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Distinct Immunohistomorphologic Changes in Periprosthetic Hip Tissues from Historical and Highly-crosslinked UHMWPE Implant Retrievals

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Abstract

Assessment of immune response to implant wear debris in periprosthetic tissue following total hip arthroplasty suggests that multiple factors are involved in the loss implant function. The current study investigated wear debris and the associated immunohistomorphologic changes in tissues from nine patients with historical (gamma air-sterilized) and nine highly-crosslinked UHMWPE implant components. Paraffin embedded tissue sections were evaluated for the presence of histiocytes, giant cells, fibrocartilage/bone and necrosis. To determine the incidence, degree and co-localization of immunohistomorphologic changes and wear, overlapping full-field tissue arrays were collected in brightfield and polarized light. The historical cohort tissues predominantly showed histiocytes associated with significant accumulations of small wear (0.5–2 μm), and giant cells associated with large wear ($\geq 2 \mu\text{m}$). Frequently, focal regions of necrosis were observed in association with wear debris. For the highly-crosslinked cohort, inflammation and associated wear debris were limited, but in tissues from patients revised after implantation times of >2 years a response was observed. Whereas significant amounts of fibrocartilage/bone were observed in patients at earlier implantation times. In both cohorts, tissue responses were more extensive in the retroacetabular or proximal femoral regions. The current findings suggest that wear debris-induced inflammation may be a major contributor to the loss of implant function for both the historical and highly-crosslinked cohorts, but it is not the primary cause of early implant loosening. This study highlights the importance of using a more quantitative and standardized assessment of immunohistomorphologic responses in periprosthetic tissues, and emphasizes differences in specific anatomical regions of individual patient tissues.

Keywords

Immunohistomorphology; highly-crosslinked UHMWPE; wear debris; periprosthetic tissue; aseptic loosening

Introduction

Implant component wear and the associated immune reaction to wear debris are among the most important factors in determining the longevity of a total joint replacement. [1–5] The major gross, clinical manifestation associated with wear debris generation is osteolysis, which has been identified as a driving force in aseptic implant loosening and the need for revision surgery.

The overall immunohistomorphologic response to ultra high molecular weight polyethylene (UHMWPE) wear debris is a complex process that involves multiple cell types, causes progressive local tissue damage and increases with implantation time. Previous studies have demonstrated that accumulation of submicron and micron-sized wear debris initiates a foreign body inflammatory reaction. This reaction involves the activation of resident cells within the tissue, such as fibroblasts and histiocytes, and the infiltration, maturation and activation of predominantly monocytic phagocytes. [1,6] The continuous influx of UHMWPE wear particles, ingestion of the particles by phagocytosis and the resulting production of pro-inflammatory cytokines and chemokines, can ultimately cause inflammatory-mediated morphologic changes in the periprosthetic tissues. In addition ingestion of wear debris causes cellular necrosis, a highly inflammatory form of cell death. The release of intracellular contents following cell death exacerbates and perpetuates inflammation, tissue necrosis and osteoclast-mediated bone resorption. [7] Collectively, the immunohistopathologic changes in response to wear debris contribute to tissue dysfunction, osteolysis, and implant component loosening.

Particle characteristics of UHMWPE wear debris, including size and number, can affect the inflammatory response in periprosthetic tissue. [8–10] Studies have shown that exposure to UHMWPE wear debris within a specific size range (0.1–1 μm) results in enhanced macrophage activation *in vitro*. [6,11–13] In particular, submicron wear debris has been implicated as a potentially important contributor to the onset of osteolysis. [6,14–17] Others have shown the importance of particle number as a major contributor to the development of tissue morphological changes and osteolysis. [11,18–19] Evaluation of clinical data showed that patients revised for osteolysis consistently had UHMWPE particle quantities in the order of 10^9 particles per gram weight of tissue. [8,18,20] Several *in vitro* studies have also shown a dependence of cytokine release to UHMWPE particle number. [21–22]

To date, studies performed on periprosthetic tissue, while informative, have predominantly been conducted on tissues from patients receiving historical gamma-air sterilized or conventional gamma-inert sterilized UHMWPE components. [23–30,35] The current generation of implants, however, is composed of highly-crosslinked UHMWPE, and an understanding of the immunohistomorphologic response to the newer implant materials is crucial. The assumption has been that the newer material would generate less wear debris, and that the debris would be smaller in size, thus the histiocyte and GC responses *in vivo* would be considerably less. This is the first quantitative study to evaluate whether this assumption is correct, despite *in vitro* studies showing that smaller wear debris induces a significantly greater release of pro-inflammatory cytokines. [31–34] To provide a context of differences between the inflammatory potential of wear debris from these materials, the current study compared immunohistomorphologic changes in hip tissues from patients with historical gamma-air sterilized to highly-crosslinked UHMWPE implant components revised for osteolysis and implant loosening. Our hypothesis was that distinct immunohistomorphologic responses would be observed in tissues from historical and highly-crosslinked UHMWPE components based on specific wear debris characteristics.

Materials & Methods

Tissue Procurement

This multi-center study used a standardized tissue retrieval protocol allowing collection and analysis of periprosthetic hip tissues from patients undergoing revision arthroplasty. Informed consent was obtained prior to tissue collection, and all procedures were performed in accordance with Institutional Review Board guidelines of the participating institutes. Tissue samples were collected from eighteen patients undergoing revision arthroplasty of uncemented, metal-on-UHMWPE hip components. UHMWPE components were classified into two cohorts: historical gamma-air sterilized (n=9) and highly-crosslinked (n=9). Tissues were excised from locations adjacent to the implant, which included the pseudocapsule for all eighteen THR revision surgeries, as well as retroacetabular tissue (acetabular loosening) or proximal femoral tissue (femoral loosening). The clinical details for these two collections of tissue samples are summarized in Tables 1 and 2 for the gamma-air sterilized and highly-crosslinked UHMWPE materials, respectively.

Brightfield, Polarized Light Microscopy and Image Analysis

Fixed tissues were embedded in paraffin and sections (6 μm) were mounted on Fisher Superfrost/Plus slides, dewaxed, rehydrated and stained with hematoxylin and eosin (Fisher Scientific; 245–678) to evaluate tissue morphology. To determine co-localization of histomorphologic changes and wear debris, overlapping full-field tissue arrays were collected in brightfield and polarized light using an Olympus BX50 microscope, equipped with a stepper motor controlled stage (± 2 μm positioning accuracy) and elliptically polarized light imaging system (EPLIS) filters. EPLIS relies on the use of a $\frac{1}{4}$ wavelength birefringent quartz retardation plate, a calibrated rotating polarizing element and a rotating interference filter, which were adjusted to achieve maximum particle/tissue contrast and to eliminate collagen and other non-particle birefringence, allowing us to ensure that only UHMWPE particles were observed. Images were captured at a magnification of 100X using a PixeLINK microscope camera both in brightfield and polarized modes. Pixel resolution was determined using a calibrated scale to be 0.5 $\mu\text{m}/\text{pixel}$ (note, this is not the optical resolution). Brightfield images were scored for the presence of histiocytes, giant cells, necrosis and fibrocartilage/bone. Histomorphologic changes were expressed as a percentage of images that contained each morphological parameter relative to the total number of images. The incidence of these morphological changes was evaluated using a set of established histologic grading criteria: 0, none; 1 *rare*, <10% of fields; 2 *occasional*, 10–30% of fields; 3 *moderate*, 30–50% of fields; 4 *frequent*, >50% of fields [24–25]. In addition, the degree of the histiocyte and giant cell response was determined for each image using a modified semi-quantitative tissue scoring classification (Table 3). [35] The corresponding polarized light images were evaluated to qualitatively determine the presence of small (<2 μm) UHMWPE particles, and to quantitatively determine the number of large ($\geq 2\mu\text{m}$) UHMWPE particles per mm^2 .

The presence of small wear debris was expressed as a percentage of images that contained particles relative to the total number of images. Images were then analyzed to determine large UHMWPE particle number and tissue area using customized image threshold operations programmed in MatlabTM (Mathworks, Inc.). To calculate particle number per mm^2 tissue area, we first applied a threshold to separate tissue from the white background (Fig. 1A). Pixels associated with the tissue were replotted in a new window as a grey layer, and pixels associated with the background were replotted as a green/gold layer (Fig. 1C). UHMWPE particles from the corresponding polarized light image (Fig. 1B) were replotted as a white layer (Fig. 1C). Note that it was necessary to replace the background with the green/gold color to distinguish it from the white UHMWPE particle layer. The average

particle number per mm² was typically analyzed from 15–20 images per section (Table 1 and 2).

To initially validate the elliptically polarized light imaging system (EPLIS), UHMWPE particles were isolated from hip tissues, imaged using environmental scanning electron microscopy (ESEM) in variable pressure mode, and then imaged with EPLIS at 100 and 400X. Particle statistics confirmed that the same particles were observed using both methods. Measurements of particle size/area were performed for the EPLIS image by manually counting pixels, and comparing the size of the same particle in the ESEM image. To calculate particle size error, the following formula was used: %AreaDiff = (ESEMarea–PolarizedLightarea) ÷ ESEMarea. The maximum %AreaDiff was determined to be –2.8%, indicating that the polarized light measurement overestimated particle size by 2.8 percent as compared to the ESEM measurement.

To further evaluate the accuracy of the EPLIS method, serial sections from two historical UHMWPE patient tissues were evaluated using polarized light at 100X and ESEM at 2000X. First, the tissue sections were analyzed using the EPLIS method described above, then serial sections from both patients were digested, filtered and analyzed using ESEM. Three regions of the filters were imaged at 2000X, and ten images were taken of each region. The particle number and size were determined for both systems using a custom macro in NIH ImageJ. For both ESEM and EPLIS, the number of particles within each equivalent circle diameter (ECD) particle size range (1 µm increments) was determined as a percentage of the total number of particles. The number of particles in each size distribution agreed within 5% of each other using a ≥0.5 µm cutoff.

Statistical Analysis

To determine whether the tissue responses (represented by the presence of histiocytes, giant cells, necrosis, fibrocartilage/bone) were proportional to implant-related variables (small UHMWPE, large UHMWPE particle number, revision reason, implantation time), statistics were performed using JMP 6.0 (SAS Institute Inc., Cary, NC). Data were collected from each of 226 images in the historical cohort, and 192 images in the highly-crosslinked UHMWPE cohort. Statistically significant correlates were evaluated using ordinal logistic regression or nominal logistic regression. Separate analyses were conducted for comparison of necrosis and fibrocartilage/bone, and for differences across implant cohort using Fisher's Exact Test. Two-factor analysis of variance (ANOVA) was conducted to evaluate whether the amount of small or large UHMWPE wear debris was significantly correlated with UHMWPE type (historical versus highly-crosslinked) or tissue region (capsular versus non-capsular tissue). There were nine patients in each cohort and gender was equally represented.

Results

Histomorphologic Changes and Wear Debris in Tissues from Historical UHMWPE Implants

The present study was conducted to compare the immunohistology of periprosthetic tissues from patients with historical or highly-crosslinked UHMWPE implant components, and to correlate these changes with wear debris, implantation time, and reason for revision. For the historical UHMWPE component revisions, the average implantation time was 13.3 years (range 9.6–15.6yr). Pseudocapsular tissue was available for all 9 patients and proximal femoral tissues and/or retroacetabular tissue was collected from patients revised for component loosening, femoral (n=3), acetabular (n=2) or both loosened components (n=1). The other reason for revision in this cohort was wear debris and associated osteolysis (n=2).

In evaluating the pseudocapsular tissue response to historical UHMWPE, histiocytes with intracellular small UHMWPE wear debris were observed in four of the nine patient tissues (Fig. 2A-D) (Table 1). The remaining five pseudocapsular tissues were completely devoid of wear debris and histiocytes. When histiocytes were present, there were correlations with increasing implantation time (ordinal logistic regression, $p=0.0036$), small UHMWPE wear debris (ordinal logistic regression, $p<0.0001$) and large UHMWPE particle number (ordinal logistic regression, $p=0.0346$). Of the four patient tissues with histiocytes, two had the maximum score of 3+ (>50 cells/field) in $>50\%$ of the tissue section images. The other two had a score of 2+ (10–49 cells/field) in at least 40% of the fields. With the exception of patient 4, histiocytes were always observed when small wear debris was present. However, some small wear debris was found in the pseudocapsular tissue from patient 4 ($<25\%$ of the fields); no large wear was observed, but 100% of the pseudocapsular fields showed histiocytic necrosis. Regions of focal necrosis were also observed in five other pseudocapsular tissues (Table 1). These regions showed correlations with the presence of small wear debris (nominal logistic regression, $p<0.0001$).

A less frequent finding in the historical cohort pseudocapsular tissue was the presence of giant cells (GCs), which were observed in only two of the nine tissues (Fig. 2A-D) (Table 1). When GCs were present, there was a significant correlation with large UHMWPE particle number (ordinal logistic regression, $p=0.0004$). In addition, the two patient tissues with GCs had the maximum histiocyte score.

For the RA and PF tissues, histiocytes were observed in all six tissues and 67% had a score of 3+ in $>50\%$ of the tissue section images (Fig. 3A). Five of the six non-capsular tissues showed the concomitant presence of GCs and necrosis (Fig. 3A). Similar to the pseudocapsular tissue, histiocytes, GCs and necrosis were observed in the presence of small and large UHMWPE wear debris (Fig. 3B). However, the number of histiocytes (ordinal logistic regression, $p<0.0001$), GCs (ordinal logistic regression, $p<0.0001$) and the amount of wear debris (ANOVA, $p<0.001$) was higher than in the pseudocapsule. Whereas, the number of fields with necrosis was significantly lower than in the pseudocapsular tissue (Fisher's exact test, $p=0.0296$). We noted a patient specific response for patient 6, in that the number of histiocytes in the pseudocapsule tissue was lower than other patient tissues with similar incidence of small wear. This phenomenon was more pronounced in the non-capsular tissue from the same patient. Fibrocartilage/bone was observed in two of the non-capsular tissues, but was not correlated with wear debris, revision reason or implantation time.

Histomorphologic Changes and Wear Debris in Tissues from Highly-crosslinked UHMWPE Implants

For the highly-crosslinked component revisions, the average implantation time was 3.3yr (range 1.5–5.9yr). Pseudocapsular tissue was available for all 9 patients and proximal femoral tissues and/or retroacetabular tissue were collected from patients revised for component loosening, femoral ($n=4$), acetabular ($n=3$) or both loosened components ($n=1$). The other reason for revision in this cohort was subluxation after 5.21 years post-implantation.

In evaluating the pseudocapsular response, seven of nine patient tissues were completely devoid of histiocytes and detectable small UHMWPE wear debris (Table 2, Fig. 4A). Of the two patient tissues with histiocytes, small wear debris was also present. Only tissue from patient 7 contained tissue fields with a histiocyte score of 2+ (10–49 cells/field), which was observed in 33% of the tissue section images. Pseudocapsular tissue from patient 3 had a score of 1+ (<10 cells/field) in 17% of the fields. Similar to the limited presence of

histiocytes, neither giant cells (GCs) nor significant amounts of large UHMWPE wear debris were observed in the pseudocapsular tissues (Table 2).

Necrosis and regions of fibrocartilage/bone were detected in three of the pseudocapsular tissues (Table 2). For patient 7, the combined presence of necrosis and small wear debris was observed; however, this was not a consistent finding for the other two patients. Regions of fibrocartilage/bone were detected in the same three pseudocapsule tissues (Table 2). When fibrocartilage/bone was present, there was a correlation with implantation time, so that patients with shorter implantation times had a higher probability of having fibrocartilage (nominal logistic regression, $p < 0.02$).

For the RA and PF tissues, histiocytes were observed in three of the four patient tissues. Of the three patient tissues with histiocytes, only the RA tissue from patient 7 was given a score of 3+, which was observed in 17% of the tissue section images. In these three non-capsular tissues, a histiocyte score of 2+ was observed in $\leq 20\%$ of the fields. (Fig. 5), and two of these three patients were revised after implantation times of > 2 years. In all non-capsular tissues, histiocytes and small wear debris were observed, except for tissue from patient 4, which contained small wear debris but no histiocytes. In contrast to histiocytes, GCs were only observed in the RA tissue from patient 7, which contained one of the higher percentages of large UHMWPE wear debris.

Necrosis was observed in all four of the non-capsular tissues in association with small UHMWPE wear debris, but unlike the pseudocapsular tissue, the association was not significant. Fibrocartilage/bone was also observed in all of the non-capsular tissues; a higher incidence was consistently observed at earlier implantation times (nominal logistic regression, $p < 0.0001$) (Fig. 5A).

Histomorphologic Changes and Wear Debris Comparison in Tissues from Historical and Highly-Crosslinked UHMWPE Implants

In comparing the responses of the pseudocapsular and non-capsular tissues from the historical cohort, there was an increased presence of histiocytes (ordinal logistic regression, $p < 0.0001$), GCs (ordinal logistic regression, $p < 0.0001$), small UHMWPE wear and particle number (ANOVA, $p < 0.001$) in the non-capsular tissues, whereas necrosis was observed more frequently in the pseudocapsule (Fisher's exact test, $p < 0.0296$). The incidence and average percentage of pseudocapsular and non-capsular tissue responses are summarized for both cohorts in Table 4.

In comparing the responses of pseudocapsular and non-capsular tissues from the highly-crosslinked cohort, one dominant histomorphologic change in non-capsular tissues was the presence of necrosis associated with small wear. The second frequently observed change was the presence of fibrocartilage/bone, which showed a correlation with decreasing implantation time (nominal logistic regression $p < 0.0001$). In general, the immunohistomorphologic responses of pseudocapsular and non-capsular tissues (for either $\leq$ the or > 2 year implants) were not distinctly different (Table 2). The exception was an increased presence of histiocytes in the non-capsular regions (ordinal logistic regression, $p = 0.0002$), which were predominantly from patients with implantation times of > 2 years. The incidence and average percentage of pseudocapsular and non-capsular tissue responses are summarized for both cohorts in Table 4.

Overall, in comparing the responses of the historical and highly-crosslinked cohorts, the quantity of UHMWPE wear debris (ANOVA, $p < 0.001$), and subsequently, the presence of histiocytes (ordinal logistic regression, $p < 0.0001$), GCs (ordinal logistic regression,

$p < 0.0001$) and necrosis (Fisher's exact test, $p < 0.001$) were significantly lower in the highly-crosslinked UHMWPE cohort.

Discussion

In this study, we present the wear debris characteristics and histomorphology of revision tissues for a cohort of patients that received highly-crosslinked UHMWPE implant components. Tissues from this cohort, showed a significant reduction in wear debris, histiocytes, and necrosis as compared to a historical implant cohort, and the presence of GCs was only observed in one patient tissue. However, the presence of small wear and a histiocytic response was evident, and this correlated with increasing times of implantation. For those patients revised at time points ≤ 2 years, there was an increased incidence of focal fibrocartilage/bone, suggesting that implant fixation was incomplete, and the contribution of wear debris was secondary. A detailed comparison of wear debris and tissue responses to a cohort implanted with historical UHMWPE components showed that the historical cohort had extensive tissue histiocytosis and GC formation associated with the presence of small and large UHMWPE wear debris, respectively, all of which were predominantly observed in retroacetabular and proximal femoral tissues.

Our findings for the historical cohort are, for the most part, in agreement with those reported by others [24–25,27,35]. However, our characterization of tissue histomorphology from loosened hip prostheses is unique in that it included a description of the incidence [24–25] and degree [27,35] to which the morphological changes and wear debris occurred. In addition, by analyzing full-field tissue sections, the distinct regional variations of morphological changes and wear debris in each tissue section could be determined. This study highlights the importance of evaluating tissues from multiple regions and emphasizes the need to quantify tissue responses in a more comprehensive manner to ascertain the predominant histomorphologic changes in localized areas, anatomical regions and individual patient tissues.

In agreement with Ito et al. (2004) we found heterogeneity in the distribution of histiocytes, GCs and wear debris within individual tissue sections. [25] In addition to heterogeneity, we separately evaluated the presence of both small and large wear debris, which were associated with histiocytes and GCs, respectively. In contrasting regional differences, our observations also agree with those of Ito et al. (2004) that the number of histiocytes and GCs and the presence of wear debris were predominantly observed in non-capsular, RA and PF tissues. In a separate study of PF tissues from uncemented hip prostheses, Kim et al. reported the presence of histiocytes with a score of 2+ or 3+ in 80% of tissues, and GCs in 40% of tissues using a modified Mirra tissue classification, and UHMWPE wear debris. [27] Similarly, in the current study, images with 2+ or 3+ histiocyte scores were observed as a dominant feature in 83% of PF tissues. However, we did not observe a similar increase in the number of GCs or the amount of wear debris, which may reflect the difference in scoring all fields versus the average of only five fields in their study.

Our finding that small, rather than large UHMWPE wear debris was localized to RA and PF tissues is consistent with the current understanding of particle migration. [36–39] Large particles are trapped in dense, collagen-rich joint connective tissue, while smaller particles are able to move more freely and migrate both locally and to more remote sites along the implant interface, as well as other organs. Multiple studies have demonstrated the migration of UHMWPE wear debris to the lymph nodes, spleen, and liver. [36–37] However, the nature and ultimate fate of the wear debris, and the implications of long-term systemic exposure, are still among the least understood aspects of joint arthroplasty. If the ability to migrate through tissue is size dependent, then it could be speculated that the submicron sized

wear debris species from highly-crosslinked components have a greater potential for diffusion, and a reduced likelihood of initiating a periprosthetic tissue reaction.

One major difference between the two patient cohorts is the implantation times. The average implantation time was 13.3 years (range 9.6–15.6 yr) for the historical cohort and 3.3 years (range 1.5–5.9yr) for the highly-crosslinked cohort. In addition, while the range of implantation times for both cohorts was similar, early revisions within 2 years of implantation for the highly-crosslinked cohort represent a distinct patient subgroup. In one of the few early cadaveric studies evaluating tissue responses for uncemented historical UHMWPE (6 patients), extensive polymeric wear and associated histiocytosis were only observed after an average of 4.8 years. [40] Similarly, Knahr et al (2007) reported the presence of histiocytes, GCs and a few submicron wear particles in pseudocapsular tissues from two patients with highly-crosslinked UHMWPE implants revised after 4 and 5 years. [41] We also observed a histiocytic response to wear debris in patients 6 and 7 from the highly-crosslinked cohort, which were revised after an implantation time of >2 years. This increase was predominantly observed in the non-capsular tissues obtained from regions adjacent to loosened implant components. Thus, similar to the previous findings for the historical implants, wear debris and inflammation are evident after implantation times longer than 2 years.

Recent clinical studies have reported that bedding-in and creep of highly-crosslinked UHMWPE occur during the first 2 years after implantation, and relatively low wear rates are observed at a five-year follow-up. [42–43]. However, the lower wear rates are associated with the generation of smaller particles, which are thought to be more biologically active. [31–34] The biological activity is a measure of cytokine release after ingestion of the small wear debris particles by macrophages, but may not be readily observed in tissues due to the decreased histiocyte swelling/hypertrophy after ingestion of nanometer-sized particles. Our findings agree, in that fewer histiocytes and particles within a range of (0.5–2 μm) were observed in tissues from the highly-crosslinked patient cohort, and that GCs and large wear debris were less frequently observed in these tissue samples.

Given the reduced amount of wear debris and inflammation in the highly-crosslinked cohort tissues, particularly those revised at ≤ 2 years, revision surgery for component loosening may be more dependent on mechanical factors. Previous studies have shown that instead of bone formation or osseointegration, a fibrous membrane can form at the implant-bone interface. [44] This membrane is characteristically thin or absent in well-fixed implants, whereas loose components generate thickened, dense membranes which contain fibrocartilage. The presence of fibrocartilage and lack of detectable wear debris in the highly-crosslinked cohort revised after implantation times of ≤ 2 years is suggestive of decreased implant fixation, and implicates implant micro-motion as a potential contributor to component loosening and/or fibrocartilage formation. While patient 5 was revised at 2.7 years, which is outside of the 2 year cutoff, the tissue response was primarily fibrocartilage, which is consistent with loosening as a result of poor implant fixation. However, for patients revised after implantation times of >2 years, the presence of detectable wear debris and undetected nanometer-sized UHMWPE wear (<0.5 μm pixel resolution) may collectively contribute to the tissue response and implant loosening.

Most previous studies have been performed during a discipline-wide focus of factors contributing to osteolysis, but do not provide a sufficient context of differences between the osteolytic potential of wear debris from new and existing UHMWPE materials. Our observations suggest that differences in size and number of wear debris particles generated from historical and highly-crosslinked implants contribute to a distinct immunohistomorphologic response that results in the loss of implant function, and that the

primary cause of early implant loosening is not due to wear-debris induced inflammation. These observations underscore the need to evaluate pseudocapsular and non-capsular tissues to characterize wear debris and immunohistomorphologic responses that may contribute to implant loosening.

Bibliography

1. Goodman S. Wear particulate and osteolysis. *Orthop Clin North Am.* 2005; 36(1):41–8. [PubMed: 15542121]
2. Abu-Amer Y, Darwech I, Clohisy JC. Aseptic loosening of total joint replacements: mechanisms underlying osteolysis and potential therapies. *Arth Res Ther.* 2007; 9 (Suppl 1):S6. [PubMed: 17634145]
3. Purdue PE, Koulouvaris P, Potter HG, Nestor BJ, Sculco TP. The cellular and molecular biology of periprosthetic osteolysis. *Clin Orthop Rel Res.* 2007; 454:251–61.
4. Looney RJ, Schwarz EM, Boyd A, O'Keefe RJ. Periprosthetic osteolysis: an immunologist's update. *Curr Opin Rheumatol.* 2006; 18(1):80–7. [PubMed: 16344623]
5. Goldring SR, Clark CR, Wright TM. The problem in total joint arthroplasty: aseptic loosening. *J Bone Joint Surg Am.* 1993; 75(6):799–801. [PubMed: 8314820]
6. Ingham E, Fisher J. The role of macrophages in osteolysis of total joint replacement. *Biomater.* 2005; 26(11):1271–86.
7. Milošev I, Antolič V, Minović A, Cör A, Herman S, Pavlovčič V, et al. Extensive metallosis and necrosis in failed prostheses with cemented titanium-alloy stems and ceramic heads. *J Bone Joint Surg Br.* 2000; 82(3):352–7. [PubMed: 10813168]
8. Margevicius KJ, Bauer TW, McMahon JT, Brown SA, Merritt K. Isolation and characterization of debris in membranes around total joint prostheses. *J Bone Joint Surg Am.* 1994; 76(11):1664–75. [PubMed: 7962027]
9. Shanbhag AS, Jacobs JJ, Glant TT, Gilbert JL, Black J, Galante JO. Composition and morphology of wear debris in failed uncemented total hip replacement. *J Bone Joint Surg Br.* 1994; 76(1):60–7. [PubMed: 8300684]
10. Revell PA. The combined role of wear particles, macrophages and lymphocytes in the loosening of total joint prostheses. *J R Soc Interface.* 2008; 5(28):1263–78. [PubMed: 18647740]
11. Green TR, Fisher J, Stone M, Wroblewski BM, Ingham E. Polyethylene particles of a 'critical size' are necessary for the induction of cytokines by macrophages in vitro. *Biomater.* 1998; 19(24):2297–302.
12. Green TR, Fisher J, Matthews JB, Stone MH, Ingham E. Effect of size and dose on bone resorption activity of macrophages by in vitro clinically relevant ultra high molecular weight polyethylene particles. *J Biomed Mater Res.* 2000; 53(5):490–7. [PubMed: 10984696]
13. Ingham E, Fisher J. Biological reactions to wear debris in total joint replacement. *Proc IME HJ Eng Med.* 2000; 214(1):21–37.
14. Shanbhag AS, Bailey HO, Hwang DS, Cha CW, Eror NG, Rubash HE. Quantitative analysis of ultrahigh molecular weight polyethylene (UHMWPE) wear debris associated with total knee replacements. *J Biomed Mater Res.* 2000; 53:100–10. [PubMed: 10634959]
15. Schmalzried TP, Campbell P, Schmitt AK, Brown IC, Amstutz HC. Shapes and dimensional characteristics of polyethylene wear particles generated in vivo by total knee replacements compared to total hip replacements. *J Biomed Mater Res.* 1997; 38(3):203–10. [PubMed: 9283965]
16. Lapcikova, M.; Slouf, M.; Dybal, J.; Zolotarevova, E.; Entlicher, G.; Pokorny, D., et al. Nanometer size wear debris generated from ultrahigh molecular weight polyethylene in vivo. In: Richter, S.; Schwedt, A., editors. *EMC 2008. Springer; Berlin Heidelberg: 2008.* p. 767-8.
17. Slouf M, Pokorny D, Entlicher G, Dybal J, Synkova H, Lapcikova M, et al. Quantification of UHMWPE wear in periprosthetic tissues of hip arthroplasty: Description of a new method based on IR and comparison with radiographic appearance. *Wear.* 2007; 265(5–6):674–84.

18. Kobayashi A, Freeman MA, Bonfield W, Kadoya Y, Yamac T, Al-Saffar N, et al. Number of polyethylene particles and osteolysis in total joint replacements. A quantitative study using a tissue-digestion method. *J Bone Joint Surg Br.* 1997; 79(5):844–8. [PubMed: 9331048]
19. Kadoya Y, Revell PA, Kobayashi A, al-Saffar N, Scott G, Freeman MA. Wear particulate species and bone loss in failed total joint arthroplasties. *Clin Orthop Rel Res.* 1997; 340:118–29.
20. Tipper JL, Ingham E, Hailey JL, Besong AA, Fisher J, Wroblewski BM, et al. Quantitative analysis of polyethylene wear debris, wear rate and head damage in retrieved Charnley hip prostheses. *J Mater Sci Mater Med.* 2000; 11(2):117–24. [PubMed: 15348056]
21. Matthews JB, Green TR, Stone MH, Wroblewski BM, Fisher J, Ingham E. Comparison of the response of primary human peripheral blood mononuclear phagocytes from different donors to challenge with model polyethylene particles of known size and dose. *Biomater.* 2000; 21:2033–44.
22. Matthews JB, Besong AA, Green TR, Stone MH, Wroblewski BM, Fisher J, et al. Evaluation of the response of primary human peripheral blood mononuclear phagocytes to challenge with in vitro generated clinically relevant HMWPE particles of known size and dose. *J Biomed Mater Res.* 2000; 52:296–307. [PubMed: 10951368]
23. Willert HG, Bertram H, Buchhorn GH. Osteolysis in alloarthroplasty of the hip. The role of ultra-high molecular weight polyethylene wear particles. *Clin Orthop Rel Res.* 1990; 258:95–107.
24. Goodman SB, Chin RC, Chiou SS, Schurman DJ, Woolson ST, Masada MP. A clinical-pathologic-biochemical study of the membrane surrounding loosened and nonloosened total hip arthroplasties. *Clin Orthop Rel Res.* 1989; 244:182–7.
25. Ito S, Matsumoto T, Enomoto H, Shindo H. Histological analysis and biological effects of granulation tissue around loosened hip prostheses in the development of osteolysis. *J Orthop Sci.* 2004; 9(5):478–87. [PubMed: 15449123]
26. Kim KJ, Rubash HE, Wilson SC, D'Antonio JA, McClain EJ. A histologic and biochemical comparison of the interface tissues in cementless and cemented hip prostheses. *Clin Orthop & Rel Res.* 1993; 287:142–52.
27. Kim KJ, Chiba J, Rubash HE. In vivo and in vitro analysis of membranes from hip prostheses inserted without cement. *J Bone Joint Surg Am.* 1994; 76(2):172–80. [PubMed: 8113250]
28. Chun L, Yoon J, Song Y, Huie P, Regula D, Goodman S. The Characterization of Macrophages and Osteoclasts in Tissues Harvested from Revised Total Hip Prostheses. *J Biomed Mater Res: Appl Biomater.* 1999; 48:5.
29. Hansen T, Otto M, Buchhorn G, Scharnweber D, Gaumann A, Delank K, et al. New aspects in the histological examination of polyethylene wear particles in failed total joint replacements. *Acta Histochemica.* 2002; 104:7.
30. Goodman SB, Knoblich G, O'Connor M, Song Y, Huie P, Sibley R. Heterogeneity in cellular and cytokine profiles from multiple samples of tissue surrounding revised hip prostheses. *J Biomed Mater Res.* 1996; 31:421–8. [PubMed: 8806069]
31. Endo M, Tipper JL, Barton DC, Stone MH, Ingham E, Fisher J. Comparison of wear, wear debris and functional biological activity of moderately crosslinked and non-crosslinked polyethylenes in hip prostheses. *Proc IME HJ Eng Med.* 2002; 216(2):111–22.
32. Ingram J, Stone M, Fisher J, Ingham E. The influence of molecular weight, crosslinking and counterface roughness on TNF-alpha production by macrophages in response to ultra high molecular weight polyethylene particles. *Biomater.* 2004; 25:3511–22.
33. Goodman SB, Goldberg V, O'Keefe R. Biology Summary; on Behalf of the Implant Wear Symposium 2007 Biologic Work Group. *J Am Acad Orthop Surg.* 2008; 16(Suppl 1):S76–8.
34. Richards L, Brown C, Stone MH, Fisher J, Ingham E, Tipper JL. Identification of nanometre-sized ultra-high molecular weight polyethylene wear particles in samples retrieved in vivo. *J Bone Joint Surg Br.* 2008; 90(8):1106–13. [PubMed: 18669972]
35. Mirra JM, Amstutz HC, MM, Gold R. The pathology of the joint tissues and its clinical relevance in prosthesis failure. *Clin Orthop.* 1976; 117:221–40. [PubMed: 776484]
36. Basle MF, Bertrand G, Guyetant S, Chappard D, Lesourd M. Migration of metal and polyethylene particles from articular prostheses may generate lymphadenopathy with histiocytosis. *J Biomed Mater Res.* 1996; 30:157–63. [PubMed: 9019479]

37. Urban RM, Jacobs JJ, Tomlinson MJ, Gavrilovic J, Black J, Peoc'h M. Dissemination of wear particles to the liver, spleen, and abdominal lymph nodes of patients with hip or knee replacement. *J Bone Joint Surg Am.* 2000; 82(4):457–76. [PubMed: 10761937]
38. Mabrey J, Afsar-Keshmiri A, Engh G, Sychterz C, Wirth M, Rockwood C, et al. Standardized analysis of UHMWPE wear particles from failed total joint arthroplasties. *J Biomed Mater Res: Appl Biomater.* 2002; 63:475–83.
39. Elfick APD, Green SM, Krikler S, Unsworth A. The nature and dissemination of UHMWPE wear debris retrieved from periprosthetic tissue of THR. *J Biomed Mater Res.* 2003; 65A:95–108.
40. Santavirta S, Kontinen YT, Bergroth V, Eskola A, Tallroth K, Lindholm TS. Aggressive granulomatous lesions associated with hip arthroplasty. Immunopathological studies. *J Bone Joint Surg Am.* 1990; 72(2):252–8. [PubMed: 1968067]
41. Knahr K, Pospischill M, Kottig P, Schneider W, Plenck H Jr. Retrieval analyses of highly cross-linked polyethylene acetabular liners four and five years after implantation. *J Bone Joint Surg Br.* 2007; 89(8):1036–41. [PubMed: 17785741]
42. Digas G, Karrholm J, Thanner J, Herberts P. 5-year experience of highly cross-linked polyethylene in cemented and uncemented sockets: two randomized studies using radiostereometric analysis. *Acta Orthop.* 2007; 78(6):746–54. [PubMed: 18236180]
43. Dorr LD, Wan Z, Shahrddar C, Sirianni L, Boutary M, Yun A. Clinical Performance of a Durasul Highly Cross-linked Polyethylene Acetabular Liner for Total Hip Arthroplasty at Five Years. *J Bone Joint Surg Am.* 2005; 87:1816–21. [PubMed: 16085624]
44. Kontinen YT, Zhao D, Beklen A, Ma G, Takagi M, Kivela-Rajamaki M, et al. The microenvironment around total hip replacement prostheses. *Clin Orthop Rel Res.* 2005; (430):28–38.

Figure 1. UHMWPE wear debris characterization in revised hip tissue

(A) Brightfield image showing the tissue area, (B) polarized light image showing UHMWPE particles, and (C) a composite of A and B generated as layers using customized threshold operations programmed in MatlabTM to determine the particle number per mm² area of tissue (100x).

Figure 2. Representative images of H&E-stained pseudocapsule tissues from historical UHMWPE THRs

(A) Normal immunohistomorphology observed in five of the nine pseudocapsular tissues, and (B) the corresponding polarized light image showing collagen fibers (pink arrow) that are only visible before filter alignment. (C) Histiocytes (blue arrow) observed in four of the nine pseudocapsular tissues, and giant cells (green arrow) observed in two of the four tissues, and (D) the corresponding polarized light image showing large (yellow arrow) and small UHMWPE wear co-localized with giant cells and histiocytes, respectively (200x).

Figure 3. Representative images of H&E-stained proximal femoral tissues from historical UHMWPE THRs

(A) Histiocytes (blue arrow) observed in all of the non-capsular tissues and giant cells (green arrow) observed in five of the six tissues, and (B) the corresponding polarized light image showing UHMWPE wear debris (yellow arrow) (200x).

Figure 4. Representative images of H&E-stained pseudocapsule tissues from highly-crosslinked UHMWPE THRs

(A) Normal immunohistomorphology observed in seven of the nine pseudocapsular tissues, and (B) the corresponding polarized light image showing no visible wear debris (200x).

Figure 5. Representative image of fibrocartilage/bone in retroacetabular tissues from highly-crosslinked UHMWPE THRs

(A) A segment of bone (red arrow) with outer regions of fibrocartilage embedded within loose connective tissue. Visible within the tissue is an osteoclast (orange arrow) in a resorption lacuna, and necrosis (inset) associated with histiocytes. Histiocytes (blue arrow) are visible near the top edge of the bone, and (B) the corresponding polarized light image showing a small UHMWPE particle (yellow arrow) (200x).

Table 1
Clinical information and tissue scoring for historical UHMWPE hip implant revision tissues

Abbreviations: C=pseudocapsule, PF=proximal femur, RA=retroacetabulum. UHMWPE wear ranged from 0.5–2 μm at 100X and included particles $>0.35\mu\text{m}$ at 400X based on the resolution limit of microscopy. The lower cutoff for large UHMWPE wear was 2 μm . To indicate samples with a limited number of particles/field, the total particle count is represented as (% -- #).

Patient #	Implantation Time (y)	Gender	Revision Reason	Implant Type	Tissue Region	Histocyte Score (%)				Small (<2 μm) UHMWPE Particles (%)		Large (>2 μm) UHMWPE Particles (Number/field)				Percentage Large Particles /Area (%)	
						0	1	2	3	0	1	0	1	2	3		
1	9.6	F	PE Wear, Osteolysis	Zimmer HGT 1	C	20	10	70	0	60	50	0	0	0	0	14,555.0	0
2	11	M	Femoral Locking	Boston	PF	100	0	0	0	0	0	0	0	0	0	0.145.1	0
3	12.7	F	Acetabular Locking	Boston	C	20	0	80	0	60	60	0	0	0	0	10,443.4	0
4	13.1	M	PE Wear, Osteolysis (femoral)	Boston	RA	100	0	0	0	100	100	0	0	0	0	181,833.4	86
5	13.6	F	Femoral Locking, PE wear	Boston	C	43	13	43	0	0	0	0	0	0	0	0.145.1	0
6	14.2	F	Femoral Locking, PE wear	Boston	PF	100	0	0	0	0	0	0	0	0	0	0.145.1	0
7	15.1	M	PE Wear, Osteolysis (acetabulum)	Zimmer HGT 1	C	43	13	43	0	63	90	0	0	0	0	0.145.1	0
8	15.4	M	PE Wear, Osteolysis (acetabulum & femoral)	Zimmer HGT 1	RA	100	0	0	0	100	100	0	0	0	0	0.145.1	0
9	15.6	F	PE Wear, Osteolysis	Boston	C	100	0	0	0	100	100	21	4	21	37	19,292.2	7

Table 2
Clinical information and tissue scoring for highly-crosslinked UHMWPE hip implant revision tissues

Abbreviations: C=pseudocapsule, PF=proximal femur, RA=retroacetabulum. UHMWPE wear ranged from 0.5–2 μm at 100X and included particles $>0.35\mu\text{m}$ at 400X based on the resolution limit of microscopy. The lower cutoff for large UHMWPE wear was 2 μm . To indicate samples with a limited number of particles/field, the total particle count is represented as (% -- #).

Patient #	Implantation Time (y)	Gender	Revision Reason	Implant Type	Tissue Region	Histiocyte Score (%)				Small ($<2\mu\text{m}$) UHMWPE Wear (%) ^a	Necrosis (%)	Giant cell Score (%)				Large ($\geq 2\mu\text{m}$) UHMWPE Particle Number/mm ²	Fibrocartilage/Bone (%)
						0	1+	2+	3+			0	1+	2+	3+		
1	1.5	F	Acetabular Loosening	Stryker Trident	C	100				0	8	100				0.7 $\pm$ 0.2	60
2	1.6	M	Femoral Loosening	Stryker Trident	C	100				0	0	100				0.7 $\pm$ 0.3	0
					PF	50	30	20		10--4	10	100				0.8 $\pm$ 0.3	60
3	1.7	F	Acetabular & Femoral Loosening	Zimmer Longevity	C	83		17		25--22	0	100				2.9 $\pm$ 0.7	0
4	2	M	Femoral Loosening	Stryker Trident	C	100				0	0	100				0.3 $\pm$ 0.1	0
					PF	100				33--13	33	100				0.4 $\pm$ 0.2	6
5	2.7	F	Acetabular Loosening	Stryker Trident	C	100				0	67	100				0.0	67
6	3.7	F	Femoral Loosening	Stryker Omnifit Ser. II	C	100				0	0	100				0.1 $\pm$ 0.1	0
					PF	85	10	5		15--6	5	100				1.9 $\pm$ 0.4	15
7	5.2	F	Acetabular Loosening	Stryker Trident	C	45	22	33		44--24	56	100				2.5 $\pm$ 0.4	22
					RA	44	28	11	17	50--10	6	94		6		2.9 $\pm$ 0.4	6
8	5.2	F	Subluxation	Stryker Trident	C	100				0	0	100				0.3 $\pm$ 0.1	0
9	5.9	M	Femoral Loosening	Stryker Trident	C	100				0	0	100				0.8 $\pm$ 0.22	0

Table 3
Histiocyte and giant cell scoring criteria based on a modification of the method by Mirra et al. (1976)

[35]

Histiocyte/Macrophage	
0	0 cells
1+	1–9 cells/low power field (100x)
2+	10–49 cells/low power field (100x)
3+	>50 cells/low power field (100x)
Giant Cell	
0	0 cells
1+	1–2 cells/low power field (100x)
2+	3–8 cells/low power field (100x)
3+	>9 cells/low power field (100x)

Table 4
Summary of pseudocapsular and non-capsular tissue responses for historical and highly-crosslinked UHMWPE implant revisions

Abbreviations: n = the incidence, N = total number of tissues. Presented is the average percentage of fields (minimum and maximum) containing histiocytes, GCs, necrosis, fibrocartilage and small UHMWPE wear for pseudocapsular and non-capsular regions. Large UHMWPE wear is presented as the average particle number/mm².

Pseudocapsular Tissue	Historical		Highly-crosslinked	
	n/N	Average (min-max)	n/N	Average (min-max)
Histiocytes	4/9	32% (0–100)	2/9	8% (0–55)
Giant Cells	2/9	8% (0–58)	0/9	0%
Necrosis	6/9	33% (0–100)	3/9	15% (0–67)
Fibrocartilage	0/9	0%	3/9	17% (0–67)
Small (<2µm) UHMWPE	5/9	33% (0–100)	2/9	8% (0–44)
Large (≥2µm) UHMWPE	8/9	6/mm ² (0–19)	8/9	1/mm ² (0–3)
Non-capsular Tissue	Historical		Highly-crosslinked	
	n/N	Average (min-max)	n/N	Average (min-max)
Histiocytes	6/6	77% (6–100)	3/4	33% (0–56)
Giant Cells	5/6	25% (0–55)	1/4	1.5% (0–6)
Necrosis	5/6	16% (0–32)	4/4	14% (5–33)
Fibrocartilage	2/6	14% (0–86)	2/4	22% (6–60)
Small (<2µm) UHMWPE	6/6	84% (6–100)	3/4	27% (10–50)
Large (≥2µm) UHMWPE	6/6	55/mm ² (3–182)	4/4	1.5/mm ² (0.4–2.9)