**Quantitative Assessment of Continuous Infusion of Submicron-Sized Particles**

Steven Ortiz, BS, SMSIII*, Ting Ma, MD, MS, R. Lane Smith, PhD, Stuart Goodman, MD, PhD
Department of Orthopedic Surgery,
Stanford University

**Statement of Purpose:** A significant problem in total joint replacements is loosening due to the biological effects of wear debris. The presence of submicron-sized particles may interfere with initial osseointegration and, over time, alter bone remodeling processes resulting in periprosthetic osteolysis and loosening. Animal models play an important role in the investigation of these processes. However, current intramedullary particle delivery models use a single bolus injection, unlike the clinical scenario in which wear particles are formed on a continuous basis. The purpose of this study was to quantitatively assess a model of continuous infusion of submicron-sized particles to enable *in vivo* intramedullary delivery at doses representing clinical conditions.

**Methods:** Three Alzet mini-osmotic pumps (0.25ul/hour delivery rate, Durect, Cupertino, CA) were filled with a suspension composed of blue-dyed polystyrene particles (0.5um diameter, Polysciences, Warrington, PA) mixed with 100% mouse serum ($3.64 \times 10^{11}$ particles/ml, 200ul per pump, $7.28 \times 10^{10}$ total particles). Three 6mm 21G hollow titanium tubes (New England Small Tube, Litchfield, NH) were inserted 2mm into one end of 5cm pieces of V3A vinyl tubing catheters (SCI, Lake Havasu, Arizona). The opposite ends of the vinyl tubing were attached to pump flow moderators. The tubing assemblies were pre-filled with polystyrene/serum suspension and the flow moderators were inserted into the pre-filled pumps. The pump assemblies were placed into sealed 5ml round bottom tubes with 3ml of phosphate buffered saline and placed on a rocker at 37° C for four weeks. After four weeks, the pump assemblies were removed from the collection tubes. Suspension color change and scanning electron microscopy were used to verify the presence of particles and the average number of particles delivered over four weeks was determined using spectrophotometry.

**Results/Discussion:** Particles were successfully transferred from the pumps to the collection vessels in all 3 samples.

A B

**Figure 1:** Figure 1A: Pump assemblies inside collection vessels on day 0. After four weeks, particles can be visualized by the blue coloration of the collection suspensions (Figure 1B).

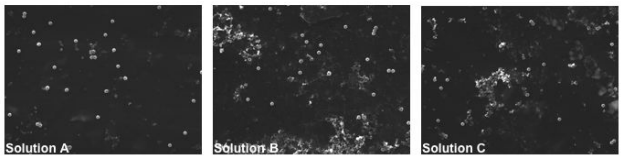


**Figure 2:** Presence of particles was further confirmed using scanning electron microscopy (dilution: 100x; magnification: 8.05kx).

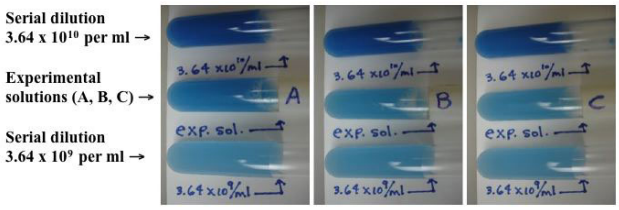


**Figure 3:** Collected suspensions were compared by color with serial dilutions of polystyrene particles. Comparison showed the experimental suspensions contained between $3.64 \times 10^9$ and $3.64 \times 10^{10}$ particles per ml.

Turbidity analysis using spectrophotometry (595nm, Bio-Rad, Hercules, CA) showed the average concentration of the collection suspensions to be $1.11 \times 10^{10}$ particles per ml. From this data, the average number of particles pumped over four weeks was calculated to be $3.52 \times 10^{10}$, or 48% of the original $7.28 \times 10^{10}$ particles initially loaded into each pump.

We have successfully pumped clinically relevant submicron-sized particles using this *in vitro* model and quantitated the average number of particles pumped over four weeks. Although wear debris particles recovered from total joint replacement simulators will ultimately be used in our *in vivo* experiments, we chose to use commercially available blue-dyed polystyrene particles in the initial *in vitro* assessment of this model because these particles are readily available, inexpensive and easily visualized without special staining procedures.

**Conclusion:** Alzet mini-osmotic pumps provide a viable solution for continuous delivery of submicron-sized particles. It is our goal to develop a system in which wear debris particles can be continuously delivered *in vivo*. In the future, time course studies will be conducted to validate the effective delivery of particles over an extended period of time.

**Acknowledgments:** This study was supported in part by NIH (Grant # R21 AR053189-01), Zimmer and Stanford Medical Scholars (SGO).

* SMSIII: Stanford Medical Student, year 3