Validation and Quantification of an In Vitro Model of Continuous Infusion of Sub-Micron Sized Particles

+*Ortiz, S; *Ma, T; *Smith, R L; *Goodman, S
+*Stanford University, Stanford, CA

Statement of Purpose:
Wear particles produced from total joint replacements have been shown to stimulate a foreign body and chronic inflammatory reaction that results in periprosthetic osteolysis. Most animal models that simulate these events have used a single injection of particles, which is not representative of the clinical scenario, in which particles are continuously generated. The goal of this study was to evaluate the feasibility of an osmotic pump for the continuous delivery of clinically relevant sub-micron sized particles over an extended period of time.

Methods:

Phase 1A: Three groups consisting of ten Alzet mini-osmotic pumps each (0.25µl/hour delivery rate, Durect, Cupertino, CA) were filled with 200µl of one of three different concentrations of blue-dyed polystyrene particles (0.5µm diameter, Polysciences, Warrington, PA) suspended in 100% mouse serum (Group 1: 6.0 x 10^9 particles/pump; Group 2: 6.0 x 10^{10} particles/pump; Group 3: 3.0 x 10^{11} particles/pump). Particle delivery tubing assemblies consisting of 5cm of V3A vinyl tubing catheters (SCI, Lake Havasu, Arizona), hollow titanium rods (6mm. 21G, New England Small Tube, Litchfield, NH) and Alzet osmotic pump flow moderators were attached to 0.5ml centrifuge tubes, used as collection vessels, and glued in place with Nusil Med-1137 adhesive (Nusil Technology LLC, Carpinteria, CA). Tubing assemblies were pre-filled with polystyrene particle suspensions and attached to the Alzet mini-osmotic pumps. Pump/tubing assemblies were placed inside 14ml round bottom tubes with 12ml of phosphate buffered saline, which were placed on their sides on a rocker at 37° Celsius (**Figure 1**). Five pump assemblies from each group were removed after two weeks and four weeks.

Phase 1B: The previous experiment was repeated with two concentrations of ultra-high molecular weight polyethylene particles (0.5µm diameter) suspended in 100% mouse serum (Group 1: 3.0 x 10^{10} particles/pump; Group 2: 1.5 x 10^{11} particles/pump).

Visualization of blue suspension in the collection vessels was used to confirm delivery of polystyrene particles. Spectrophotometry (595nm, BioRad, Hercules, CA) was used to perform turbidity analysis for determination of the concentration of particles in the collected suspensions.

Figure 1:
Pump/tubing/collection assembly after completion of polystyrene experiment.

Results:

Successful pumping of blue polystyrene and polyethylene particle suspensions occurred in all groups in both the two-week (**Fig. 2a**) and four-week (**Fig. 2b)** experiments.

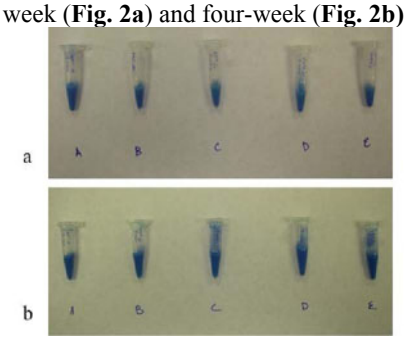

Figure 2:
a. Polystyrene Group 3, 2-week;
b. Polystyrene Group 3, 4-week.

The concentration and volume of suspension delivered allowed calculation of the average number of particles pumped for each initial concentration for both two and four-week time periods (see **Tables 1 and 2**).

Table 1: Average number of particles pumped, percent of original particles pumped, and range of particles pumped for each initial polystyrene particle concentration and time period.

Exp. group	Ave. # particles pumped	% original particles	Range
Group 1, 2-week	1.25 x 10^9	21%	9.10 x 10^8–1.63 x 10^9
Group 2, 2-week	7.54 x 10^9	13%	5.45 x 10^9–9.64 x 10^9
Group 3, 2-week	1.82 x 10^{10}	6%	1.23 x 10^{10}–2.14 x 10^{10}
Group 1, 4-week	2.73 x 10^9	46%	2.02 x 10^9–3.54 x 10^9
Group 2, 4-week	1.39 x 10^{10}	23%	7.65 x 10^9–1.89 x 10^{10}
Group 3, 4-week	4.53 x 10^{10}	15%	3.24 x 10^{10}–5.26 x 10^{10}

Table 2: Average number of particles pumped, percent of original particles pumped, and range of particles pumped for each initial polyethylene particle concentration and time trial period.

Exp. group	Ave. # particles pumped	% original particles	Range
Group 1, 2-week	3.31 x 10^9	11%	2.85 x 10^8–5.71 x 10^9
Group 2, 2-week	2.91 x 10^{10}	19%	2.45 x 10^{10}–3.37 x 10^{10}
Group 1, 4-week	8.94 x 10^9	30%	6.79 x 10^9–1.28 x 10^{10}
Group 2, 4-week	4.78 x 10^{10}	32%	3.34 x 10^{10}–7.63 x 10^{10}

Conclusions:
The present study has confirmed that submicron particle delivery system using the apparatus described is feasible and quantifiable. This system may be utilized in the future for in vivo investigations to develop a more clinically relevant murine model of continuous particle infusion.

Acknowledgements:
NIH Grant # R21 AR053189-01, Zimmer and Stanford Medical Scholars Program (SO).