Chemical eluates from ultra-high molecular weight polyethylene and fibroblast proliferation

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ABSTRACT

Although polyethylene wear particles have been implicated in osteolysis and implant loosening, this study is the first to test whether chemical eluates extracted from ultra-high molecular weight polyethylene (UHMWPE) could also be involved in this process. Eluates were prepared from UHMWPE bar stock and examined for their effects on 3H-thymidine incorporation by human foreskin fibroblasts grown in 96-well culture plates. Low concentrations of eluates stimulated 3H-thymidine uptake; whereas, high concentrations inhibited uptake in a dose-dependent manner. Maximum inhibition of proliferation for eluates (87 ± 0.03% inhibition, n = 45 paired wells) was greater than that observed for particles (54 ± 0.07% inhibition, n = 45 paired wells). Ethylene oxide sterilization of UHMWPE reduced 3H-thymidine uptake at low eluate concentrations relative to sterilization by gamma-irradiation. It was concluded that leachable eluates from UHMWPE implants contribute to the osteolytic process at the bone-implant interface.

Key Words: polyethylene, fibroblast, proliferation, eluate, osteolysis

INTRODUCTION

Osteolysis is a pathological process characterized by an aggressive, progressive, three dimensional resorption of trabecular bone and endocortex immediately adjacent to a prosthesis (Buechel et al. 1994; Harris 1994; Kadoya et al. 1998). Consistent identification of particulate debris at sites of osteolysis has led investigators to consider the physical effects of metallic, polymethylmethacrylate (PMMA) cement, and polyethylene wear debris on bone resorption and loosening (Harris 1994; Kadoya et al. 1998). Metallic debris has been found in areas of osteolysis around loose prostheses, and its role in the lytic process and loosening has been investigated along with that of
PMMA cement particles (Glant et al. 1993; Haynes et al. 1993; Maloney et al. 1993). With the advent of cementless arthroplasty, attention has focused on polyethylene particles, also shown to accumulate in areas of osteolysis around both loose and stable components (Ingham and Fisher 2000; McKellop et al. 1995; Murray and Rushton 1990).

Studies on inflammatory cell involvement in the osteolytic process have implicated macrophages, monocytes, osteoblasts, osteoclasts, foreign body giant cells, and fibroblasts in the process (Murray and Rushton 1990; Howie et al. 1993; Lassus et al. 1998). These studies indicate that macrophages and monocytes phagocytose particulate debris, become activated, and release a number of cytokines and proteolytic enzymes that contribute to bone resorption in vivo and in vitro (Glant et al. 1993; Jiranek et al. 1993; Lassus et al. 1998). Fibroblasts have also been implicated in the osteolytic process, regulating their own proliferation and that of other inflammatory cells, including macrophages and osteoclasts (Hogaboam et al. 1998; Lind et al. 1998). In the past, some studies have been conducted on the effect of artificially produced or retrieved particles from the synovial fluid of failed implants on fibroblast activity in vitro (Mostardi et al. 1997; Shanbhag et al. 1997; Vale et al. 1997). It seems likely that both the fibroblastic phenotype and the macrophage/osteoclast phenotype play a critical role in the loosening process. The thickened fibrous membrane that forms at the bone implant interface contains fibroblasts, macrophages, and osteoclasts as its main cellular constituents (Jiranek et al. 1993; Kadoya et al. 1998), and polyethylene wear particles are also known to accumulate in this membrane.

Although researchers have focused on the effect of phagocytosed wear particles on inflammatory cell activities, it is possible that UHMWPE particles induce osteolysis through a diffusible, chemical eluate, and that both the physical and chemical effects may independently lead to osteolysis. There is evidence from other studies that eluates from plastics leach out and are cytotoxic to cells in surrounding tissues (Northup 1989). Although leachable chemical eluates from UHMWPE may be involved in the osteolytic process, no studies have directly tested this hypothesis. In addition, there is evidence to suggest that the method used to sterilize implants may generate chemical substances that can affect cell viability in the region of the implant (Costa et al. 1998).

The aim of the present study was to test the effects of UHMWPE particles on human fibroblasts in culture and to investigate the possibility that leachable substances from UHMWPE components contribute to the osteolytic process. This is the first reported study to directly test the effects of soluble eluates of UHMWPE on cells known to participate in the osteolytic process in failed joint transplants.

MATERIALS AND METHOD

Preparation Of UHMWPE Slices

Medical grade UHMWPE in North America is manufactured according to ASTM (American Society for Testing and Materials) F-648 standards. The most common medical grade UHMWPE used is 415. Recently Hylamer (DePuy — Du Pont Orthopaedics, Warsaw, IN) has been marketed as an upgrade of 415. A similar regulatory body in Europe, the International Standards Organisation (ISO), controls UHMWPE production there. The most commonly used ISO grade of polyethylene in Europe is RCH1000. Five grades of UHMWPE were tested in the present study: 415, Hylamer, RCH1000, 1900CM, and 412. Slices of UHMWPE measuring 25 mm long, 16 mm wide, and either 4 µm or 30 µm thick were cut. The slices were washed with Milli-Q water (Milli RQ, Millipore Corporation, Bedford, MA) and sterilized by either ethylene oxide (ETO) or gamma irradiation, followed by aeration for 7 to 10 days before use.

Preparation Of UHMWPE Particles

UHMWPE particles were prepared in an artificial hip simulator consisting of a 32 mm diameter stainless steel head (provided by Synthes & Protek (NZ) Ltd, Auckland, NZ) articulated with a polyethylene cup. The movement of the hip simulator was by flexion and extension, with a small component of rotation (5 degrees), such that the resultant motion of the ball against the cup was linear. This motion is slightly different from the cross-path motion experienced by a hip arthroplasty in vivo. Two weights on the simulator produced a static axial load of 490 Newtons, and the vertical movements of the metal blocks at 70 cycles/ min generated an impact load of approximately 650 Newtons. An electrical fan was used to maintain the hip simulator at room temperature. The simulator was run continuously for 13 days (1.3 million cycles). This is approximately equivalent to a person walking for 16 months. The arthroplasty components of the hip simulator were enclosed in a non-toxic silicon bellows containing 100 ml sterile Medium 199 (Gibco BRL, Grand Island, NY) containing 10% fetal calf serum (FCS) (Gibco) and 136 mg/L penicillin plus 117 mg/L streptomycin. Greater than 95% of the particles
produced in the simulator were 5 µm in diameter or less, although particles of less than 1.4 µm were below the limit of detection of the Coulter Counter used for determining size. Particles were prepared in the hip simulator from three different UHMWPE cups (1900CM, 412, and 415) and diluted as appropriate with standard culture medium consisting of RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 10% fetal calf serum (FCS) (Gibco), 136 mg/L penicillin, and 117 mg/L streptomycin. Particle suspensions were sonicated in a ultra-sonicator bath for 1 min before being tested for their effects on fibroblast activity.

Preparation Of UHMWPE Conditioned Medium

A single slice of three different grades of UHMWPE (415, Hylamer, and RCH1000) was incubated for 72 hr at 37°C with 3 ml of standard culture medium. Low concentration eluates were prepared by extracting 4 µm thick slices without agitation in plastic culture dishes. High concentration eluates were prepared from 30 µm thick slices using a shaking incubator (New Brunswick Scientific Co., Model G24, New Brunswick, NJ) set at moderate shaking speed for the whole of the 72-hr incubation. In the shaking incubator, the UHMWPE surface was continually subjected to new medium, thus promoting maximal extraction of eluates from the bar stock. The shaking speed was kept low enough to prevent excessive surface agitation in order to minimise oxidation of the UHMWPE. After incubation, the slices were removed and the UHMWPE ‘conditioned medium’ was collected for subsequent testing. The conditioned medium was filtered through a 0.2 µm membrane filter (Millipore Corporation, Bedford, MA) to ensure that no UHMWPE particles were inadvertently included in the samples.

Fibroblast Cell Culture

A human foreskin fibroblast cell line, kindly provided by the New Zealand Communicable Disease Centre (Porirua, New Zealand), was established in culture flasks in standard culture medium (RPMI 1640 plus 10% FCS plus antibiotics). The cells were cultured at 37°C in a humidified 5% CO₂ incubator until the cells were near confluence as monitored by phase contrast microscopy. The cells were then trypsinized, seeded into 96-well culture plates at a concentration of 2.5 × 10⁵ cells/well and incubated for 16 hr. Fibroblast viability was assessed by 0.2% trypan blue exclusion, and cell number was determined in a haemocytometer. The cells were used only if viability was 98% or greater.

At the end of a 16-hr pre-incubation, the medium was removed by aspiration. For UHMWPE particle toxicity, 40 µl of particle suspension collected from a hip simulator was added to each test well of a 96-well plate. Paired control wells received 40 µl of standard culture medium. All wells were brought up to 200 µl final volume with standard culture medium. Different concentrations of particles from three different UHMWPE stocks were tested for their effects on ³H-thymidine uptake by the cells.

For tests of the effects of UHMWPE eluates on fibroblast proliferation, three different volumes of UHMWPE conditioned medium (50 µl, 100 µl or 200 µl) were added to the test wells of a 96-well plate, and the final volume of each well was brought up to 200 µl with standard culture medium. Paired control wells were prepared in the same way but with medium in which the ‘conditioning’ procedure had been carried out in the absence of UHMWPE slices. Negative controls were set up by adding 5% formalin to the medium. The 96-well plate was then incubated for 72-hr at 37°C in a humidified 5% CO₂ incubator. After exposure of the fibroblasts to UHMWPE eluates, the cells were examined for morphological changes by phase contrast microscopy.

³H-Thymidine Uptake Assay

After 3 days exposure to UHMWPE particles or eluates in culture, fibroblasts in each well of a 96-well plate were pulsed for 3 hours with 5 µl/well (0.5 µCi/well) of ³H-thymidine (Amersham Laboratories, Buckinghamshire, England). After the 3-hr incubation at 37°C, the attached cells were washed twice with 200 µl of ice cold 1.5% perchloric acid to remove unincorporated isotope. Finally, 150 µl of 5% perchloric acid was added to each well and the plates were incubated at 60°C for one hour. Once the plates had cooled down, 100 µl of the lysate was transferred from each well into a vial containing 1 ml of scintillation fluid, and radioactivity was determined in a Beckman LS3801 scintillation counter (Beckman Instruments, Fullerton, CA). Fibroblast proliferation was calculated from the amount of ³H-thymidine uptake into acid-insoluble DNA (Nacey et al. 1986) and a test/control ratio was determined. All uptake measurements were corrected for background uptake in the presence of 5% H₂O₂ (<5% of total control uptake).

Statistics

Particles and eluates from each grade of UHMWPE were tested three times (three culture preparations) with a minimum of 15 replicate paired wells (test and control) in each preparation (total n = 45). This experimental design had an 80% power to find a
difference of 10% between the test and the control. The logarithms of the ratio of test to control were analysed for statistical significance by analysis of variance (ANOVA). UHMWPE types, eluate or particle concentrations, and sterilization methods were the variables used in the statistical analyses.

RESULTS

UHMWPE Particle Effects on Fibroblast Proliferation

The effects of exposure of fibroblasts to different concentrations of UHMWPE particles on 3H-thymidine uptake are presented in Fig. 1. Uptake was significantly inhibited in a dose-dependent manner by all three types of UHMWPE particles. Type 412 was about 10-fold more potent an inhibitor of uptake than types 1900CM or 415. Fibroblast morphology was normal when cells were examined by phase contrast microscopy.

Low Concentration Eluates and Method of Sterilization

The effects of exposure to low concentrations of eluates from UHMWPE on fibroblast proliferation are presented in Table 1. Eluates were extracted from bar stock for 3 days into conditioned medium without agitation. The effect of the procedure used to sterilize the UHMWPE slices was also tested, comparing ethylene oxide (ETO) (Table 1a) with gamma irradiation (Table 1b). Of the 5 UHMWPE grades tested after sterilization with ethylene oxide, 412, 415, and RCH1000 stimulated 3H-thymidine uptake by fibroblasts, Hylamer eluates inhibited uptake at the highest eluate concentration, and 1900CM had no effect on uptake. Of the three grades tested that had been sterilized by gamma irradiation, both 415 and RCH1000 again stimulated uptake and Hylamer again had no significant effect on uptake (Table 1b). Although all UHMWPE tested except Hylamer showed greater stimulation at the 100% eluate concentration than the 50% concentration, only RCH1000 was statistically significant (P<0.0001).

Comparisons from the data of Table 1 between the method of sterilization showed that stimulation of uptake by RCH1000 eluates was greater for gamma irradiated UHMWPE (128% increase) than for ETO-treated material (35% increase) (P<0.0001). Uptake was also greater for Hylamer eluates prepared from gamma-irradiated UHMWPE stock (17% increase) than for ETO-treated stock (17% decrease) (P<0.05).

Type 415 UHMWPE eluates produced the same stimulation of uptake regardless of the method of sterilization used.

High Concentration Eluates

The effects of exposure to high concentrations of eluates from UHMWPE on fibroblast proliferation are presented in Fig. 2. Eluates were extracted from UHMWPE for 3 days with continual agitation in a shaking incubator to generate conditioned medium. In all cases, this conditioned medium caused a significant inhibition of 3H-thymidine uptake, and the inhibition was dose-dependent. Eluates from type 415 UHMWPE showed the most potent anti-proliferative activity compared with other UHMWPE types, with 86% inhibition of 3H-thymidine uptake at the highest eluate concentration tested. Interestingly, bar stock 415 and RCH1000 showed the greatest stimulatory effect on fibroblast proliferation at low eluate concentrations (Table 1). UHMWPE 1900CM and 412 were not tested in the high concentration eluate study.
Table 1
Effects of low concentration eluates from UHMWPE on fibroblast proliferation.

<table>
<thead>
<tr>
<th>UHMWPE Grade</th>
<th>³H-Thymidine Uptake Fraction of Control</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Ethylene Oxide Sterilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1900CM</td>
<td>50%</td>
<td>1.07</td>
<td>0.91–1.26</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>1.08</td>
<td>0.92–1.27</td>
</tr>
<tr>
<td>412</td>
<td>50%</td>
<td>1.18</td>
<td>1.00–1.39</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>1.21</td>
<td>1.03–1.43</td>
</tr>
<tr>
<td>415</td>
<td>50%</td>
<td>1.21</td>
<td>1.01–1.44</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>1.28</td>
<td>1.08–1.53</td>
</tr>
<tr>
<td>Hylamer</td>
<td>50%</td>
<td>0.95</td>
<td>0.79–1.13</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.83</td>
<td>0.69–0.98</td>
</tr>
<tr>
<td>RCH1000</td>
<td>50%</td>
<td>0.86</td>
<td>0.72–1.02</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>1.35</td>
<td>1.13–1.61</td>
</tr>
<tr>
<td>b. Gamma Irradiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>415</td>
<td>50%</td>
<td>1.15</td>
<td>0.97–1.37</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>1.39</td>
<td>1.17–1.66</td>
</tr>
<tr>
<td>Hylamer</td>
<td>50%</td>
<td>0.98</td>
<td>0.83–1.17</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>1.17</td>
<td>0.98–1.40</td>
</tr>
<tr>
<td>RCH1000</td>
<td>50%</td>
<td>1.32</td>
<td>1.12–1.58</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>2.28</td>
<td>1.91–2.72</td>
</tr>
</tbody>
</table>

Eluates were extracted without agitation for 3 days from UHMWPE slices sterilized by ethylene oxide (a) or by gamma irradiation (b) and used undiluted (100%) or diluted by half with medium (50%). Values are presented as medians and 95% confidence intervals. P values (ANOVA) test whether uptake was significantly different from the control (ns = not significant). Sample size equals 45 paired wells from three preparations. There was no significant difference between controls given standard medium pre-incubated for 72 hrs without UHMWPE and those given fresh medium without UHMWPE.

Figure 2. Dose-response effects of UHMWPE eluates on fibroblast proliferation. High concentration eluates were prepared from ETO-sterilized UHMWPE slices with agitation and diluted in standard culture medium. 100% Eluate Concentration represents no dilution of the conditioned medium. Uptake of ³H-thymidine is expressed as the fraction of control uptake (median ± 95% confidence limit). Sample size equals 45 paired wells from three different preparations. * P<.05; § P<.001; † P<.0001 compared with controls (ANOVA)
To determine if the effects of eluates from different types of UHMWPE were different from each other in their anti-proliferative potency, each type of UHMWPE was statistically compared with the other two types. There was no significant difference between types RCH1000 and Hylamer; however, type 415 was more potent than either RCH1000 (P<0.002) or Hylamer (P<0.001).

Phase contrast microscopy of fibroblasts exposed to high concentrations of UHMWPE eluates revealed that at the lowest of the three concentrations tested, all cells were attached and had normal morphology in culture. At the highest and middle concentrations tested, the number of adherent fibroblasts was reduced and some floating dead cells were observed; however, the morphology of cells that remained attached was similar to the controls, with no evidence of membrane disruption (blebbing), changes in shape, swelling or shrinking, or lipid droplet accumulation.

**DISCUSSION**

Fibroblasts have an important role in the osteolytic process at the bone implant interface. Histocytological studies of the distribution of cell types at the bone implant interface indicate that the majority of cells are members of the mononuclear phagocyte system, including osteoblasts, osteoclasts, macrophages, and monocytes but that fibroblasts are also present in significant numbers (Kadoya et al. 1998; Jiranek et al. 1993; Neale and Athanasou 1999). Synovial or interstitial fibroblasts are directly involved in three main osteolytic processes: 1) self-activation and stimulation of other inflammatory cells of the mononuclear phagocytic family (Hogaboam et al. 1998); 2) degradation of bone organic matrix via production and release of metalloproteinases (collagenases) (Imai et al. 1998; Lind et al. 1998; Pap et al. 1999); and 3) deposition of a fibrous membrane (Kadoya et al. 1998; Jiranek et al. 1993). Fibroblasts are activated by or activate macrophages, monocytes, osteoblasts, and osteoclasts through the release of cytokines and other intercellular mediators (Hogaboam et al. 1998), in particular interleukin-6 (IL-6) (Jones et al. 1999; Lind et al. 1998; Manlapaz et al. 1996), prostaglandin E2 (PG_{E2}) (Manlapaz et al. 1996), interleukin 1-β (IL-1β) (Lind et al. 1998; Manlapaz et al. 1996; Moreschini et al. 1997), tumour necrosis factor-α (TNFα) (Jones et al. 1999; Lind et al. 1998; Xu et al. 1996), and platelet-derived growth factor (PDGF) (Manlapaz et al. 1996). The chemical mediator TNFα is expressed in fibroblasts (Xu et al. 1996), is present in synovial fluid (Jones et al. 1999), and plays a major role in the differentiation of osteoclasts from monocyte/macrophage cells (Azuma et al. 2000; Merkel et al. 1999; Takayanagi et al. 2000). Fibroblasts are themselves responsive to cytokines released by macrophages and by other fibroblasts, and a role for fibroblast/macrophage interactions in the regulation of osteolytic processes at the bone-implant interface has been postulated (Lind et al. 1998).

The degradation of the organic matrix of bone by fibroblasts involves release of matrix metalloproteinase (MMP) by the cells (Imai et al. 1998; Lind et al. 1998; Pap et al. 1999). MMP-1 and membrane-type-1-MMP are found at sites of bone resorption and at the thickened interface membrane (Lind et al. 1998; Pap et al. 1999), and MMP-13 mRNA expression is higher than normal in osteoarthritis implants (Imai et al. 1998). The deposition of a thickened fibrous membrane at the bone-implant interface follows collagen deposition by fibroblasts (Iwanaga et al. 2000; Windhager et al. 1998). The observed thickening of the fibrous membrane at the bone-implant interface may be a result in part of increased fibroblast invasion as well as chemical cross-linking of the collagen as a result of the generation of free-oxygen radicals by metal particles (Windhager et al. 1998) or PMMA particles (Vale et al. 1997). Membrane-type-1 MMP collagenase expression by fibroblasts and osteoclasts correlates with the thickness of the fibrotic membrane (Pap et al. 1999).

Metal particle wear debris at high concentrations inhibits fibroblast activity in vitro, although at low particle concentrations, stimulation is more commonly reported (Manlapaz et al. 1996; Maloney et al. 1993; Mostardi et al. 1997). PMMA cement particles (Frondoza et al. 1993; Lind et al. 1998; Vale et al. 1997) and polyethylene particles McKellog et al. 1995; Shanbhag et al. 1997) both stimulate and inhibit fibroblast proliferation in cell cultures, depending on their concentration. Because the size distribution of particles is critical to their effect on cell-mediated osteolysis (Ingham and Fisher 2000; Kadoya et al. 1998), some investigations have used particles retrieved from synovial fluid at the time of re-implantation surgery, although the yield of particles is low (Merkel et al. 1999; Mostardi et al. 1997; Shanbhag et al. 1997). In our study, we observed a dose-dependent inhibition of 3H-thymidine uptake by UHMWPE wear particles collected from a hip simulator (Fig. 1). At the concentrations tested, no stimulation of 3H-thymidine uptake was observed, and the morphology of the cells appeared normal. The focus in the literature on the role of particulate debris in periprosthetic osteolysis and loosening raises a number of questions about the migration of particles from the weight bearing surface.
to the periprosthetic bone interface. It has been suggested that particles migrate between the prosthesis and cement, entering the cement bone interface through defects in the cement mantle (Anthony et al. 1990). It is also possible that cracks in the cement mantle may allow the egress of particles into the cement bone interface. It has been reported, however, that biopsy of osteolytic cavities does not always demonstrate the presence of particles (Maloney et al. 1990). The mechanics of particulate migration in the bone cement or cement prosthesis interface of a cemented joint replacement, or the prosthesis bone interface of an uncemented prosthesis, appears poorly understood. The movement of fluid at these interfaces has been described in detail (Howie et al. 1993). The uncertainty of the mechanism of particle migration is heightened by the observation that osteolytic cavities are found most frequently at a considerable distance from the site of generation of high density polyethylene particles (Tanzer et al. 1992). Thus, diffusible eluate release from either solid hip components or wear-generated particles could help explain the lack of correlation between the presence and sites of particle generation and the sites of osteolytic destruction.

Generally biomaterials are insoluble, but it is recognised that low molecular weight chemicals may be released from either the surface or the matrix of biomaterials (Northup 1989). Nacey et al. (1986) showed that eluant collected following incubation of a variety of urinary catheter types was toxic to mouse fibroblasts and human prostate epithelial cells in vitro. Other authors have considered the effect of leachable chemicals that are released from plasticisers, catalysts, and heat stabilisers used for fabrication of medical devices (Marchant 1989). Chemical components may also leach out of polyethylene components of implants. For example, rat tail fibroblasts grown on UHMWPE surfaces synthesise significantly less DNA and attachment is reduced compared with cells grown on other orthopedic materials, suggesting that UHMWPE is possibly toxic to the cells (Hunter et al. 1995). Our study presents the first direct evidence for the existence of chemical eluates from UHMWPE that can affect cell proliferation in vitro. Unlike the study by Hunter et al. there was no direct contact of cells with UHMWPE surfaces or particles. The proliferative response was biphasic with low eluate concentrations in most cases stimulating fibroblast proliferation (Table 1) and high concentrations of eluate inhibiting proliferation (Fig. 2). It is possible, therefore, that low concentrations of eluates could activate fibroblasts and cause release of inflammatory mediators that alter bone resorption at the implant bone interface and increase collagen deposition in synovial membranes. The surface to volume ratio of a polyethylene cup in situ and the synovial fluid in a joint is not known; however, it seems reasonable that the extraction procedure used in our study would be physiologically relevant, given the confines of the in vivo synovial space. Information on synovial fluid turnover in the implant-bone region would help answer this question. Since fluids can migrate along biological interfaces more readily than particles, it is possible that eluates play a role in the destruction of periprosthetic bone. Despite the fact that particle size distribution is critical for osteolytic effects (Kadaya et al. 1998, Ingham and Fisher 2000), Glant et al. (1993) showed that particles with the same size distribution sometimes had differing effects on macrophages. This anomaly may be partly explained by a difference in the response of macrophages and fibroblasts to phagocytosis of particles and direct effects of eluates released from the particles on the cells.

Currently, two techniques, gamma irradiation and ethylene oxide (ETO), are used to sterilize most UHMWPE components in total joint replacement. There is substantial literature on the effects of sterilization, packaging, and ageing on the in vitro physical and mechanical properties of UHMWPE (Besong et al. 1998; Lewis 1997). ETO is being used more frequently as an alternative to gamma irradiation because of the cross-linking and oxidation of polyethylenes known to occur following gamma irradiation. ETO residues, however, such as esters, acids, and hydroperoxides (Costa et al. 1998), may also be responsible for some of the toxic effects of polyethylene wear particles and may have contributed to the anti-proliferative effects of eluates seen in the present study. Gunther et al. (1993) demonstrated that fibroblasts have a reduced rate of in-growth into porous implant materials sterilized by ETO. Thorén and Aspenberg reported that following ETO sterilization, bone in-growth rate of an allograft implanted in rat tibia was impaired by 68% compared with that sterilized by gamma irradiation. In the present study, conditioned medium from UHMWPE sterilized by ETO showed significantly reduced 3H-thymidine uptake compared to conditioned medium from polyethylene sterilized by gamma irradiation (Table 1). These results suggest that following ETO sterilization, ETO residues may still be present within polyethylene, despite the fact that the recommended aeration time before use of 1 to 2 weeks was followed, and these residues may be partially responsible for the observed inhibitory effects on fibroblast activity.

From the clinical point of view, it could be speculated that at an early stage after implantation, the concentration of eluates from UHMWPE is low and the eluates may stimulate fibroblast proliferation,
leading to enhancement of inflammatory responses, bone resorption, and synovial membrane deposition. At a later stage following implantation, higher eluate concentrations could reach cytotoxic levels in the synovial fluid. It is possible that even when proliferation has been inhibited, inflammatory mediators could be released from the cells, thus, contributing to further acute, osteoclastic bone resorption. This conclusion is supported by the fact that fibroblasts increase their release of collagenase and the inflammatory mediators IL-6 and PGE2 by up to 16 fold when exposed to metal particles in vitro (Manlapaz et al. 1996). These results differed from ours in that the particles had no effect on ³H-thymidine uptake by the fibroblasts whereas our UHMWPE particles and eluates inhibited or stimulated uptake. In no case, however, were the fibroblasts morphologically damaged in our investigation, based on observations by phase contrast microscopy.

This study is the first to demonstrate that stimulatory and inhibitory chemical substances can leach out of UHMWPE bar stock. These eluates displayed a characteristic biphasic effect on fibroblast proliferation, stimulating proliferation at low concentrations and causing a dose-dependent inhibition at high concentrations. It is postulated that these effects on fibroblast activity from leachable chemical eluates of UHMWPE cups may be significant, along with direct particle effects and micromovement effects, in the osteolytic degeneration process observed in failed joint replacements.

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