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# Polyethylene wear particles do not induce inflammation or gelatinase (MMP-2 and MMP-9) activity in fibrous tissue interfaces of loosening total hip arthroplasties

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ABSTRACT

*In vitro* and *in vivo* studies have suggested that polyethylene wear particles are the main cause for osteolysis in prosthetic loosening. Elevated amounts of proteases including gelatinases (or matrix metalloproteinases MMP-2 and MMP-9) have been found in fibrous tissue interfaces of loosened total hip arthroplasties suggesting that proteolysis plays a role in osteolysis. The presence of proteases does not mean that they are active, because activity of proteases is highly regulated at the post-translational level. We investigated whether the activity of two major proteases that are active extracellularly and have been associated with loosening, MMP-2 and MMP-9, is involved in loosening of non-cemented hip implants with polyethylene acetabular components. Eight interface tissues retrieved during revision were studied with light and electron microscopy and by *in situ* zymography to localize MMP-2 and MMP-9 activity in combination with immunohistochemistry to localize MMP-2 and MMP-9 proteins. All interface tissues contained large amounts of polyethylene wear particles, either in large accumulations or dispersed in the extracellular matrix or intracellularly in fibroblasts. Particles were not encountered in association with MMP-2 or MMP-9 activity or leukocytes. Inflammation was never found. MMP-9 activity was restricted to macrophages and MMP-2 activity was restricted to microvascular endothelial cells mainly outside areas where particles were present. Our data indicate that wear particles do not induce activation of leukocytes or MMP-2 or MMP-9 activity. Therefore, aseptic loosening may not be particle induced but initiated by other mechanisms such as mechanical stress.



## Introduction

Processes leading to aseptic loosening of hip prostheses have not yet been completely unraveled (Purdue et al., 2006). Osteolysis has been suggested to be wear particle induced, especially wear particles of the polyethylene acetabular components (Harris et al., 1976; Goldring et al., 1986; Howie et al., 1993; Mjoberg, 1994). Polyethylene particles are considered to cause an inflammatory reaction and have been found to activate macrophages *in vitro* (Davis et al., 1993; Archibeck et al., 2001) and *in vivo* (Ingham and Fisher, 2005; Purdue et al., 2006). *In vivo* animal studies have indicated that osteoclastic bone resorption is induced by activated macrophages (Howie et al., 1993; Kim et al., 1998). On the other hand, studies in animal models have shown reduced bone formation only in the presence of polyethylene wear particles, suggesting that osteolysis is not merely induced by an inflammatory reaction to wear particles (Van der Vis et al., 1997; Aspenberg and Van der Vis, 1998a,b; Waris et al., 2004). Takagi et al. (2001) also found that defective bone formation may contribute to loosening of hip protheses. Localization in relation to osteolysis in aseptic loosening of hip protheses has been performed with regard to many proteolytic enzymes such as: MMP-1, -2, -3, -9, -10, -12 and -13, and membrane type 1 (MT-1)-MMP (Takagi et al., 1994a,b, 1995c; Hembry et al., 1995; Yokohama et al., 1995; Nawrocki et al., 1999; Takei et al., 1999; Wagner et al., 2008), the cysteine proteinases cathepsin B (Schuller et al., 1993), cathepsin K (Kontinnen et al., 2005), cathepsin L (Kido et al., 2007), and the serine proteinases cathepsin G (Takagi et al., 1995a), elastase (Takagi et al., 1995b), urokinase-type and tissue-type plasminogen activators (Diehl et al., 2004) either or not in relation to their endogenous inhibitors (Takagi et al., 1998a,b). All these studies, except that of Schuller et al. (1993) on cathepsin B, localized the proteases in fibrous tissue interfaces obtained at revision surgery at the mRNA level (*in situ* hybridization or PCR) or at the protein level

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**Table 1**
List of total hip revision patients.

| Case | Gender | Age (years) | Type of fixation | Revised components | Time to revision (years) |
|---|---|---|---|---|---|
| 1 | F | 86 | Cemented | Stem | 11 |
| 2 | F | 90 | Cemented | Both | 20 |
| 3 | F | 77 | Cemented | Both | 16 |
| 4 | F | 76 | Cemented | Both | 6 |
| 5 | F | 73 | Cemented | Stem | 4 |
| 6 | M | 63 | Cemented | Both | 15 |
| 7 | M | 54 | Cementless | Both | 11 |
| 8 | M | 56 | Cementless | Stem | 2 |
| | Mean | 72 | | Mean | 11 |

(immunohistochemistry, ELISA). Activity was determined only in tissue homogenates (Takagi et al., 1998a,b) thus providing data on activity without a relation to specific cell types or processes such as osteolysis.

Activity of many enzymes and particularly of proteases is highly regulated at the post-translational level (Nawrocki et al., 1999; Boonacker and Van Noorden, 2001; Van Noorden, 2009, 2010). Therefore, localization of mRNA or protein of proteolytic enzymes in tissue sections or the determination of activity in tissue homogenates is of limited value (Boonacker et al., 2004) for understanding the exact role of proteolytic enzymes in loosening of total hip arthroplasties. *In situ* localization of activity of proteases, in association with osteolysis, can give a better insight on their exact role in the process of loosening of artificial joints (Baruch et al., 2004; Bogyo and Cravatt, 2007; Van Noorden, 2009, 2010).

Therefore, activity of two proteases frequently associated with proteolysis in pathologies, the gelatinases or MMP-2 and MMP-9 (Takagi et al., 1994a,b, 1998a,b; Nawrocki et al., 1999; Mook et al., 2004), was analyzed histochemically using *in situ* zymography in interface tissue retrieved during revision of loosened femoral components in relation to the presence of wear particles and macrophages as well as periprosthetic osteolysis.

## Material and methods

### Sampling

After removal of loosened femoral components, the soft tissue interpositioned between the cement/prosthesis and bone was collected during revision surgery of 8 failed total hip arthroplasties of 3 male and 5 female patients. All prostheses had a metal/ceramic head and polyethylene socket joint and an either cemented or non-cemented femoral component. Septic loosening was ruled out by several means, using the erythrocyte sedimentation rate, determination of the C-reactive protein levels, macroscopic examination, tissue culturing and joint aspirates. The mean age of the patients was 72 years and the mean time between implantation and revision was 11 years. All patients were primarily operated for osteoarthritis of the hip (Table 1). Tissue samples were frozen in liquid nitrogen and then kept in a storage freezer at −80 °C until used. Consent of individual patients for this specific project was waivered by the ethics committee of the Academic Medical Center because research was performed on 'waste' material, stored in a coded fashion.

### Histochemical analysis

Cryostat sections (8 μm thick) were cut at −25 °C for histochemical, immunohistochemical and *in situ* zymography staining. Sections were dried for 30 min at room temperature and either fixed for 30 min at room temperature in 4% paraformaldehyde (Merck, Darmstadt, Germany) in phosphate-buffered saline (PBS) and rinsed in distilled water, or fixed for 12 min in acetone at 4 °C and dried for 10 min at room temperature. For orientation in the fibrous tissue interfaces, sections were stained with a Giemsa solution (Merck) and subsequently rinsed in distilled water, ethanol, and xylene. Then, sections were mounted in Euparal (Chroma, Stuttgart, Germany). Serial sections were used for immunohistochemistry and *in situ* zymography.

Immunohistochemical staining was performed according to Mook et al. (2003) to analyze the cellular composition of the fibrous tissue interfaces. Fibroblasts were localized with the ASO2 primary antibody (dilution, 1:400; Dianova, Hamburg, Germany), monocytes with the anti-CD64 primary antibody (dilution, 1:400; Sanquin, Amsterdam, The Netherlands), macrophages with the anti-CD68 primary antibody (dilution, 1:100; Dako, Glostrup, Denmark), B cells with the anti-CD20 antibody (dilution, 1:10; Sanquin), and T cells with the anti-CD3 antibody (dilution, 1:100; BD, Mountain View, CA, USA). For negative controls, sections were incubated with mouse IgG (dilution, 1:1000; Dako) instead of the primary monoclonal antibody.

Air-dried sections were rinsed three times in PBS containing 1% (w/v) fetal calf serum (FCS; Hyclone, Logan, UT, USA). Then, sections were incubated with primary antibody dissolved in PBS for 2 h at room temperature and rinsed again three times in PBS containing 1% FCS and incubated with rabbit anti-mouse antibodies conjugated with horseradish peroxidase (dilution, 1:50; Dako) for 60 min at room temp. Sections were rinsed again three times in PBS containing 1% FCS and peroxidase activity was visualized by incubation for 10 min at room temp in a solution containing 1 mM 3-amino-9-ethylcarbazole (AEC; Sigma–Aldrich, St. Louis, MO, USA), 5% (v/v) dimethylformamide, 0.05% (v/v) hydrogen peroxide (Merck) and 50 mM acetate buffer (pH 4.9). AEC was dissolved first in dimethylformamide. Hydrogen peroxide was added to the solution immediately before incubation. After incubation, sections were rinsed in distilled water, and nuclei were counterstained with a hematoxylin solution for 3 s. After rinsing in tap water and finally distilled water, sections were mounted in glycerin–gelatin.

MMP-2 and MMP-9 were localized immunohistochemically (Mook et al., 2003). Primary monoclonal antibodies were anti-human MMP-2 and MMP-9 (each in dilution 1:400; Neomarkers; Fremont, CA, USA). The immunohistochemical procedure was the same as described above for the detection of specific cell types.

Gelatinase activity was localized by *in situ* zymography as described by Mook et al. (2003) and Frederiks and Mook (2004). Unfixed cryostat sections were dried for 30 min at room temperature. The substrate dye quenched (DQ)-gelatin (Molecular Probes, Leiden, The Netherlands) was dissolved in agarose solution and poured onto sections. After 60 min incubation at room temp, fluorescein isothiocyanide (FITC) fluorescence was present at sites of gelatinase activity. Control incubations were performed by adding 20 mM EDTA to the DQ-gelatin solution, which inhibits activity of MMPs (Mook et al., 2003).

Digital photomicrographs were made using standard light and fluorescence microscopy.