Direct subcutaneous injection of polyethylene particles over the murine calvaria results in dramatic osteolysis

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Abstract

Purpose The murine calvarial model has been widely employed for the in vivo study of particle-induced osteolysis, the most frequent cause of aseptic loosening of total joint replacements. Classically, this model uses an open surgical technique in which polyethylene (PE) particles are directly spread over the calvarium for the induction of osteolysis. We evaluated a minimally invasive modification of the calvarial model by using a direct subcutaneous injection of PE particles.

Methods Polyethylene (PE) particles were injected subcutaneously over the calvaria of C57BL6J ten-week-old mice

(“injection” group) or were implanted after surgical exposure of the calvaria (“open” group) ($n=5/\text{group}$). For each group, five additional mice received no particles and served as controls. Particle-induced osteolysis was evaluated two weeks after the procedure using high-definition microCT imaging.

Results Polyethylene particle injection over the calvaria resulted in a $40\% \pm 1.8\%$ decrease in the bone volume fraction (BVF), compared to controls. Using the “open surgical technique”, the BVF decreased by $16\% \pm 3.8\%$ as compared to controls ($p < 0.0001$).

Conclusions Direct subcutaneous injection of PE particles over the murine calvaria produced more profound resorption of bone. Polyethylene particle implantation by injection is less invasive and reliably induces osteolysis to a greater degree than the open technique. This subcutaneous injection method will prove useful for repetitive injections of particles, and the assessment of potential local or systemic therapies.

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Introduction

Aseptic loosening is the main cause of failure of total joint replacements in the mid and long term [1–3]. Aseptic loosening appears as the end point of a complex biological response to wear particles generated by using the artificial joint [1–5]. Wear particles generally originate from the bearing couple, and have been identified as polyethylene (PE) in most conventional implants [6]. These particles initiate an inflammatory response driven by macrophages, resulting in chronic inflammation and resorption of the bone adjacent to the implant, defined as periprosthetic osteolysis [7]. This process is a function of wear of the PE component [1–3, 5], and often develops after extensive use of the

implant. It has been estimated that 8–20 % of patients develop periprosthetic osteolysis within ten years after a total joint replacement with conventional polyethylene [6].

To study the effects of wear particles on bone and the inflammatory cascade, numerous *in vitro* [1, 2, 4, 8, 9] and *in vivo* models have been developed [10]. *In vivo* models aim to closely simulate the relevant aspects of periprosthetic osteolysis in humans by using wear particles similar to those present in the periprosthetic environment. *In vivo* models also facilitate the modulation of the biological response to particles, by using various compositions of particles [11–13], potential therapeutic molecules [12, 14–17], or by using genetically modified animals [18–21]. Ideally, an animal model of particle-induced osteolysis should reproduce osteolysis observed in the clinical setting with comparable biological, histological, and radiological features [10, 22]. In addition, the model should be convenient, reproducible, cost-effective and yield the desired results in a timely manner. Mouse models used to evaluate particle-induced osteolysis include the air-pouch model, the calvarial model, load and non-load-bearing implants, and those associated with particle infusion pumps [10, 22–24]. Despite some drawbacks, including specific local biological and mechanical environment, the calvarial model fulfills most of the advantages of an *in vivo* model as listed above.

We postulated that particle administration by direct injection subcutaneously over the periosteum of the calvarium would induce a focal osteolytic response, comparable to the one observed using a conventional open surgical technique. Thus, we evaluated a polyethylene (PE) particle injection technique in comparison to a conventional open surgical technique, and evaluated subsequent osteolysis using a microCT imaging technique.

Methods

Animals

A total of 20 C57BL/6 male mice (Jackson Labs, Bar Harbor, ME) aged eight to ten weeks were housed and fed in our Animal Facility under local vivarium conditions (24 °C and 12 h/12 h light/dark cycle). Animals were housed in quarantine 48 hours prior to the experiment to allow for acclimatisation. The experimental design was approved by the Institutional Administration Panel for Laboratory Animal Care of our institution. We strictly followed university guidelines for care and use of laboratory animals. Four groups were maintained with two different particle implantation techniques, with five animals randomly assigned to each group: the “open surgery” groups with saline or PE particles administered by an open surgical technique and the “injection” groups with saline or PE

particles administered by subcutaneous injection over the calvarium. Animals were sacrificed two weeks after the procedure by cervical dislocation following isoflurane anaesthesia. Then, the calvaria were harvested and fixed in paraformaldehyde prior to micro-computed tomography (microCT) analysis.

Polyethylene particles

We used conventional ultra high molecular weight polyethylene (UHMWPE) particles (a gift from Dr. Timothy Wright, Hospital for Special Surgery, New York, NY) obtained from joint stimulator tests. The particles were isolated by density gradient centrifugation and sterilised by incubation with 95 % ethanol overnight. Then frozen aliquots of the particles containing serum were lyophilised for four to seven days. The dried material was digested in 5 M sodium hydroxide at 70 °C for two hours. The digested particle suspension was centrifuged through a 5 % sucrose gradient at 40 K rpm at 10 °C for three hours. The collected particles at the surface of the sucrose solution were ultrasonicated and centrifuged again through an isopropanol gradient (0.96 and 0.90 g/cm³) at 40 K rpm at 10 °C for one hour. The purified particles at the interface between the two layers of isopropanol were harvested and the isopropanol was evaporated from the particle mixture until dry. Particles were then re-suspended in 95 % ethanol, which was evaporated completely. Then, UHMWPE particles were washed in 70 % ethanol and tested for the presence of endotoxin using a Limulus Amoebocyte Lysate Kit (BioWhittaker, Walkersville, MD). Ultimately, particles were re-suspended in sterile phosphate buffered saline (PBS) prior to implantation. The mean diameter of the particles was 1.0±0.1 µm (mean±SE) measured by electron microscopy. The concentration of UHMWPE stock solution was 30 mg/ml, and using the density value of 0.94 g/ml, we calculated the appropriate volume to administer 4×10⁸ particles to the calvaria of each animal. Prior to use, the particle solution was sonicated on ice for one minute to homogenise the suspension. Previous studies of the calvarial model have used particle concentrations ranging from 10⁷ to 10²¹ in dry and suspended forms [12, 25–28]. This particle volume was chosen based on prior experimentation which showed an osteolytic effect after two weeks [29, 30].

Subcutaneous injection of particles

Animals were anaesthetised with 2–3 % isoflurane in 100 % oxygen at a flow rate of 1 L/min and were operated upon on a warm, small animal surgery station. A 1×1 cm² area of skin overlying the skull was carefully shaved in order to visualize the injection point and sterilised with Betadine Scrub® (Purdue Products, Stamford, CT). The injection point was located on the midline, one millimetre anterior to the line connecting the ears. A 25-gauge needle was used

to sterily inject 100 µl of saline or PE particle suspension contained in a 1-ml syringe, in five mice each. The needle was introduced subcutaneously over the calvarial bone, at a 45°-angle, until contact with the periosteum was obtained. The solution was then slowly injected in the subcutaneous space without any pressure, which resulted in a temporary subcutaneous bubble overlying the skull. Following the procedure, animals were returned to their cages until euthanasia.

Open surgical technique

The open surgical technique was employed as previously described [15, 21, 31–33]. Briefly, mice were operated upon under general anaesthesia via inhaled isoflurane 2–3 % at 1 L/min. A 0.5×0.5 cm² area of periosteum was exposed by making a one centimetre midline sagittal incision over the shaved and cleaned calvarium. The periosteum was preserved in all cases. Five animals received 100 µl of the PE particle suspension, and five animals received saline alone (100 µl) as a control. The incision was closed using 3-0 Ethylon sutures (Ethicon Inc., Somerville, NJ). Following the procedure, the animals were returned to their cages with water and food ad libitum until euthanasia.

MicroComputed tomography imaging

MicroComputed Tomography (microCT) Imaging was performed in the Small Animal Imaging Facility at our Institution. A microCT scan was performed ex vivo for all animal groups to detect changes in the bone volume (BV) relative to the total volume (TV), defined as the bone volume fraction (BVF), and compared to the controls. We used a phantom made of an epoxy-based resin, which mimics hydroxyapatite and contains water and air inclusion for calibration. Calvaria were placed vertically in the MicroCAT microCT scanner (ImTek, Inc, Knoxville, TN) with a 40-µm resolution and a 35-minute acquisition time. Before the procedure, scout images were made to confirm that the calvarial bone was entirely scanned. After scanning, we used the MicroCAT software (Imtek, Inc) for acquisition and COBRA Reconstruction interface software (Exxim computing corporation, Pleasanton, CA) for reconstruction. For BVF assessment, we used the MicroView software (GE Medical Systems, Waukesha, WI) and a 3D region of interest was created at the level of the parietal bones. A 6 mm × 2 mm ($d \times h$) cylindrical area of interest was created after threshold normalisation for all images. Information on the bone volume fraction was collected in the above-defined region of interest (Fig 1a).

Statistical analysis

All values are presented as mean±standard error of the mean (SEM). We used a one-way analysis of variance with

Newman-Keuls correction to assess for statistical significance between all four groups. We then used a two-tailed unpaired *t*-test to compare groups with their respective controls. A level of significance was set at $p < 0.05$. Data is presented as BVF for saline and PE treatment using the open surgical technique and injection technique. To compare the two techniques, we compared the BVF of the PE treated group relative to the saline treated group for each technique.

Results

MicroCT analysis revealed osteolytic lesions with both the “open” and “injection” methods on the frontal and parietal bones centred over the coronal suture at the intersection of the metopic and sagittal sutures (Fig. 1a,b). Using a one-way ANOVA, we found an overall significance level of $p < 0.0001$. Using the injection technique, we found that the bone volume fraction for the saline injected group was 0.13 ± 0.002 compared to the PE injected group 0.09 ± 0.003 ($p < 0.0001$) (Fig. 2a). Using the open surgical technique, we found that the bone volume fraction (BV/TV) in the saline treated group was 0.11 ± 0.002 compared to the PE particle treated group 0.08 ± 0.004 ($p = 0.0043$) (Fig. 2a). We found that using the open surgical technique, the ratio of the BVF for PE divided by saline treatment was 0.84 ± 0.037 . In contrast, using the injection technique, the ratio was 0.62 ± 0.018 , $p < 0.0001$ (Fig. 2b). This correlated to a 40 % decrease in the BVF by the injection technique, compared to a 16 % decrease by the conventional surgical technique ($p < 0.0001$).

Discussion

The purpose of this study was to investigate a novel, simple technique for the study of particle-induced osteolysis of the murine calvaria. We postulated that particle administration by direct injection would result in localised osteolysis, and in osteolysis comparable to the open surgical technique of PE particle implantation. Potential strengths of the injection method are that it is quick, less invasive, and potentially allows for re-injection of particles or biological compounds of interest. Animals can be injected repeatedly to more closely mimic the continuous production of wear particles in humans or a drug of interest could be injected. The model also permits the study of drugs or compounds that require a longer period of administration for modulation of osteolysis.

Traditionally, particle implantation on the murine calvaria has used an open surgical technique to place particles directly onto the calvaria [8, 15, 21, 23–25, 31, 32, 34–37]. Numerous versions of the murine calvarial model have been

Fig. 1 MicroCT analysis of murine calvaria treated with saline or polyethylene particles by a surgical or injection technique. **a** Polyethylene particle-induced osteolysis in C57BL6J mice treated with saline or polyethylene (PE) particles, by a surgical or injection technique, was assessed within the volume of interest by longitudinal 3D micro-computed

tomography (μ CT). The volume of interest is indicated by the *yellow shaded region* as a 6 mm $\times$ 2 mm ($d \times h$) cylinder. **b** Representative microCT images of calvaria treated with saline or PE particles administered by a surgical or injection technique. Both techniques were sufficient to induce an osteolytic reaction in response to PE particles

developed using different particle types [11–13, 33, 37, 38], either dry or diluted [26, 33, 39], different implantation techniques (preserving the periosteum or not), different strains of mice [18–21], and different times of sacrifice [16, 32, 35], all appearing to be effective in inducing osteolysis [15, 21, 31, 32]. However, the open surgical approach to particle administration carries

the risk of complications, mainly from infection, particle leakage out of the incision site, and possible inflammatory reactions due to surgery and sutures. In addition, the open surgical technique does not allow for multiple administrations of wear particles as occurs during constant wear particle generation in the clinical setting. The surgical technique also does not easily facilitate particle

Fig. 2 Graphical representation of μ CT quantifying the bone volume fraction (BVf) 14 days after PE particle implantation onto calvaria. **a** Using an open surgical technique and injection technique, we found that polyethylene treatment resulted in a significant reduction in the BVf compared to the saline control. **b** Comparison of the surgical technique

to the injection technique of polyethylene particle implantation showed that there was a 40 % decrease in the BVf by the injection technique, compared to only a 16 % decrease by the conventional surgical technique. * Indicates $p < 0.05$

administration with simultaneous delivery of potential therapeutic agents locally.

We first tested our hypothesis in a preliminary unpublished study using methylene blue dye injected subcutaneously over the periosteum compared to an open surgical technique. We found that the injection technique produced a more focal area of staining compared to the surgical technique. We initially tested multiple volumes for injection from 20–100 μ l and found that 100 μ l resulted in the greatest staining over the entire calvarium. After perfecting and validating the method, we proceeded to our study. This study reports that a subcutaneous injection of PE particles suspended in PBS produced a greater osteolytic response as evaluated by microCT after two weeks compared to the open surgical implantation. Possible explanations for these findings include a greater sustained particle load due to reduced leakage of particles in the injection technique compared to the open technique, and the addition of pressure with injection due to non-disruption of the soft tissues enclosing the particles. Pressure has been previously shown to produce osteolysis [17, 40–42].

Thus, the injection model allows for accurate implantation of a pre-determined quantity of particles, without leakage. However, care must be taken when using the injection method as well as the open method to adequately quantify the particles delivered. The injection model was able to elicit a more focal and profound area of polyethylene particle-induced osteolysis as assessed by the change in bone volume fraction by microCT imaging. Wedemeyer et al. showed that microCT is a reliable method for morphological analysis of particle-induced osteolysis as compared to histological evaluation [32]. Additionally, prior histological examination by this group using the injection technique has confirmed a significant decrease in the bone volume fraction by haematoxylin and eosin staining [30].

There are numerous strengths of the murine calvarial model as an *in vivo* model for the study of osteolysis. The calvarial model is simple and cost-effective, allows for the use of transgenic and knockout mice, is a rapid model for the development of osteolysis, and can be used to screen large numbers of compounds and doses [10]. Some deficiencies include the fact that the calvarium is a flat bone and not a long bone that sees significant load. The calvarium undergoes primary bone formation as opposed to endochondral ossification for long bones. Continuous infusion of particles to the calvarium is also difficult. The addition of an implant (in addition to particles) is difficult with the calvarial model. It is not possible to remove the periosteum with the injection technique, so studies that require the periosteum to be removed would not be possible. Additionally, clinically, the primary site of osteolysis is the endosteal surface of the metaphysis and diaphysis on the long bone and the cancellous bone region, which do not have a periosteal membrane. Also, the calvarial model is representative of an acute rather than a chronic inflammatory reaction to

particles, as occurs in the clinical setting. Finally, other non-biological factors that may contribute to osteolysis such as oscillatory fluid pressures or mechanical forces cannot be mimicked in the calvarial model [10].

In summary, this study presents a novel minimally invasive technique for modelling PE particle-induced osteolysis in the murine calvaria. The so-called “injection method” is simple, reliable and produced more profound osteolysis than the open surgical technique in our hands. In theory, the calvarial particle injection method may be used repeatedly for the injections of particles, and the assessment of potential local or systemic therapies.

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References

1. Wooley PH, Schwarz EM (2004) Aseptic loosening. *Gene Ther* 11(4):402–7
2. Purdue PE, Koulouvaris P, Nestor BJ, Sculco TP (2006) The central role of wear debris in periprosthetic osteolysis. *Hss J* 2(2):102–13
3. Goodman S (2005) Wear particulate and osteolysis. *Orthop Clin North Am* 36(1):41–8
4. Purdue PE, Koulouvaris P, Potter HG, Nestor BJ, Sculco TP (2007) The cellular and molecular biology of periprosthetic osteolysis. *Clin Orthop Relat Res* 454:251–61
5. Marshall A, Ries MD, Paprosky W (2008) How prevalent are implant wear and osteolysis, and how has the scope of osteolysis changed since 2000? *J Am Acad Orthop Surg* 16(Suppl 1):S1–6
6. Ma T, Goodman SB (2008) Biological effects of wear debris from joint arthroplasties. In: Wnek GE, Bowlin GL (eds) *Encyclopedia of biomaterials and biomedical engineering*. Informa Healthcare, London
7. Ingham E, Fisher J (2005) The role of macrophages in osteolysis of total joint replacement. *Biomaterials* 26(11):1271–86
8. Taki N, Tatro JM, Lowe R, Goldberg VM, Greenfield EM (2007) Comparison of the roles of IL-1, IL-6, and TNF α in cell culture and murine models of aseptic loosening. *Bone* 40(5):1276–83
9. Shimizu S, Okuda N, Kato N, Rittling SR, Okawa A, Shinomiya K, Muneta T, Denhardt DT, Noda M, Tsuji K et al (2010) Osteopontin deficiency impairs wear debris-induced osteolysis via regulation of cytokine secretion from murine macrophages. *Arthritis Rheum* 62(5):1329–37
10. Langlois J, Hamadouche M (2011) New animal models of wear-particle osteolysis. *Int Orthop* 35(2):245–51
11. von Knoch M, Jewison DE, Sibonga JD, Sprecher C, Morrey BF, Loer F, Berry DJ, Scully SP (2004) The effectiveness of polyethylene versus titanium particles in inducing osteolysis *in vivo*. *J Orthop Res* 22(2):237–43
12. Zhou X, Zhang C, Wang X, An B, Zhang P, Zhu Z (2012) Berberine inhibits lipopolysaccharide- and polyethylene particle-induced mouse calvarial osteolysis *in vivo*. *J Surg Res* 173(2):e47–52
13. Warashina H, Sakano S, Kitamura S, Yamauchi KI, Yamaguchi J, Ishiguro N, Hasegawa Y (2003) Biological reaction to alumina,

- zirconia, titanium and polyethylene particles implanted onto murine calvaria. *Biomaterials* 24(21):3655–61
14. Ren W, Wu B, Peng X, Mayton L, Yu D, Ren J, Chen BD, Wooley PH (2006) Erythromycin inhibits wear debris-induced inflammatory osteolysis in a murine model. *J Orthop Res* 24(2):280–90
 15. von Knoch M, Wedemeyer C, Pingsmann A, von Knoch F, Hilken G, Sprecher C, Henschke F, Barden B, Loer F (2005) The decrease of particle-induced osteolysis after a single dose of bisphosphonate. *Biomaterials* 26(14):1803–8
 16. Zhang W, Zhao H, Peng X, Cheng T, Zhang X (2011) Low-dose captopril inhibits wear debris-induced inflammatory osteolysis. *J Int Med Res* 39(3):798–804
 17. Johansson L, Edlund U, Fahlgren A, Aspenberg P (2009) Bone resorption induced by fluid flow. *J Biomech Eng* 131(9):094505
 18. Takahashi K, Onodera S, Tohyama H, Kwon HJ, Honma K, Yasuda K (2011) In vivo imaging of particle-induced inflammation and osteolysis in the calvariae of NF κ B/luciferase transgenic mice. *J Biomed Biotechnol* pii:727063
 19. Ren W, Wu B, Peng X, Hua J, Hao HN, Wooley PH (2006) Implant wear induces inflammation, but not osteoclastic bone resorption, in RANK(-/-) mice. *J Orthop Res* 24(8):1575–86
 20. von Knoch M, Jewison DE, Sibonga JD, Turner RT, Morrey BF, Loer F, Berry DJ, Scully SP (2004) Decrease in particle-induced osteolysis in obese (ob/ob) mice. *Biomaterials* 25(19):4675–81
 21. Wedemeyer C, Neuerburg C, Pfeiffer A, Heckeleei A, Bylski D, von Knoch F, Schinke T, Hilken G, Goshager G, von Knoch M et al (2007) Polyethylene particle-induced bone resorption in alpha-calcitonin gene-related peptide-deficient mice. *J Bone Miner Res* 22(7):1011–9
 22. Schwarz EM, Benz EB, Lu AP, Goater JJ, Mollano AV, Rosier RN, Puzas JE, O'Keefe RJ (2000) Quantitative small-animal surrogate to evaluate drug efficacy in preventing wear debris-induced osteolysis. *J Orthop Res* 18(6):849–55
 23. Merkel KD, Erdmann JM, McHugh KP, Abu-Amer Y, Ross FP, Teitelbaum SL (1999) Tumor necrosis factor-alpha mediates orthopedic implant osteolysis. *Am J Pathol* 154(1):203–10
 24. Schwarz EM, Lu AP, Goater JJ, Benz EB, Kollias G, Rosier RN, Puzas JE, O'Keefe RJ (2000) Tumor necrosis factor-alpha/nuclear transcription factor-kappaB signaling in periprosthetic osteolysis. *J Orthop Res* 18(3):472–80
 25. Greenfield EM, Tatro JM, Smith MV, Schnaser EA, Wu D (2011) PI3Kgamma deletion reduces variability in the in vivo osteolytic response induced by orthopaedic wear particles. *J Orthop Res* 29(11):1649–53
 26. Nich C, Langlois J, Marchadier A, Vidal C, Cohen-Solal M, Petite H, Hamadouche M (2011) Oestrogen deficiency modulates particle-induced osteolysis. *Arthritis Res Ther* 13(3):R100
 27. Kauter MD, Bachmann HS, Neuerburg L, Broecker-Preuss M, Hilken G, Grabellus F, Koehler G, von Knoch M, Wedemeyer C (2011) Calcitonin substitution in calcitonin deficiency reduces particle-induced osteolysis. *BMC Musculoskelet Disord* 12:186
 28. Landgraaber S, Jaeckel S, Loer F, Wedemeyer C, Hilken G, Canbay A, Totsch M, von Knoch M (2009) Pan-caspase inhibition suppresses polyethylene particle-induced osteolysis. *Apoptosis* 14(2):173–81
 29. Nich C, Rao AJ, Valladares RD, Li C, Christman JE, Antonios JK, Yao Z, Zwillingenberger S, Petite H, Hamadouche M et al (2013) Role of direct estrogen receptor signaling in wear particle-induced osteolysis. *Biomaterials* 34(3):641–50
 30. Rao AJ, Nich C, Dhulipala LS, Gibon E, Valladares R, Zwillingenberger S, Smith RL, Goodman SB (2012) Local effect of IL-4 delivery on polyethylene particle induced osteolysis in the murine calvarium. *J Biomed Mater Res A*. Dec 5. doi:10.1002/jbm.a.34486
 31. von Knoch F, Heckeleei A, Wedemeyer C, Saxler G, Hilken G, Brankamp J, Sterner T, Landgraaber S, Henschke F, Loer F et al (2005) Suppression of polyethylene particle-induced osteolysis by exogenous osteoprotegerin. *J Biomed Mater Res A* 75(2):288–94
 32. Wedemeyer C, Xu J, Neuerburg C, Landgraaber S, Malyar NM, von Knoch F, Goshager G, von Knoch M, Loer F, Saxler G (2007) Particle-induced osteolysis in three-dimensional micro-computed tomography. *Calcif Tissue Int* 81(5):394–402
 33. Pearl JJ, Ma T, Irani AR, Huang Z, Robinson WH, Smith RL, Goodman SB (2011) Role of the Toll-like receptor pathway in the recognition of orthopedic implant wear-debris particles. *Biomaterials* 32(24):5535–42
 34. Taki N, Tatro JM, Nalepka JL, Togawa D, Goldberg VM, Rimnac CM, Greenfield EM (2005) Polyethylene and titanium particles induce osteolysis by similar, lymphocyte-independent, mechanisms. *J Orthop Res* 23(2):376–83
 35. Tatro JM, Taki N, Islam AS, Goldberg VM, Rimnac CM, Doerschuk CM, Stewart MC, Greenfield EM (2007) The balance between endotoxin accumulation and clearance during particle-induced osteolysis in murine calvaria. *J Orthop Res* 25(3):361–9
 36. Bi Y, Nielsen KL, Kilts TM, Yoon A, Kardsal MA, Wimer HF, Greenfield EM, Heegaard AM, Young MF (2006) Biglycan deficiency increases osteoclast differentiation and activity due to defective osteoblasts. *Bone* 38(6):778–86
 37. Kaar SG, Ragab AA, Kaye SJ, Kilic BA, Jinno T, Goldberg VM, Bi Y, Stewart MC, Carter JR, Greenfield EM (2001) Rapid repair of titanium particle-induced osteolysis is dramatically reduced in aged mice. *J Orthop Res* 19(2):171–8
 38. Ren K, Purdue PE, Burton L, Quan LD, Fehring EV, Thiele GM, Goldring SR, Wang D (2011) Early detection and treatment of wear particle-induced inflammation and bone loss in a mouse calvarial osteolysis model using HEMA copolymer conjugates. *Mol Pharm* 8(4):1043–51
 39. Zhang C, Tang T, Ren W, Zhang X, Dai K (2008) Influence of mouse genetic background on wear particle-induced in vivo inflammatory osteolysis. *Inflamm Res* 57(5):211–5
 40. Skoglund B, Aspenberg P (2003) PMMA particles and pressure—a study of the osteolytic properties of two agents proposed to cause prosthetic loosening. *J Orthop Res* 21(2):196–201
 41. Fahlgren A, Bostrom MP, Yang X, Johansson L, Edlund U, Agholme F, Aspenberg P (2010) Fluid pressure and flow as a cause of bone resorption. *Acta Orthop* 81(4):508–16
 42. Johansson L, Edlund U, Fahlgren A, Aspenberg P (2011) Fluid-induced osteolysis: modelling and experiments. *Comput Methods Biomech Biomed Engin* 14(4):305–18