Macrophages Systemic Recruitment Induced by Continuously Infused UHMWPE Particles in Nude Mice

[1]Ren, Pei-Gen; [1]Irani, Afraaz; [1]Huang, Zhinong; [1]Ma, Ting; [2]Biswal, Sandip; [1]Smith, Robert L.; [1]Goodman, Stuart B.
1Orthopaedic surgery, Stanford University, and 2 Radiology, Stanford University, Stanford, CA.

Statement of purpose:

Aseptic loosening and periprosthetic osteolysis due to wear particles are the most common sequela after total joint replacement. Phagocytic cells engulf particulate debris and release proinflammatory cytokines, chemokines, and other substances which stimulate osteoclasts to undermine the prosthetic bed. Macrophage (MΦ) is a key cell in the foreign body and chronic inflammatory response to wear particles. To investigate the systemic migration of MΦs in the presence of clinically relevant ultra high molecular weight polyethylene (UHMWPE) particles, a novel mouse model which uses an osmotic pump to infuse particles continuously was used. Exogenous MΦs (RAW264.7 cell) tagged with firefly luciferase (Fluc) and green fluorescence protein (GFP) were injected intravenously. The systemic migration and localization of reporter MΦs to implanted polymer particles in the femur were tracked by non-invasive in vivo bioluminescence imaging (BLI) and immunofluorescence staining.

MEHODS and Materials:

Adult male nude mice (12 week old) were used. UHMWPE particles from mechanical testing simulator studies were isolated by ultracentrifugation. The size of the UHMWPE particles (1.0 ± 0.1 μm) was determined by SEM. The particles were found to be endotoxin free using the LAL kit. Particle suspended in saline (15 mg/ml, 250 ul) or saline alone were placed into an Alzet mini-osmotic pump. Silicon tubing (6 cm) and a hollow titanium rod (6 mm) were connected to the pump. Under anesthesia, the intercondylar notch of the distal femur was exposed sterilely through a medial parapatellar arthrotomy. A series of needles from 25-gauge to 21-gauge were used to manually drill through the intercondylar notch to access the medullary cavity of left femur progressively. The hollow titanium rod was implanted into the distal 5mm of the medullary cavity of the femur and connected to tubing and the pump located in the subcutaneous interscapular region. Five groups were included in the experiment: infusion of particles with or w/o reporter cells, infusion saline with or w/o reporter cells, and normal animals without intervention.

10 to 14 days after surgery, reporter MΦ s (5×10^5 cells) suspended in 0.1 ml HBSS were injected through the lateral tail vein of the mice. D-luciferin (3 mg/mouse) was injected intraperitoneally, and images were taken with an Xenogen's IVIS200. Animals were imaged at 2-day intervals post-MΦ injection. BLI signal was quantified by drawing uniformly sized regions of interest (ROI) over the thigh on the prone images of the mice, and the data were collected as to photon/cm^2/sec/steradian. Non-parametric Mann-Whitney U tests were used to compare the ratio of bioluminescence from the operated (left) side to the non-operated (right) side for particle-injected and saline injected (control) animals. After imaging experiments, the femora were collected and frozen sectioned for immunofluorescence staining. Images were taken using Nuance multispectral imaging systems (Woburn, MA).

RESULTS:

Upon the completion of the experiment, 30 successful animals were obtained from 41 operated ones (Table 1). Most animals failed due to dislodged tubing at the rod-tubing interface (10 out of 11) during the first week.

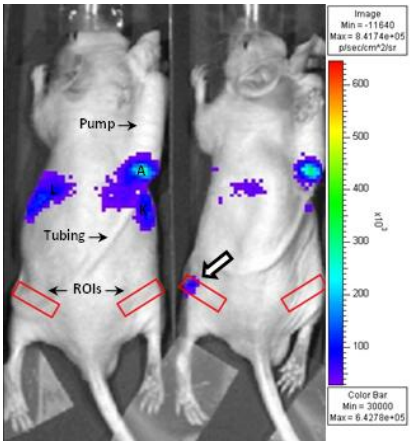

Fig. 1 Non-invasive in vivo BLI of nude mice with implanted pump system at day 6 post-injection of macrophages. Left: Saline in the pump, Right: UHMWPE particles in the pump. A: autoluminescence from pump; L & K: signal from liver & kidney respectively. The big arrow indicated the BL from implanted femur with UHMWPE particles. The unit of the signal in the scale bar is p/sec/cm^2/sr.

BLI was taken from day 0 (the day reporter MΦs injected intravenously) to day 10 (Fig. 1). Starting from day 4 post-macrophage injection, higher bioluminescent signal was detected from UHMWPE particle injected femora. The ratios of UHMWPE + cell group were 1.78 ± 0.61, 6.19 ± 3.84, and 13.95 ± 5.65 at day 4, 6 and day 8 ($p < 0.01$), whereas the values for the saline +cell group were 1.01 ± 0.09, 1.52 ± 0.39, and 2.60 ± 1.14, respectively. The other three control groups all had the ratios close to 1.0 from day 0 to day 10 (Fig. 2).

Overlapping of the images stained for general MΦ marker and GFP reporter gene indicated the existence of infused reporter MΦs in femoral cavity (Fig.3).

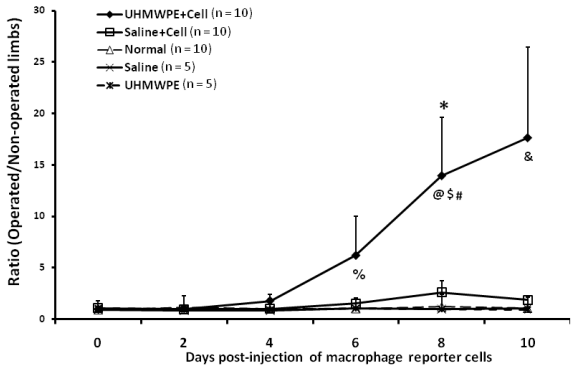

Fig. 2 Curve of ratio of bioluminescence signal from UHMWPE + cell or saline + cell femora versus the signal from corresponding non-operated contralateral femur from day 0 to 10 post-injection of MΦs. The values are mean ± SE. The ratio of UHMWPE + cell group compared with all the control groups is statistically significant. *: PE vs. Saline ($p = 0.009$); #: PE vs. Normal ($p = 0.001$); @, &: PE vs. PE no cell ($p = 0.004$, $p = 0.028$); $, %: PE vs. Saline no cell ($p = 0.002$, $p=0.038$)

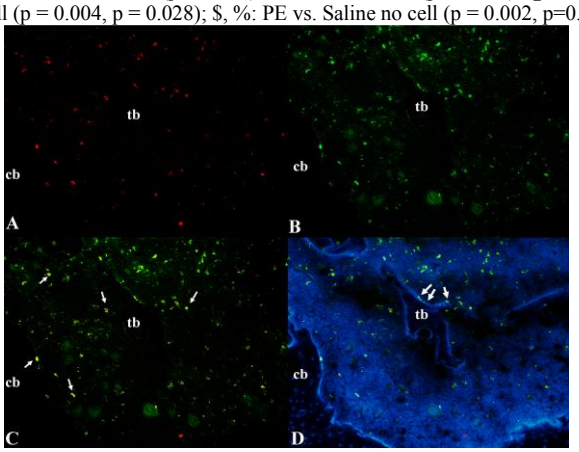

Fig. 3 General MΦ marker MOMA-2 and reporter cell marker GFP double immuno-fluorescent staining of femoral sections. Primary antibodies (Abs) were rat anti-mouse MOMA-2 and rabbit anti-GFP; secondary Abs were goat anti-rat IgG-AF594 and goat anti-rabbit IgG-AF488, respectively. DAPI containing mount medium was used. A) Image taken under TRITC filter with MOMA-2 staining demonstrating total number of MΦs. B) Image taken under FITC filter demonstrating the MΦs containing GFP. C) Overlay of images A and B using Photoshop (yellow color, arrowed). D) Overlay of images C and image taken under DAPI filter (showing total cell number and bone). Arrows show the double stained cells along trabecular bone. Magnification rate: 20 ×. **cb** and **tb** refer to cortical bone and trabecular bone respectively.

CONCLUSSION:

In this study, we infused UHMWPE particles continuously into the femur and non-invasively observed the migration of exogenous reporter MΦs by using an in vivo imaging system (IVIS). Bioluminescent signals were significantly higher in the femoral cavity of nude mice continuously infused with UHMWPE particles than those infused with saline (day 8, $p < 0.01$). Immunofluorescence staining results indicated the existence of the exogenous reporter MΦs. These findings demonstrated that systemic MΦ trafficking to the site of UHMWPE particle infusion occurred and may contribute to the processes of particle-induced osteolysis.

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