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# The effect of simvastatin on polyethylene particle-induced osteolysis ☆

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**Abstract**

This study aimed to investigate the effects of the HMG-CoA reductase inhibitor simvastatin on ultra-high molecular weight polyethylene (UHMWPE) particle-induced osteolysis.

The murine calvarial osteolysis model was used in 21 C57BL/J6 mice randomized to three groups. Group I underwent sham surgery only, group II received UHMWPE particles, and group III, particles and simvastatin treatment. After two weeks, calvaria were processed for histomorphometry. Bone resorption was measured as resorption within the midline suture using Giemsa staining. Osteoclast numbers were determined per high-power field using TRAP-staining. Statistical analysis was performed using one-way ANOVA and Student's *t*-test.

Bone resorption in midline suture was $0.094 \pm 0.007\,mm^2$ in sham controls (group I), $0.25 \pm 0.025\,mm^2$ after particle implantation without further intervention (group II), and $0.131 \pm 0.02\,mm^2$ with particle implantation and additional simvastatin treatment (group III) ($p = 0.00003$). Osteoclast numbers were $15.3 \pm 3.6$ in group I, $48.7 \pm 7.1$ in group II and $6.2 \pm 3.1$ in group III ($p = 0.00002$).

In conclusion, simvastatin treatment markedly decreased UHMWPE particle-induced osteolysis in a murine calvarial model. This finding suggests that simvastatin may have a role for noninvasive prevention and treatment of wear debris-mediated periprosthetic osteolysis after total joint arthroplasty.


*Keywords:* Osteolysis; Wear debris; Polyethylene; Calvarium; Osteoclast

## 1. Introduction

Periprosthetic osteolysis with subsequent aseptic loosening remains the most frequent and relevant long-term complication associated with total hip arthroplasty (THA) [1–3]. It is well understood that particulate wear debris such as ultra-high molecular weight polyethylene (UHMWPE) particles from the articulating surfaces induce an activation of phagocytic cells at the bone–implant interface which initiates an osteolytic cascade involving peri-prosthetic granulomatous inflammation and subsequent osteoclastic bone resorption [1–6]. Enormous efforts have been directed at preventing osteolysis, including development of improved bearing materials for example crosslinked UHWMPE and alternative articulations [7–9].

In the face of these challenges to THA surgery, noninvasive treatment alternatives are becoming

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increasingly desirable both for prevention and treatment of periprosthetic osteolysis. An increasing understanding of the molecular mechanisms behind wear debris-induced osteolysis has opened up potential pharmacological interventions [10–12]. Recently, the effects of bisphosphonates on particle-induced osteoclastic bone loss with subsequent aseptic loosening of total joint prostheses have been evaluated since the key pharmacologic action of bisphosphonates is the inhibition of osteoclastic bone resorption. Shanbhag et al. have demonstrated that oral alendronate treatment can effectively inhibit particulate debris-mediated bone resorption in a canine THA model [13]. Our group recently introduced a murine calvarial model of polyethylene particle-induced osteolysis [14]. Utilizing this model, we showed that the third-generation bisphosphonate zoledronate can significantly decrease particle-induced bone resorption [15]. These findings are supported by recent clinical studies which report beneficial effects of bisphosphonate in improving fixation and durability of total joint replacements in humans [16,17].

The pharmalogical mechanism of action in aminobisphosphonates relies on interference with the mevalonate pathway by inhibiting the farnesyl pyrophosphate (FPP) synthase enzyme ultimately resulting in the inhibition of osteoclast formation and osteoclast function [12,18]. Interestingly, statins, another class of drugs, act also on the mevalonate pathway by inhibiting the more upstream 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in hepatic cholesterol biosynthesis [20]. As a consequence, statins are clinical widely prescribed to reduce serum cholesterol levels and the ultimate risk of heart attacks [19]. There are convincing lines of evidence that statins also cause effects on bone metabolism. Mundy et al. first demonstrated that statins such as simvastatin, and lovastatin, are capable of increasing osteoblastic bone formation both in vitro as well as in vivo [20]. Further, it has been reported that statins can also suppress osteoclastic bone resorption in vitro [12,21,22] as well as in vivo [20].

In consideration of these findings, we have hypothesized that statins, similar to bisphosphonates, could suppress particle-induced osteolysis through effects on osteoclastic bone resorption. This study aimed to investigate the effects of simvastatin on UHMWPE particle-induced osteolysis in a murine calvarial model.

## 2. Materials and methods

### 2.1. Study design

A murine calvarial model of UHMWPE particle-induced osteolysis, recently introduced by our group [14] and based on the original model of calvarial osteolysis [23], was applied in 21 healthy 14-week-old female and male C57BL/J6 mice in accordance with the official guidelines and following approval by the University and the local government. Animals were equally randomized to three groups. In group I animals underwent sham surgery only, in groups II and III animals were treated with UHMWPE particles (about $6 \times 10^6$ particles per animal), with animals from group III receiving additional simvastatin (Zocor, Merck, Rahway, NJ). Simvastatin was administered through a diet prepared professionally (Altromin 1324, Altromin, Lage, Germany), so that each mouse received a daily dose of ~120 mg/kg of body weight from day 0 until sacrifice. An average dose of simvastatin per mouse could be determined since the diet consumed was measured on day 5, 10 and 14, and the mice were weighed at surgery and time of death. This dose regime and application mode was based on a dosage successfully applied in a previous study on fracture healing [24]. Animals from group I and II received the same diet except for simvastatin supplementation. The group size of seven was determined in a power analysis indicating that for a two-sided test at alpha = 0.05 there was 85% power to detect a significant difference in the groups.

### 2.2. Particles

Commercially pure UHMWPE polyethylene particles were obtained from the manufacturer (Ceridust VP 3610, Clariant, Gersthofen, Germany). The particle size was described by the manufacturer as 50% of the particles being smaller than 5 μm and 90% smaller than 9 μm. The particle size was confirmed using size-independent morphological description [25]. The samples were coated with 10 nm gold/palladium (Au/Pd 80/20%) to enable detection in the scanning electron microscope (Hitachi FESEM S-4100, Hitachi, Kyoto, Japan) (Fig. 1). The area and perimeter of approximately 2000 particles were measured with PC-Image (Version 2.2.03, Foster Findlay Associates Ltd, Newcastle upon Tyne, UK) and calculated in Excel (Version 2000, Microsoft, NY, USA). The mean particle size (given as equivalent circle diameter) was $1.74 \pm 1.43$ μm (range 0.05–11.06) with more than 34% of the particles smaller than 1 μm.

The particles were processed under sterile conditions under a cell culture hood and meticulous care was taken to avoid contamination with endotoxins. The particles were washed in 70 percent ethanol for 48 h (ethyl alcohol, absolute, 200 proof, for molecular biology, Aldrich Co., Inc., Milwaukee, WI) at room temperature using a rocking device (Thermal Rocker, Lab Line Instruments, In., Melrose Park, IL). This treatment resulted in negative testing for endotoxin using a quantitative Limulus Amebocyte Lysate (LAL) Assay