Treatment of MG63 Cells With UHMWPE Particles After Fractionation by Vacuum Filtration Into Three Different Size Ranges, Including a Nanometer Size Fraction

[1,2]Musib, MK; [1]Marshall AD; [3]Oxley J, [1]Sylvia VL; [2]Agrawal CM; [1]Dean DD
[1]University of Texas Health Science Center at San Antonio, San Antonio, TX; [2]University of Texas at San Antonio, San Antonio, TX; [3]Southwest Research Institute, San Antonio, TX

STATEMENT OF PURPOSE:

Ultra-high molecular weight polyethylene (UHMWPE) is widely used to fabricate components of orthopaedic implants. Despite good mechanical properties, it produces wear debris particles that have been implicated in implant loosening. Cell response to submicron size UHMWPE particles has been well documented[1,2], while reports of response to particles smaller in size (<0.2µm) are virtually absent. In the present study, UHMWPE resin was fractionated into 3 size ranges and added to cultures of human osteoblasts (MG63). At harvest, the effect of particle treatment on cell viability and membrane integrity was assessed.

METHODS:

Fractionation of UHMWPE particles. UHMWPE (GUR 1050) particles were suspended in water, pH 5.5, containing 500ppm Pluronic. The suspension was vortexed for 15mins, sonicated for 2hrs and then stored at 4ºC for 7 days. Earlier studies in our lab demonstrated that this solvent minimizes particle aggregation and facilitates fractionation. After 7 days, the suspension was filtered through a 10µm pore size filter to remove large resin particles. The suspension was then fractionated into 3 size ranges (1.0-10.0µm, 0.2-1.0µm and 0.01-0.2µm) by filtration through filters of varying pore size. Representative images were obtained using a Zeiss EVO50 SEM and analyzed according to ASTM F-1877 using software which determines equivalent circle diameter (ECD) and various shape descriptors[3,4].

Effect of particle size on MG63 cell viability and membrane integrity. MG63 cells (ATCC) were cultured in DMEM containing 10% FBS. Cells were seeded into 24 well plates and allowed to attach. After 12hrs, media were changed and fresh media containing 10^5 to 10^7 particles/well for each of the 3 size ranges added. Control cultures only received fresh media. Treatment continued for 4 to 120hrs. At harvest, the number of viable cells was measured using the CellTiter-Blue Assay (Promega), while release of LDH (a marker of membrane integrity and cytotoxicity) was measured using a CytoTox-ONE kit (Promega).

Statistical Interpretation of Data: ANOVA was used to analyze the data; post-hoc testing was performed using Student's t-test with Bonferonni correction. P values $\leq$ 0.05 were considered significant.

RESULTS: All particle suspensions were endotoxin-free. Equivalent circle diameter (ECD) for each of the 3 preparations is shown in Table 1. Minimum and maximum particle size in each fraction was predictable based on the pore size of the filter (Table 1). Up to 24hrs, particles had a dose-dependent stimulatory effect on the number of viable cells; the magnitude of the effect was greatest with the nanoparticle fraction. After 24hrs, particles dose-dependently inhibited cell viability and the effect was greatest for the nanoparticle fraction. Particle treatment also had a dose-, size-, and time-dependent effect on LDH release (Figure 1). As seen for cell viability, the nanoparticle fraction produced the greatest release of LDH. Unlike viability, the most pronounced effect of particles on LDH release was seen after 120hrs of treatment.

Table 1: Mean ECD (µm) of Fractionated Particle Suspensions

Particle Fraction	Mean (µm)	S.E.M.	Min.	Max.
10.0µm filtrate (collect on 1µm)	2.240*#	0.084	0.220	7.650
1.0µm filtrate (collect on 0.2µm)	0.513*	0.012	0.017	0.909
0.2µm filtrate (collect on 0.01µm)	0.056	0.002	0.015	0.190

A total of 300 particles in each fraction were analyzed. *P<0.05, vs 0.2µm filtrate; #P<0.05, vs. 1.0µm filtrate.

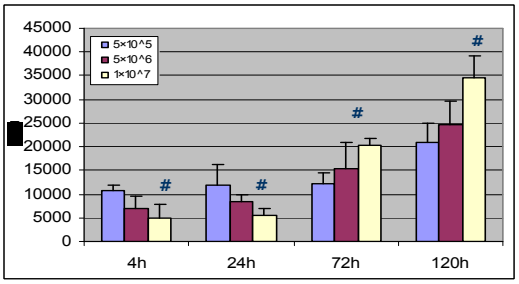

Figure 1. Effect of UHMWPE nanoparticles on LDH release [above control] by MG63 cells. (#P<0.05, vs treatment with 10^5 particles).

CONCLUSIONS: A method for fractionating UHMWPE particles has been developed. Fractionated particles were found to have size-, dose-, and time-dependent effects on osteoblast cell viability and membrane integrity. With 24hrs of treatment, nano-size particles demonstrated a 1.3 to 2.5-fold stimulatory effect on the number of viable cells compared to submicron- and micron-size particles. Cell viability decreased with longer treatment times. Similarly, LDH release was dose-dependently increased, suggesting a cytotoxic effect of the nanoparticles. These results suggest that nano-sized particles may elicit more adverse affects on osteoblasts than previously reported for submicron-sized particles.

REFERENCES

[1]Dean DD, et al., *J Orthop Res* 17:9-17, 1999.
[2]Dean DD, et al., *J Bone Joint Surg* 81A:452-461, 1999.
[3]Landry M, et al., *J Biomed Mat Res* 48:61-69, 1999.
[4]McMullin BT, et al., *Biomaterials* 27:752-757, 2006.
Supported by DePuy Orthopaedics.