# Systemic Homing of Osteoprogenitor Cells to Continuously Infused UHMWPE Particles In Vivo

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**Introduction**:
Osteolysis due to excessive wear particle production is a common problem after total joint arthroplasty. However the role of multipotent mesenchymal stromal cells (MSCs) in particle-induced osteolysis is unknown. These cells can differentiate into multiple mesenchymal lineages, including osteoblast, chondrocyte, adipocyte, muscle cell, etc. However, MSCs are a very rare cell population, but can be expanded after isolated from bone marrow, peripheral blood and other sources. The pre-osteoblast cell line MC3T3 (subclone 14) which derived from mouse calvaria is widely used in osteoblast differentiation and bone generation studies. For in vivo studies, MC3T3 cells have similar characteristics as MSCs; their tendency to aggregate and their relatively big size, both can induce severe pulmonary embolism (PE) when introduced systemically into the venous circulation. In this study, we used MC3T3 cells to investigate the systemic homing of osteoprogenitor cells in response to continuously infused UHMWPE particles in a mouse model. We also tested different in vivo cell delivery methods of these large size cells, such as MSCs and MC3T3 cells. In preclinical cell therapy models, the method of infusion of MSCs is important for safety and efficiently applying MSCs when working with mouse models.

**Materials and Methods**:
Adult male nude mice (10 week) were used for these studies. Ultra high molecular weight polyethylene (UHMWPE) particles from mechanical testing simulator studies were isolated (size: 1.0 ± 0.1 μm). The endotoxin level (< 0.1 EU) in the particles was determined by using the LAL kit. Particles suspended in saline (15 mg/ml) or saline alone were filled into Alzet mini-osmotic pumps. A hollow titanium rod (6 mm long) was implanted sterily into the femoral marrow cavity and connected to the outlet of the pump. MSCs were isolated from C57BL/6 wild mice, cultured and expanded. MC3T3 cells were bought from ATCC and cultured as recommended. Stable MC3T3 cell line which expresses Fluc and Tomato reporter genes was established by FACS method. Reporter MSCs were prepared by transducing cells with lentiviral vector containing Fluc and Tomato reporter genes. Heparin (MP Biomedicals, LLS) concentration used was 100 unit/ml.
Three different methods were tried to delivery MSCs or MC3T3 cell into the systemic circulation of mice as shown in Table 1. Intra-arterial delivery methods through common carotid artery (CCA) and ultrasound guided intracardiac injection (ICI) were evaluated by injection of PBS but not cells. ICI (left sided of heart) was facilitated using ultrasound (Vevo 2100, VisualSonics) or blindly. Bioluminescence imaging (BLI) was performed with an in vivo imaging system IVIS200 (Xenogen) for up to 4 weeks. BL signal was quantified by drawing uniformly sized regions of interest (ROI) over the thigh on the prone images of the mice.

**Results**:

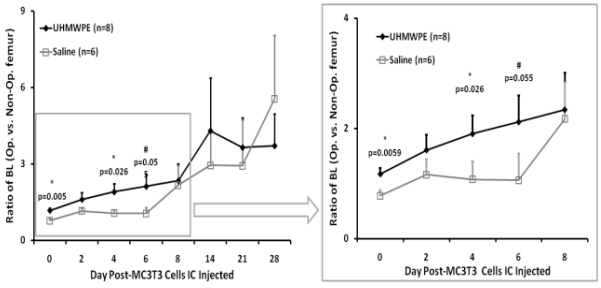


**Fig. 1**. UHMWPE particles induced significant MC3T3 cell migration from the systemic circulation (when the cells were introduced in the left heart) to the area where particles had been introduced. The ratio of BL from the operated femur divided by the non-operated contralateral femur indicated an increase of the bioluminescence in animals in which particles were infused conpared to saline controls. Right panel is enlarged from left panel to show days 0-8.

*Result 1*. UHMWPE particles infused into the femoral cavity of nude mice induced significant homing of reporter MC3T3 cells compared to controls (day 0 and day 4, a trend at day 6, Fig. 1). Background BL signals increased 1-week post cell injection, mainly from cells located in the kidneys, which led to distortion of the signal from femora.
*Result 2*. Reporter MSCs or MC3T3 injected intravenously are trapped in the pulmonary vasculature (Fig. 1C). These lung signals will stay for up to 5-7 days. When heparin was added to the cell suspension, 1 x $10^6$ cells can be injected through the tail vein satisfactorily, but more cells will cause severe pulmonary embolism (Table 1). Compared to intravenous injection, cells injected via the Carotid Artery, or left ventricle of heart can ensure systemic arterial cell distribution. Blind ICI was selected as the basic method for systemic delivery of MSCs or MC3T3. This method is quick and minimizes animal morbidity (Fig.2 A,B). and allows delivery of sufficient numbers of reporter cells into the arterial circulation in mice (Table 1).

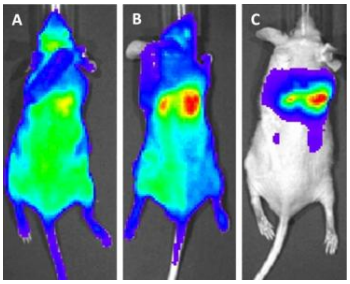


**Fig. 2**. Bioluminescent signals from mice with different delivery methods of MSCs or MC3T3 cells (5E6 cells/mouse) at day 0. A) successful IC injection (note widespread reporter signal) B) successful IC injection, but still have some cells are trapped in the lung; C) intravenously injected cells show pulmonary entrapment.

**Table 1**. Three methods used to deliver reporter MSCs or MC3T3 cells into the systemic circulation of the mouse.

| Cell delivery way | | Animal survive rate | |
|---|---|---|---|
| | | MC3T3 | MSC |
| Intravenous | Without heparin | 70% (1E6)* | 60% (1E6) |
| | With heparin | 100% (1E6)<br>0% (3E6) | 100% (1E6)<br>0% (2E6) |
| Intra-arterial | CCA | NA | NA |
| Intracardiac | Ultrasound guided | NA | NA |
| | blind | >90% (5E6) | >90% (2E6) |

CCA: common carotid artery; *: cell number injected; NA: not applicable.

**Discussion**:
Osteoprogenitor cells can be a powerful tool to regenerate damaged musculoskeletal tissues. Methods of systemic delivery of these cells are of great interest for diagnostic and therapeutic purposes. MSCs are relatively large cells that cannot easily pass through the capillary system in the lung. This pulmonary entrapment occurs with intravenous injection of MSCs and can have serious consequences. In this study, continuously infused UHMWPE particles induced potent systemic migration of osteoprogenitor cell MC3T3 cells delivered by IC injection. Also, we compared different delivery methods for MSCs and MC3T3 cells in the mouse; we eventually selected intracardiac injection for this in vivo study. This may be assisted by ultrasound, but this is cumbersome and expensive. Blind left ventricular injection of MSCs, when practiced and perfected can lead to successful introduction of cells into the arterial vasculature without extensive surgical dissection or special equipment. In this study, this technique was used to confirm systemic trafficking of MSCS to polymer particles.

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