Epidural application of spinal instrumentation particulate wear debris: a comprehensive evaluation of neurotoxicity using an in vivo animal model

Laboratory investigation

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Object. The introduction and utilization of motion-preserving implant systems for spinal reconstruction served as the impetus for this basic scientific investigation. The effect of unintended wear particulate debris resulting from micromotion at spinal implant interconnections and bearing surfaces remains a clinical concern. Using an in vivo rabbit model, the current study quantified the neural and systemic histopathological responses following epidural application of 11 different types of medical-grade particulate wear debris produced from spinal instrumentation.

Methods. A total of 120 New Zealand White rabbits were equally randomized into 12 groups based on implant treatment: 1) sham (control), 2) stainless steel, 3) titanium alloy, 4) cobalt chromium alloy, 5) ultra–high molecular weight polyethylene (UHMWPe), 6) ceramic, 7) polytetrafluoroethylene, 8) polycarbonate urethane, 9) silicone, 10) polyethylene terephthalate, 11) polyester, and 12) polyetheretherketone. The surgical procedure consisted of a midline posterior approach followed by resection of the L-6 spinous process and L5–6 ligamentum flavum, permitting interlaminar exposure of the dural sac. Four milligrams of the appropriate treatment material (Groups 2–12) was then implanted onto the dura in a dry, sterile format. All particles (average size range 0.1–50 μm in diameter) were verified to be endotoxin free prior to implantation. Five animals from each treatment group were sacrificed at 3 months and 5 were sacrificed at 6 months postoperatively. Postmortem analysis included epidural cultures and histopathological assessment of local and systemic tissue samples. Immunocytochemical analysis of the spinal cord and overlying epidural fibrosis quantified the extent of proinflammatory cytokines (tumor necrosis factor–α, tumor necrosis factor–β, interleukin [IL]–1α, IL–1β, and IL–6) and activated macrophages.

Results. Epidural cultures were negative for nearly all cases, and there was no evidence of particulate debris or significant histopathological changes in the systemic tissues. Gross histopathological examination demonstrated increased levels of epidural fibrosis in the experimental treatment groups compared with the control group. Histopathological evaluation of the epidural fibrous tissues showed evidence of a histiocytic reaction containing phagocytized inert particles and foci of local inflammatory reactions. At 3 months, immunohistochemical examination of the spinal cord and epidural tissues demonstrated upregulation of IL–6 in the groups in which metallic and UHMWPe debris were implanted (p < 0.05), while macrophage activity levels were greatest in the stainless-steel and UHMWPe groups (p < 0.05). By 6 months, the levels of activated cytokines and macrophages in nearly all experimental cases were downregulated and not significantly different from those of the operative controls (p > 0.05). The spinal cord had no evidence of lesions or neuropathology. However, multiple treatments in the metallic groups exhibited a mild, chronic macrophage response to particulate debris, which had diffused intrathecally.

Conclusions. Epidural application of spinal instrumentation particulate wear debris elicits a chronic histiocytic reaction localized primarily within the epidural fibrosis. Particles have the capacity to diffuse intrathecally, eliciting a transient upregulation in macrophage/cytokine activity response within the epidural fibrosis. Overall, based on the time periods evaluated, there was no evidence of an acute neural or systemic histopathological response to the materials included in the current project.

Abbreviations used in this paper: ABC = avidin-biotin complex; HAM = human alveolar macrophage; IL = interleukin; PCU = polycarbonate urethane; PEEK = polyetheretherketone; PET = polyethylene terephthalate; PTFE = polytetrafluoroethylene; TNF = tumor necrosis factor; UHMWPe = ultra–high molecular weight polyethylene; ZTA = zirconium toughened alumina.

The effect of unintended wear particulate debris resulting from micromotion between the interconnection mechanisms in spinal instrumentation

This article contains some figures that are displayed in color online but in black-and-white in the print edition.
Epidural application of particulate wear debris remains a clinical concern. Recently, there have been a number of retrospective clinical studies describing the histological response to wear particle generated from spinal implants and the clinical consequence of this material in posterolateral arthrodesis procedures.\textsuperscript{4,10,15,34,42,56,60} Dubousset et al.\textsuperscript{15} were among the first to describe a late “infection” complication in patients in whom posterior Cotrel-Dubousset instrumentation had been placed (n = 18; average 34 months postoperatively). Histopathological examination of the local tissue samples revealed an acute and chronic inflammation with granuloma formation at the instrumentation transverse connector site, necessitating hardware removal. The etiology of this complication was considered a sterile, inflammatory reaction caused by fretting corrosion of the instrumentation. Cook et al.\textsuperscript{10} retrospectively compared the reoperation rate in 190 consecutive patients following primary posterior instrumentation for idiopathic scoliosis. Evaluating 3 different instrumentation systems, the authors introduced the concept of “late operative site pain” of unknown etiology as the most frequent indication for reoperation (occurring in 8% or 14 patients). The most frequently noted surgical observation at the time of implant removal was corrosion at the level of the interconnection mechanisms, which occurred in 9 of 14 patients and was associated with tissue discoloration and intracellular metallic debris. Moreover, Wang et al.\textsuperscript{59} described the incidence of metal debris associated with the use of titanium implants in 9 patients. They observed that tissue concentrations of titanium were highest in patients with a pseudarthrosis, whereas patients with solid fusions had negligible levels of titanium. According to Wang et al., these particles activated a macrophage cellular response in the spinal tissues similar to that seen in total-joint prostheses. A number of other retrospective clinical studies have documented an inflammatory, foreign-body reaction in soft-tissue structures adjacent to spinal implants used for spinal arthrodesis.\textsuperscript{4,18,34,60} Moreover, the introduction of motion-preserving spinal implants, including disc replacements and dynamic posterior stabilization systems, introduces a new area of concern regarding the potential for unintended wear debris and its effect on local osseous and epidural soft-tissue structures. Interestingly, despite these aforementioned studies, there have been no comprehensive, controlled basic science studies undertaken to determine if particulate wear debris from spinal instrumentation has a deleterious effect on the local epidural structures, spinal cord, or systemic tissues.

With these issues in mind, the current study was undertaken to investigate the biological response of local and systemic tissues following epidural application of 11 types of particulate wear debris derived from materials commonly used in the production of spinal instrumentation. Using an in vivo rabbit model, and based on postmortem histological and immunocytochemical analyses, the fundamental objectives of this study were 3-fold: 1) to compare and quantify the extent of macrophage activity and proinflammatory cytokine production within the epidural fibrosis and spinal cord itself following epidural application of 11 types of particulate wear debris; 2) to determine if there is a significant histopathological reaction to the wear debris created by particle challenge, and what histomorphological responses, if any, are specific to the local epidural fibrosis and spinal cord; and 3) to characterize the histomorphological features of the systemic/reticuloendothelial tissues in response to the wear particulates applied epidurally.

**Methods**

**Animal Research Permission**

All surgical and experimental animal procedures commenced following protocol approval by the University of Maryland Biotechnology Institute’s Institutional Animal Care and Use Committee.

**Animal Model and Treatment Groups**

A total of 120 skeletally mature Harlan Sprague-Dawley New Zealand White rabbits (weight range 3.8–4.2 kg) were included in this study and randomized into 12 groups based on treatment procedure (n = 10 per group). Five animals from each group were sacrificed at postoperative time intervals of 3 months (n = 60) and 6 months (n = 60). The treatment groups were as follows: 1) sham (control); 2) stainless steel 316LVM; 3) titanium alloy Ti-6AL-4 V; 4) cobalt chromium alloy; 5) UHMWPE; 6) ZTA ceramic; 7) PTFE; 8) PCU; 9) silicone; 10) PET; 11) polyester; and 12) PEEK.

**Particulate Debris Material Specifications and Methods of Characterization**

The 11 types of biomaterial particles represent the most commonly used materials in orthopedic spinal implants (Table 1). The metal-based materials (stainless steel, titanium alloy, and cobalt chromium alloy) were commercially sourced as gas-atomized spherical stock particles of acceptable size and shape distribution for implantation, and the remaining 8 composite ceramic and polymeric materials were commercially sourced (BioEngineering Solution Inc.) and produced using proprietary techniques involving custom “cryomilling” and “cryopulverization” in liquid nitrogen followed by size exclusion separation using ultrasound-facilitated filtration through polystyrene membrane filters. Briefly, the ceramic was cryomilled using < 20-μm diamond grit high-speed abrasion, and bulk polymeric materials were sectioned into small pieces (volume approximately 1 mm\(^3\)) and placed in a cryogenic pulverization chamber containing liquid nitrogen. Polymeric samples were pulverized at a frequency of greater than 10 Hz, followed by size exclusion sedimentation/filtration, to obtain samples in which more than 99% were less than 10 μm in size (number based). Particle imaging and characterization were performed based on ASTM Standard F1877–10 guidelines, Standard Practice for Characterization of Particles, and used the following techniques: 1) Low-angle laser light scattering was performed on the generated particles. A Microtrac x100 Analyzer (Microtrac, Inc.) used laser light diffraction and dynamic light scattering to quantify particulate size ranges from 10 nm to 2000 μm. The low-angle laser light scattering data produced a volume-
number-based analysis of particle size ranges in microns with comparison of mean particle diameter, particle size ranges, and percentage distribution of particulates (Fig. 1; Table 1). Using the number-based data distributions and known density (in g/cm$^3$) of materials, the approximate number of particles implanted per milligram of material was calculated according to standard technique (Table 1). Scanning electron microscopy and energy-dispersive x-ray energy analysis permitted characterization of the chemical components of each material. The x-ray data obtained under scanning electron microscopy were used to quantify the percentage of each measured element present in the individual particles (Fig. 2).

Following preparation, all particle treatments were washed (depyrogenated) with endotoxin-specific cleaning detergent (PyroCLEAN, Millipore) and verified as endotoxin free (< 0.05 EU/ml) prior to implantation using the detergent (PyroCLEAN, Millipore) and verified as endotoxin-free (Fig. 2). Prior to implantation, 4 mg of each material was packaged in Eppendorf vials and sterilized either by steam at 273°C for 30 minutes (metallic based debris), by ethylene oxide (ceramic or polymeric based materials), or by gamma irradiation (ceramic or polymeric based materials), as recommended by the manufacturer.

**Surgical Procedures**

Following determination of normal health status, each animal was sedated by subcutaneous injection of ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (0.75 mg/kg) anesthetic medications, followed by endotracheal intubation and general anesthesia with 0.5%–1.0% isoflurane. With the animal positioned prone, its posterior lumbar region was shaved, aseptically prepared, and draped in sterile fashion. A single midline skin incision of 4–5 cm was centered over the L5–6 operative level. The L-6 spinous process and L5–6 supraspinous/interspinous ligation and ligamentum flavum were then exposed and excised, permitting interlaminar exposure of the meningeous coverings and neural structures of the spinal canal (5-mm-diameter surface area). Following thorough wound irrigation, the experimental treatment materials were implanted directly on the dura in a sterile, dry format; the sham (control) group consisted of epidural exposure alone (Fig. 3). Following the surgical procedure, all wounds were closed in an interrupted fashion using 2.0 Vicryl sutures. Ambulatory activities and wound healing were monitored daily, and all animals received analgesics (intramuscular butorphanol, 0.125 mg/kg) and prophylactic antibiotics (oral trimethoprim sulfadiazine, 0.2 mg/kg) for the first 3 and 10 days postoperatively, respectively. Following the appropriate postoperative survival periods (3 or 6 months), all animals were humanely sacrificed using an overdose (150 mg/kg) of concentrated pentobarbital solution (390 mg/ml). Externals and internal examination of the surgical site, as well as gross histopathological examination of the lumbar spine, was conducted at the time of sacrifice.

**Methods of Histopathological Analysis**

**Epidural Cultures.** Sterile swabs were used to obtain samples from the operative epidural region at the time of autopsy for culture and sensitivity testing. Microbiological specimens were cultured aerobically and anaerobically using unselective media by both direct and broth enrichment cultures for 1 week. The collection media were examined for growth of high-grade pathogens (for example, *Propionibacterium*, *Corynebacterium*, coagulase-negative *Staphylococcus*) (IDEXX Laboratories, Inc.).

**Systemic Tissue Analysis.** In all animals, a total of 9
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Fig. 1. A: Low-angle laser light scattering histogram demonstrating the volume-based size distribution for PEEK material. Particles exhibited a mean diameter of 14.6 µm (range 0.5–88 µm) with 24% of particles less than 5 µm and 3% less than 1 µm. B: Low-angle laser light scattering histogram demonstrating the number-based size distribution for PEEK material. Particles had a mean diameter of 0.8 µm (range 0.5–18.5 µm); 99% of particles were less than 5 µm and 84% were less than 1 µm.

Spinal Cord and Overlying Epidural Fibrous Tissue. To evaluate the postmortem histomorphological features of the dura, spinal cord, and nerve roots, the operative level laminae were excised and the spinal canal was exposed. The spinal cord and overlying epidural fibrosis were then dissected using meticulous technique. After gross histopathological examination of the epidural fibrosis and membranous coverings, the spinal cord was transversely sectioned though the operative site to produce 2 specimens. The first underwent routine paraffin embedding, thin-sectioning microtomy (3–5 µm thick), and staining using 2 techniques: 1) H & E, and 2) HAM-56 staining method (MyBioSource, Inc.), which highlights the presence of activated macrophages. Using plain and polarized light microscopy, histopathological readings of the slide-mounted tissue sections included quantification of activated macrophages, comments regarding spinal cord morphology, and presence of wear debris, as well as any signs of foreign body giant cells, granulomatous inflammatory reactions, degenerative changes, or autolysis.

Using standard immunohistochemical techniques, the second section was processed to characterize and quantify the extent of proinflammatory cytokine activity within the local overlying epidural fibrosis, dural sac, and spinal cord. The specimens were trimmed and excess bone fragments were removed to produce a 2-cm² piece of tissue. Tissue samples were then placed in cassettes and covered with TissueTek Optimal Cutting Temperature embedding
compound (Sakura Finetek BV), placed in cooled isopentane, sectioned with a cryostat, fixed in anhydrous acetone for 20 seconds, and stored at –70°C. The endogenous peroxides within the samples were blocked with peroxide (H₂O₂), using a 2-step method previously documented. Using primary and secondary antibodies combined with the ABC–horseradish peroxidase technique, macrophage intracellular and membrane-bound cytokines were stained and included the following: TNF-α and TNF-β, IL-1α and IL-1β, and IL-6 (Dako North America, Inc.).

**Histomorphometric Evaluation**

Quantification of activated macrophages and proinflammatory cytokines was performed using an Olympus BX 41 microscope (Olympus America, Inc.) using both plain and polarized light microscopy. Each specimen, which included those from the spinal cord and overlying epidural fibrosis, was manually analyzed using 50 consecutive fields per sample at 100× magnification. At this magnification and field number, the number of activated macrophages and proinflammatory cytokines within the spinal cord and overlying epidural fibrosis tissue areas were quantified.

**Statistical Analysis**

Histomorphometric data represent the mean number of activated macrophages and cytokine-expressing macrophages within the spinal cord and overlying epidural tissues for each treatment per time interval. Standard 2-tailed Student t-test comparisons were used to demonstrate differences between the 3- and 6-month time intervals for each treatment group. A 1-way ANOVA test with a post hoc Student-Newman-Keuls test was used for comparison across treatments within each time interval (3 or 6 months). All data are presented as the mean ± 1 SD, and comparisons at p < 0.05 were considered significant.

**Results**

**Particulate Wear Debris Characterization**

Particle imaging and characterization were performed based on ASTM Standard F1877–10 guidelines and used low-angle laser light scattering, scanning electron microscopy, and energy-dispersive x-ray energy analysis. Volume-based low-angle laser light scattering assays indicated the mean particle diameters to be less on average for the metallic based groups (range 2.6–7.3 μm) than the ceramic and polymeric treatments (range 4.5–28.5 μm). Moreover, the percentage distribution in size range of the metallic groups exhibited an array from 60% to 95% of the particles being less than 5 μm, whereas 14%–19% were less than 1 μm. In contrast, the ceramic and polymeric treatments showed that 12%–68% were less than 5 μm, whereas 0%–42% were less than 1 μm (Table 1).

The number-based low-angle laser light scattering...
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**Assays** indicated that the mean particle diameters for the metal-based groups (range 0.1–0.2 μm) were slightly smaller than those of the ceramic and polymeric based groups (range 0.2–3.6 μm). The percentage distribution in size range in the metallic groups showed 100% of the particles to be less than 5 μm and 99% to be less than 1 μm. The percentage distribution in size range in the ceramic and polymeric treatment groups showed 85%–100% to be less than 5 μm and a very disperse range of particles, spanning 0%–99%, to be less than 1 μm (Table 1). Scanning electron microscopic images obtained for the metallic material groups characterized the particles as spherical in shape, whereas it characterized polymeric and ceramic materials as having a rough, irregular, granular, or flaky morphology, without evidence of fibers at any size range. The corresponding energy dispersive x-ray energy analysis signatures showed a chemical composition characteristic for each material, demonstrating the lack of contamination across treatments.

Using the number-based data distributions and known density (g/cm3) of materials, the actual number of particles implanted per milligram of material was estimated. The ranges in actual numbers were very disperse and depended significantly on the low-angle laser light scattering assays. The range was from a low of 7 million particles per milligram for the silicone to a high of 20 billion particles per milligram for the ceramic ZTA treatment (Table 1). This demonstrates how closely the dose depends on particle sizes, where the numbers of particles vary exponentially (cube) with size of the particles (for a given total mass). In the case of the silicone treatments, in particular, considerable agglomeration was observed following preparation and filtration, whereas the ceramic materials indicated a very small size range based on number distribution, accounting for the large number of particles deposited per milligram. Interestingly, despite the disparity in the calculated particle numbers per milligram, there were no observable differences in the amount of dural covering noticed at the time of the index surgical procedure.

**Surgical Procedures and Perioperative Outcomes**

There was no incidence of intra- or perioperative neurological, infectious, or vascular complications in any of the 120 cases. However, there were 6 anesthesia-related complications secondary to esophageal intubation or early extubation at the time of recovery, leading to animal death and necessitating replacement animals. Despite direct epidural exposure and application of particulate wear debris, all animals were characterized as having a normal recovery throughout the 3- and 6-month postoperative periods. By 48 hours postoperatively, all animals were fully ambulating and exhibiting normal behavior.

**Gross Macroscopic Findings**

Following multilevel laminectomies to expose the spinal canal, gross histopathological findings of the spinal cord specimens in the 11 experimental groups were compared with those of the control treatment group at 3- and 6-month time intervals. The control (sham) animals showed normal appearance of the dura mater and vascular structures, with limited epidural fibrosis. Conversely, all experimental animals, particularly those in which metallic materials (stainless steel, cobalt chrome, or titanium alloy) were used, exhibited markedly greater amounts of epidural fibrosis compared with the operative sham treatment and polymeric treatment groups (Fig. 4). Moreover, the metallic particulate debris was clearly embedded within the epidural fibrosis tissue layer and intrathecal in many cases. At the time of scheduled necropsy, there were no gross signs of infection in any animals.

**Epidural Cultures**

Sterile swabs and tissue specimens obtained from the epidural region at the time of autopsy were culture negative in 114 of 120 cases. There were 4 cases of *Propionibacterium* at the 6-month interval for the control (n = 2) and PCU (n = 2) treatments. At the 3-month interval, there was 1 case each of *Enterococcus* and alpha *Streptococcus* for the PTFE and polyester treatments, respectively. These nonpathogenic inhabitants were most likely introduced from the skin at the time of scheduled necropsy, and importantly, all culture-positive cases had no gross signs of infection, significant upregulation of cytokines, or macrophages based on histopathological examination.

**Histopathology**

**Systemic Tissue Analysis.** Histopathological analysis of the systemic tissues at the 3- and 6-month intervals demonstrated no significant pathological changes induced by any of the 11 experimental treatments or sham procedure. Pathological assessment characterized all systemic organs and tissues as having no significant histopathological changes and no foreign-body materials, foreign-body giant cell/granulomatous inflammatory reactions, degenerative changes, or autolysis. Although lymphoreticular dissemination of the metallic and polymeric particulates probably occurred, there was no histological evidence of particulates in any of the tissues analyzed.

**Immunohistochemical Analyses.** Using immunohistochemistry techniques, the spinal cord and overlying epidural fibrosis were processed to quantify the levels of local cytokines and macrophages in response to particle challenge. Using the HAM-56 specialty stain, activated mac-
rophages were indicated by a bright red chromogen label against a blue background. The membrane-bound or intracellular cytokines (antigens) localized on macrophages produced a yellow to brown chromogen label in response to the primary and secondary antibodies when combined with the ABC–horseradish peroxidase technique. Activated cytokines levels compared between the 3- and 6-month time intervals demonstrated significance in all cytokine categories except TNF-β. Activity levels of TNF-α were downregulated in 3 treatment groups when the 3- with 6-month intervals were compared (ceramic at 3 vs 6 months, 29.3 ± 21.3 vs 1.33 ± 3.99, respectively [p = 0.035]; and PET at 3 vs 6 months, 66.0 ± 34.9 vs 0 ± 0, respectively [p = 0.003]), and they were upregulated in the PTFE group (at 3 vs 6 months, 8.0 ± 10.3 vs 42.0 ± 18.1, respectively [p = 0.007]). For IL-1α, the PCU treatment exhibited an upregulation at 6 months (49 ± 36.1) versus 3 months (10.5 ± 5.6) (p = 0.046). In the case of IL-1β, a significant upregulation in activity was observed for both the stainless-steel (at 3 vs 6 months, 18.7 ± 32.0 vs 72.2 ± 34.5, respectively [p = 0.035]) and PCU (at 3 vs 6 months, 15.2 ± 9.7 vs 52.5 ± 31.3, respectively [p = 0.034]) treatments, whereas the ceramic group exhibited a reduction in activity levels of TNF-α activity (35.3 ± 24.3 at 3 months and 0.33 ± 1.29 at 6 months [p = 0.012]). Of the 3 ILs assayed, IL-6 exhibited the most pronounced changes in activity levels compared with all other cytokines. Six of (50%) the 12 treatments exhibited a significant downregulation in IL-6 activity levels when we compared samples at the 3- and 6-month intervals (for stainless steel 1395 ± 393 and 11.1 ± 175, respectively [p = 0.003]; for titanium alloy 1460 ± 381 and 41.3 ± 86.55, respectively [p = 0.000]; for cobalt chromium alloy 912 ± 642 and 120 ± 228, respectively [p = 0.032]; for UHMWPe 1639 ± 393 and 11.1 ± 10.0, respectively [p = 0.000]; for ceramic 153 ± 101 and 1.67 ± 3.62, respectively [p = 0.010]; and for polyester 70.0 ± 35.2 and 20.0 ± 17.7, respectively [p = 0.022]) (Table 2). The quantity of activated macrophages localized within the spinal cord and overlying epidural fibrosis tissues was reduced in 8 of 11 treatments when comparing the 3- with 6-month intervals, although these observations were significant only for the UHMWPe treatment (71.3 ± 56.3 at 3 months vs 10.1 ± 11.7 at 6 months [p = 0.044]) and PET treatment (61.0 ± 41.1 at 3 months vs 3.1 ± 6.36 at 6 months [p = 0.014]). No other comparisons were significantly different (p > 0.05) (Table 2). Statistical comparisons across treatment groups demonstrated highly significant findings, particularly at the 3-month interval. Activated levels of TNF-α cytokines were significantly higher for the PET treatment than for all other groups except ceramic, silicone, and polyester (p < 0.05). No significant differences were observed when comparing the control or remaining experimental treatment groups with respect to concentrations of TNF-α, TNF-β, IL-1α, or IL-1β at this time interval (p > 0.05). The most significant findings were in the levels of activated IL-6 cytokines, which were significantly higher for the stainless-steel, titanium alloy, cobalt chrome alloy, and UHMWPe treatments compared with nearly all other groups (p < 0.05) (Table 3). The corresponding macrophage counts were greatest for the stainless-steel treatment, which was significantly different from all other treatments except titanium alloy, cobalt chrome, UHMWPe, PCU, and PET (p < 0.05) (Table 3; Figs. 5 and 6).

At the 6-month interval, similar trends were observed for the activity levels of TNF-α cytokines in the PTFE, PCU, and polyester groups, which were significantly higher from nearly all other groups except for the cobalt chrome group (p < 0.05) and exhibited marked increases compared with the corresponding 3-month levels. No significant differences were observed when comparing the control or remaining experimental treatment groups with respect to concentrations of TNF-α, TNF-β, IL-1α, IL-1β, or IL-6 at this time interval (p > 0.05). Importantly, the levels of IL-6 exhibited significant or marked decreases in nearly all cases when compared with the 3-month interval and were not significantly different from each other at this 6-month postoperative period (p > 0.05). These trends were most pronounced for the stainless-steel, titanium alloy, cobalt chrome alloy, UHMWPe, and ceramic groups (p < 0.05), whereas the remaining treatment exhibited levels of IL-6 consistent with the 3-month observations. The corresponding macrophage counts were greatest for the stainless-steel and cobaltchrome treatments, which were significantly different from all other groups (p < 0.05) (Table 4; Figs. 5 and 6).

Histomorphological Analyses

Operative Control (Sham). Plain and polarized light microscopy of the histological cross-sections from the control groups indicated a very mild reaction to the duro mater and spinal cord. The surgical procedure resulted in increased concentrations of cytokines and macrophages; however, the extent of epidural fibrosis was limited. Despite the transient histiocytic reaction, histopathological evaluation indicated normal distribution of myelin and intracellular neurofibillary networks and characterized all control specimens as having no significant pathological changes at both the 3- and 6-month time intervals (Fig. 7).

Metallic Treatment Groups. Histomorphological characterization of the spinal cord and overlying epidural fibrosis indicated a significant histiocytic macrophage reaction and phagocytosis of the particulate debris from the metal-based groups (stainless steel, titanium alloy, and cobalt chrome alloy), particularly at the 3-month time interval. In cases at both time intervals, particulate debris was present in the histological sections and not only localized to the area of epidural particulate application but also exhibiting evidence of intrathecal dissemination. Plain and polarized light microscopy indicated that particulate debris from the metal-based treatment groups spanned from the site of application to within the spinal cord itself. In many cases, an epidural particulate layer formed along the dura mater consisting of “unphagocytosable” particles, which were encapsulated by an organized fibrous connective tissue layer. Despite the epidural fibrosis regions heavily laden with metallic particulate and exhibiting a transient upregulation of macrophage and cytokine activity, the spinal cord specimens showed a normal distribution of myelin and intracel-
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**TABLE 2: Proinflammatory cytokine and macrophage counts in epidural fibrosis and the spinal cord at 3 and 6 months postimplantation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activated Cytokine Cell Counts</th>
<th>Macrophages (HAM-56)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF-α</td>
<td>TNF-β</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo control</td>
<td>8.000 (9.380)</td>
<td>0.690 (1.890)</td>
</tr>
<tr>
<td>6-mo control</td>
<td>12.37 (10.36)</td>
<td>0.450 (1.470)</td>
</tr>
<tr>
<td>p value</td>
<td>0.504</td>
<td>0.828</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo SST</td>
<td>9.14 (10.75)</td>
<td>19.40 (23.79)</td>
</tr>
<tr>
<td>6-mo SST</td>
<td>6.70 (19.34)</td>
<td>0.19 (0.75)</td>
</tr>
<tr>
<td>p value</td>
<td>0.811</td>
<td>0.109</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo titanium</td>
<td>7.22 (4.55)</td>
<td>8.20 (9.32)</td>
</tr>
<tr>
<td>6-mo titanium</td>
<td>7.47 (17.07)</td>
<td>1.60 (4.22)</td>
</tr>
<tr>
<td>p value</td>
<td>0.976</td>
<td>0.187</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo cobalt chrome</td>
<td>7.25 (9.90)</td>
<td>0.13 (0.34)</td>
</tr>
<tr>
<td>6-mo cobalt chrome</td>
<td>16.30 (28.09)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.516</td>
<td>0.417</td>
</tr>
<tr>
<td>Group 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo UHMWPe</td>
<td>5.13 (5.87)</td>
<td>13.17 (16.04)</td>
</tr>
<tr>
<td>6-mo UHMWPe</td>
<td>0 (0)</td>
<td>0.33 (1.15)</td>
</tr>
<tr>
<td>p value</td>
<td>0.086</td>
<td>0.112</td>
</tr>
<tr>
<td>Group 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo ceramic</td>
<td>29.33 (21.32)</td>
<td>12.67 (27.64)</td>
</tr>
<tr>
<td>6-mo ceramic</td>
<td>1.33 (3.99)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.020</td>
<td>0.335</td>
</tr>
<tr>
<td>Group 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo PTFE</td>
<td>8 (10.33)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>6-mo PTFE</td>
<td>42 (18.14)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.007</td>
<td>N/A</td>
</tr>
<tr>
<td>Group 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo PCU</td>
<td>24.07 (8.0)</td>
<td>4.2 (9.3)</td>
</tr>
<tr>
<td>6-mo PCU</td>
<td>42.8 (24.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.136</td>
<td>0.342</td>
</tr>
<tr>
<td>Group 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo silicone</td>
<td>42.0 (37.01)</td>
<td>2.0 (4.47)</td>
</tr>
<tr>
<td>6-mo silicone</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.035</td>
<td>0.346</td>
</tr>
<tr>
<td>Group 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo PET</td>
<td>66.0 (34.94)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>6-mo PET</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.003</td>
<td>NA</td>
</tr>
<tr>
<td>Group 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo polyester</td>
<td>46.0 (43.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>6-mo polyester</td>
<td>43.75 (24.46)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.921</td>
<td>NA</td>
</tr>
<tr>
<td>Group 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-mo PEEK</td>
<td>10.21 (19.35)</td>
<td>0.25 (0.45)</td>
</tr>
<tr>
<td>6-mo PEEK</td>
<td>8.0 (12.52)</td>
<td>2.50 (4.63)</td>
</tr>
<tr>
<td>p value</td>
<td>0.836</td>
<td>0.311</td>
</tr>
</tbody>
</table>

* The 3- and 6-month cell counts are presented as the mean (± 1 SD). SST = stainless steel.
lular neurofibrillary network without evidence of lesions and were characterized as without significant histopathological changes (Fig. 8).

Polymeric and Ceramic Groups. The polymeric and ceramic treatment groups (PTFE, PCU, silicone, PET, polyester, PEEK, and ZTA ceramic) as a whole produced less reactivity in macrophages and cytokine response at both the 3- and 6-month postoperative intervals than the corresponding metallic treatment groups. In contrast, the UHMWPe treatment exhibited significant histiocytic infiltration and phagocytosis of particulate debris comparable to that of the metallic particle challenge. In all cases, histomorphological evaluation of the spinal cord and overlying fibrosis indicated a chronic-type histiocytic macrophage reaction with evidence of particulate phagocytosis. At both 3- and 6-month examinations, polymeric and ceramic particulate debris was present within the sections and remained localized to the area of epidural application without evidence of intrathecal dissemination. In many cases, an epidural particulate layer formed along the dura mater, consisting of unphagocytosable particles, which were encapsulated by an organized fibrous connective tissue layer. Of particular interest, the polymeric

![Graph](image-url)

**Fig. 5.** Comparative levels of activated IL-6 cytokines in the epidural fibrosis and spinal cord at the 3- and 6-month intervals following implantation of the materials. Statistical significance between the 3-month treatments is as follows: ~ versus all except titanium alloy and UHMWPe; ** versus all except stainless steel and UHMWPe; # versus all except stainless steel, titanium alloy, and UHMWPe; * versus all except stainless steel and titanium alloy (p < 0.05). Bar height indicates mean value and error bars minus 1 SD (1-way ANOVA, F = 19.8, p = 0.000).
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treatment groups demonstrated pronounced agglomeration of particles histopathologically. In the case of treatment with silicone, significant agglomeration was noted at the time of particle preparation and filtration, prior to implantation (Table 1). Moreover, despite the average particle size at the time of implantation (for example, 1.2 μm for UHMWPe, 1.2 μm for PTFE, and 1.0 μm for PCU), particulate aggregates ranged in size from 25 to 300 μm, and each contained a dense macrophage reaction along the periphery (Fig. 9). Similar to the metallic debris, the increased levels of histiocytic and cytokine activity were not deleterious to the local fibrous or spinal cord tissues. There was no evidence of polymorphonuclear giant cell reaction or other significant pathological changes.

Discussion

The implementation of motion-preserving spinal instrumentation systems for total disc arthroplasty, dynamic posterior “soft” stabilization, and fusionless correction of spinal deformity necessitates improved understanding of the neurohistopathological response to particulate wear debris. The current study provides an experimental model and technique to assess the local and systemic response to 11 types of particulate wear debris derived from materials commonly used in the production of spinal instrumentation. The issue of unintended wear particulate from orthopedic implants is not new. Review of the joint prosthesis literature highlights many articles describing local

<table>
<thead>
<tr>
<th>6-Mo Treatment</th>
<th>Proinflammatory Cytokines (mean ± 1 SD)</th>
<th>Macrophages (HAM-56) (mean ± 1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF-α</td>
<td>TNF-β</td>
</tr>
<tr>
<td>control</td>
<td>12.37 (10.36)</td>
<td>0.450 (1.470)</td>
</tr>
<tr>
<td>stainless steel</td>
<td>6.700 (19.34)</td>
<td>0.190 (0.750)</td>
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<td>titanium alloy</td>
<td>7.470 (17.07)</td>
<td>1.600 (4.220)</td>
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<tr>
<td>cobalt chrome</td>
<td>16.30 (28.09)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td>UHMWPe</td>
<td>0.000 (0.000)</td>
<td>0.330 (1.150)</td>
</tr>
<tr>
<td>ceramic</td>
<td>1.330 (3.990)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td>PTFE</td>
<td>42.00 (18.14)</td>
<td>0.000 (0.000)</td>
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<tr>
<td>PCU</td>
<td>42.80 (24.00)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td>silicone</td>
<td>0.000 (0.000)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td>PET</td>
<td>0.000 (0.000)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td>polyester</td>
<td>43.75 (24.46)</td>
<td>0.000 (0.000)</td>
</tr>
<tr>
<td>PEEK</td>
<td>8.000 (12.52)</td>
<td>2.500 (4.630)</td>
</tr>
</tbody>
</table>

* Versus all except cobalt chrome.
† Versus all except stainless steel.
‡ Versus all except cobalt chrome, PCU, and polyester.
§ Versus all except cobalt chrome, PCU, and polyester.
¶ Versus all except cobalt chrome, PTFE, and polyester.

Fig. 6. Comparative levels of activated macrophages in the epidural fibrosis and spinal cord at the 3- and 6-month time intervals following implantation of the materials. Significance between the 3-month treatments is as follows: ° versus all except titanium alloy, cobalt chrome, UHMWPe, PCU, and PET. Significance between the 6-month treatments is as follows: † versus all except cobalt chrome; ~ versus all except stainless steel. Bar height indicates mean value and error bars minus 1 SD (1-way ANOVA, F = 3.76, p = 0.000).
tissue reaction to metallic wear debris, as well as the in vitro human macrophage and fibroblast responses to retrieved titanium alloy particles. The use of the New Zealand White rabbit for neurotoxicity investigations is well documented in the literature. Numerous studies have used this in vivo model for studies investigating pharmaceutical agents for neuronal protection and repair, neuronal injury/degeneration resulting from the administration of aluminum maltolate and aluminum chloride via the intracisternal route, and the neurotoxic effects following intrathecal administration of anesthetic medications. The postoperative time intervals were quite variable for these studies and ranged from 48 hours for the spinal anesthesia studies to 267 days for the aluminum toxicity studies. We consider the resultant neurotoxicity effects produced in the New Zealand White rabbit in the current study to be representative of what can be expected in the human population and to correlate with previous clinical studies. The current epidural study complements previous studies by Rivard et al. and Hallab et al., which investigated the in vivo biocompatibility of PEEK and cobalt chromium/nickel, respectively, following epidural application in an experimental animal 12 and 24 weeks postoperatively. Both studies were limited in scope regarding the number of materials investigated and focused primarily on immunoreactivity to particle dose. Of importance, Hallab et al. demonstrated the efficacy of nickel as a positive control in this model because of its immunohistochemical reactivity. Other studies have indicated that corrosion is continually changing the shape, size, and

![Fig. 7. Histological axial sections from 6-month control specimens prepared with H & E (A) and HAM-56 macrophage (B) stains. Histopathological evaluation indicated normal distribution of myelin and intracellular neurofibrillary networks and characterized all control specimens as without significant pathological changes at both the 3- and 6-month time intervals. Original magnification 10×.](image)

![Fig. 8. A and B: Histological sections demonstrating the epidural layer and spinal cord from a 6-month postoperative cobalt chromium (CoCr) specimen. In many cases, a particulate layer formed along the dura mater and consisted of unphagocytosable particles, which were encapsulated by an organized fibrous connective tissue layer. Note the particulate debris (B) and intrathecal dissemination of particles localized subdurally (A). This diffusion process was coupled with histiocytic macrophage response consistent with a localized, chronic inflammatory reaction (macrophages indicated by red regions). Original magnification 40× (A) and 200× (B). C and D: Histological sections demonstrating membrane-bound or intracellular IL-6 cytokines, which produced a yellow to brown chromogen label localized in the epidural fibrosis layer as shown in these 3-month postoperative cobalt chrome treatments. Note the proximity of particulate debris to IL-6–expressing macrophages. ABC–horseradish peroxidase technique for interleukin-6, original magnification 100× (C) and 200× (D).](image)
chemical composition of implanted alloys and that this may alter the biochemical tissue environment surrounding an implant.\textsuperscript{2,22,30,47} As we enter an era of dynamic spinal stabilization using motion-preserving anterior and posterior spinal implant systems, it becomes increasingly important to characterize the neurohistopathological effects of the wear particulates that will eventually be produced. The 11 materials selected for the current project can be stratified into metallic-, polymeric-, and ceramic-based categories. In terms of particle production, the low-angle laser light scattering assays demonstrated reasonable consistency with regard to average particle diameter and size ranges. However, the number-based analysis of particles for the metallic and ceramic groups indicated percentage distributions of 99\% as being less than 1 \( \mu \)m in size. Thus, the actual numbers of particles implanted per gram of material were inordinately high (billions) for the metallic and ceramic treatments compared with the remaining groups (millions of particles per gram). This demonstrates how closely the dose depends on particle sizes, where the numbers of particles vary exponentially (cube) with size of the particles (for a given total mass). These observations may offer some insight into the transient upregulation of macrophage and cytokine activity observed in these treatment groups at the 3-month time interval. The particulate load chosen (4 mg) for each treatment group represented, in our opinion, a worst-case scenario for the spinal cord and membranous coverings in that the particles completely covered the available dura (5-mm-diameter area) at the index surgical procedure. In contrast, the in vivo particulate load that would be produced secondary to in vivo interconnection bearing surfaces or third-body wear would represent a cumulative load with time versus a 1-time bolus application as performed in the current study. The method of particle administration could have been performed using a slow release of material via an epidural catheter because this would mimic the clinical scenario. However, previous studies documenting this technique in the New Zealand White rabbit were fraught with complications, including neurologic deficits, epidural hematomas associated with signs of trauma, and subcutaneous abscesses\textsuperscript{46} at the time of scheduled necropsy. We therefore concluded that a 1-time bolus dose directly to the epidural structures would create a worst-case scenario and minimize experimental artifact (for example, infection and abscess) due to pin track infection and animal husbandry issues related to an external catheter and cage housing.

Moreover, particles of phagocytosable size (\( \leq 10 \mu \)m) used in the current study were found to be the most reactive based on previous investigations\textsuperscript{2} and were applied directly to the dura in dry, sterile condition, without fibrous tissue or ligamentous barriers. Histopathological review of tissues from the reticuloendothelial system indicated no significant pathological changes, and those that occurred were characterized as unremarkable. It is speculated that lymphoreticular dissemination of the metallic, polymeric, and ceramic particulates probably occurred due to the dose and route of administration; however, there was no evidence of particulates in any systemic tissues analyzed.

Immunohistochemical analysis of the spinal cord and overlying epidural fibrosis tissues revealed consistent trends across treatment groups. At the 3-month postoperative interval, there were no significant differences in levels of TNF-\( \beta \), IL-1\( \alpha \), or IL-1\( \beta \) when comparing the operative control and 11 treatment groups. However, the PET treatment exhibited increased levels of TNF-\( \alpha \), and IL-6 was significantly higher for stainless steel, titanium, cobalt chromium, and UHMWPe groups. At 6 months, the levels of TNF-\( \beta \), IL-1\( \alpha \), and IL-1\( \beta \) remained essentially unchanged, whereas TNF-\( \alpha \) increased significantly for the PTFE, PCU, and polyester groups. Most noticeably at the 6 months was the significant reduction in IL-6 activity, which occurred in 6 of 12 treatments. The presence of TNF-\( \alpha \) in local tissues is consistent with an “innate” immune response. Specifically, the local release of TNF-\( \alpha \) stimulates recruitment of neutrophils and monocytes to the site of infection (for example, particulate activation) through 2 mechanisms: 1) stimulation of vascular cells to express new surface receptors and 2) stimulation of endothelial cells and macrophages to secrete chemokines.\textsuperscript{1,31}

In previous investigations using an in vivo rabbit model, Cunningham et al.\textsuperscript{11,12} demonstrated increases in TNF-\( \alpha \) localized within the tissue mass overlying the posterospinal fusion area following application of titanium particulates. A previous clinical study by Takahashi et al.\textsuperscript{44} demonstrated significant increases in serum levels of the proinflammatory cytokine IL-6 following spinal surgery with instrumentation. However, the duration of the increase was limited to 1 week postoperatively, whereas the current study showed elevated levels of IL-6 in local tissues 3 months postoperatively. The long-term presence of IL-6 may be secondary to a continual cycle of particulate phagocytosis by fresh histiocytes and subsequent cytokine release resulting in high concentrations of cytokines within these tissues. At 6 months, however, the levels of...
IL-6 were not significantly greater than those in the control group. Importantly, the transient upregulation in IL-6 may be of beneficial importance. Recent studies suggest that upregulation of both TNF-α and IL-6 expression helps protect neuronal populations from irreversible injury;29,41,42,50 trigger CNS wound repair and regeneration,52 and act as a modulator of neuropathic pain.26 In fact, Klusman and Schwab28 reported that a combination of IL-1β, TNF-α, and IL-6 administered to the lesioned spinal cord of adult mice increased recruitment and activation of macrophages and microglial cells in the lesion area, minimizing tissue loss 7 days after trauma compared with operative controls. Variations in cytokine activity induced by particles from different prosthetic materials has been documented.29,49,50 Haynes et al.50 challenged in vitro cell cultures of human monocytes and compared the cytokine response to 4 different materials: stainless steel, cobalt chrome (forged), cobalt chrome (cast), and titanium alloy particulates. All 4 materials increased cytokine release, but the reactivity levels differed considerably. Stainless steel produced the greatest IL-1β response, whereas forged cobalt chrome increased TNF-α. Titanium alloy particles precipitated the most significant release of IL-6 and prostaglandin E2 compared with all other materials. The macrophage response to particulate debris is dependent on particle size, composition, and dose as given by surface area ratio. To quantify these effects, Shanbhag et al.29 challenged P388D, macrophages with titanium and polystyrene particles (<3 μm). One of the consistent findings in this study was that with a constant surface area ratio, particles of smaller size (<0.45 μm) were less inflammatory, produced fewer cytokines, and resulted in less cellular injury than did larger particles (>1.76 μm). The hypothesis behind this was that larger particles (>0.5 μm) require active phagocytosis, whereas smaller particles can be internalized with the extracellular fluid in a constitutive manner through pinocytosis. This pinocytic uptake may attenuate the histiocytic response. Based on these findings, we used particulate ranges larger than 1 μm (1–10 μm) in the current study to represent a phagocytosable size range and ideally maximize the histiocytic/cytokine response. Statistical comparisons of activated macrophages demonstrated a higher concentration for the metal-based treatments at the 3- and 6-month intervals than other treatments. Importantly, there was no evidence of an acute inflammatory reaction with polymorphonuclear cell response observed in any specimens. Plain and polarized light microscopic analyses provided definitive evidence of particulate phagocytosis by the infiltrating monocytes (macrophages) without evidence of spinal cord lesions or neuropathology in any experimental treatment groups. Histomorphological review of the polymeric treatments, in particular, demonstrated evidence of particulate agglomeration in the epidural tissues. These observations have been reported clinically from previous explant studies of total disc replacements and neurotoxicity studies involving polymeric compounds.17,50 Hence, the polymeric aggregates formed in the current study are likely to be clinically representative of those produced from total disc prostheses or dynamic posterior spinal stabilization hardware.

Conclusions

With the introduction of dynamic spinal stabilization, modular artificial disc replacements, and new materials for orthopedic spinal implants, the effects of implant fretting corrosion on local spinal and systemic tissues will remain a clinical concern. The current project demonstrates the use of a viable animal model to quantify the neural and systemic histopathological responses following epidural application of particulate wear debris. Direct epidural application of spinal instrumentation particulate wear debris elicits a chronic histiocytic reaction localized primarily within the epidural fibrous layers, producing a transient upregulation in macrophage activity and IL-6 cytokine expression at the 3-month time interval. However, corresponding downregulation in macrophage and cytokine activity was observed by 6 months postoperatively. Multiple treatments from the metallic groups demonstrated intrathecal dissemination of particulate to the innermost spinal meninx—pia mater—and spinal cord itself. Despite the worst-case scenario created with the particulate loads we used, there was no significant evidence of an acute neural or systemic histopathological response to the 11 materials included in the current investigation.

Disclosure

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