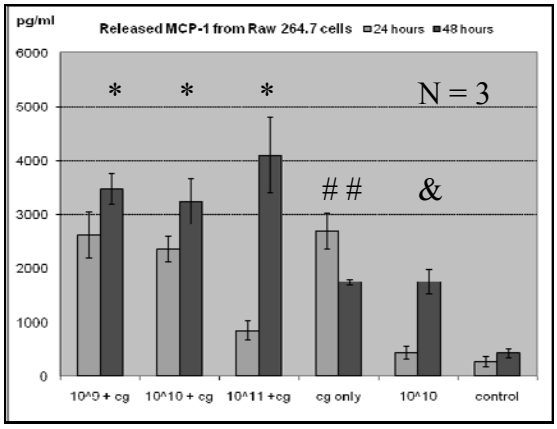

Fig. 2. MCP-1 release by macrophages challenged with UHMWPE.

*: $p < 0.05$ compare to collagen only (cg only); #: $p < 0.05$ compare to control w/o collagen, w/o particles (control); &: $p<0.05$ compare to control.

Conclusions: UHMWPE particles coated directly on the culture dishes can effectively stimulate macrophages to release MCP-1. Although collagen is commonly used as an in vitro coating material for UHMWPE particles, the stimulatory effect of the collagen coating alone may lead to MCP-1.

References:

[1] Xing S, et al. Biomaterials. 2002;23(17):3595-602.
[2] Fang HW, et al. J Biochem Biophys Methods. 2006;31;68(3):175-87.
[3] Campbell P, et al. J Biomed Mater Re 1995;29(1):127-31.

Acknowledgements:

NIH 1RO1ARO55650-01