
Analysis of polyethylene wear debris using micro-Raman spectroscopy: A report on the presence of beta-carotene

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This paper describes micro-Raman spectroscopy of ultra-high molecular weight polyethylene wear debris isolated from revised knee replacements. The novel application of micro-Raman spectroscopy to the analysis of *in vivo*-generated wear debris was used to evaluate the chemical nature of individual, retrieved polyethylene particles. The analysis revealed the presence of β -carotene on particles from both synovial fluid and tissue samples. Raman analysis

of retrieved polyethylene tibial inserts also revealed localized β -carotene signals within the primary wear region. In this paper, a mechanism is suggested that may account for the coupling of β -carotene and polyethylene wear debris. We also discuss the origin of β -carotene within the implanted joint and the implications that β -carotene, an anti-oxidant, has for the overall host response to polyethylene orthopedic components. © 1997 John Wiley & Sons, Inc.

INTRODUCTION

Premature failure of implant systems remains a significant problem in total joint replacement. In particular, wear of nonmetallic load-bearing surfaces, notably ultra-high molecular weight polyethylene, remains a limiting factor in device longevity. Accordingly, many research activities have focused on polymer wear and the subsequent host response to wear debris, which have enhanced our understanding of these complex interactions. Recent studies suggest that wear debris may play a fundamental role in a cascade of events with consequent osteolysis and bone resorption.¹⁻⁵ It has been reported that retrieved periprosthetic tissues contain significant volumes of submicron-sized, ultra-high molecular weight polyethylene (UHMWPE) particles.⁶⁻¹⁰ This is consistent with an emerging view that polymer wear debris, particularly ultra-high molecular weight polyethylene, plays an important role in the development of osteolysis and premature device failure.¹¹⁻¹³

The characterization of *in vivo*-generated wear debris typically involves extracting particles from tissue surrounding the implanted joint, although particles from synovial fluid also may be successfully sepa-

rated. The concentration of debris is much higher in tissue than in synovial fluid because of the continuous filtering action of the synovial tissue. Once wear particles are isolated for analysis, characterization is usually accomplished through light microscopy and scanning electron microscopy. Such studies have revealed much information about the morphologies, prevalence, and size distributions of wear debris, UHMWPE in particular.^{8,14-16} A large body of the characterization research is summarized in a recent review paper concerning the size and shape of biomaterial wear debris.⁹ However, although the focus on physical features of wear debris has elucidated many aspects of *in vivo* wear processes, what has been lacking up to now is a parallel effort to provide complementary information about the *chemical* nature of *in vivo*-generated wear debris. Such information would be useful in assessing wear mechanisms, such as oxidation processes, and could provide new insight into the resulting histiocytic response to wear debris.

Chemical analysis of the UHMWPE components of total joint replacements is not a wholly new approach, but it is one that has been limited to bulk samples and within those samples has focused primarily on oxidation processes. A number of studies have utilized Fourier transform infrared spectroscopy (FTIR) to examine the *in vivo* degradation of UHMWPE components and the role of oxidation.¹⁷⁻²⁰ In another study, the presence of free radicals in retrieved UHMWPE ace-

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tabular cups and tibial inserts was determined using electron spin resonance.²¹ To date, these analytical techniques have not been used for the analysis of UHMWPE wear particulates, although FTIR has been used for identification of large assemblies of UHMWPE particles.^{8,14,16} We report on a technique, micro-Raman spectroscopy, that enables quantitative chemical analysis of individual wear particles and present results from our study of UHMWPE wear debris from revised total knee implants.

Raman spectroscopy, like infrared (IR) spectroscopy, is a vibrational spectroscopy technique. However, rather than continuous scanning through a range of wavenumbers, as with IR spectroscopy, Raman spectroscopy utilizes the shift in wavenumber with respect to a single incident wavelength. Typically, a laser beam is the single excitation source, which offers the option of focusing the beam within an optical microscope. Micro-Raman spectroscopy allows analysis of materials with a spatial resolution of several microns, which is well suited for the examination of individual wear particulates, as described in the present study.

MATERIALS AND METHODS

Synovial fluid, tissue samples, and UHMWPE tibial inserts were collected from patients who underwent revision surgery of cementless total knee replacements. The clinical data for the seven patients included in this study are summarized in Table I. The implants were *in situ* between 39 and 96 months, with an average of 69 months. The primary reasons for revision included osteolysis, failed metal-backed patella, polyethylene wear, and implant instability. All implants had a tibial insert and patella made of ultra-high molecular weight polyethylene.

At the time of revision surgery, the synovial fluid samples were drawn through the synovium into a syringe and dispensed through an air-tight rubber stopper into a glass test tube or sterile plastic vial. Synovial fluid samples were stored under refrigeration prior to

analysis. Tissue samples were collected and stored in formalin in a sterile specimen container.

Polyethylene wear particles were isolated from both synovial fluid and tissue samples using a protocol involving digestion in sodium hydroxide.²² Complete details of the separation procedure are provided elsewhere.²³ Briefly, the samples were placed in two parts 5N NaOH solution, by weight, and heated in a 65°C water bath until digested, typically 24 h. UHMWPE wear debris then was separated out using a series of centrifugation and washing steps. One hundred microliters from the top of the final washed samples were dispersed onto a glass coverslip and allowed to dry under a Class II laminar flow hood. One synovial fluid sample also was separated out using only centrifugation and washing, with the NaOH digestion omitted.

Bulk UHMWPE samples (approximately 1 cm × 3 mm pellets) were cut from 1/2"-thick sheets of UHMWPE, as supplied from a specialty plastics firm. The UHMWPE powder used in this study was 415 GUR (Hoechst/Celanese), with a nominal particle diameter of 100 μm.

The micro-Raman spectrometer consisted of an argon ion laser beam (496.5 nm) focused through a microscope objective to a 3 μm beam diameter. Raman back-scattered light was collected through the objective and resolved with a triple-monochromator (double monochromator filter stage and 2400 line/mm spectrometer stage). The spectra were recorded with a software-controlled CCD array. The laser power was approximately 1 mW, and sampling times were varied from 10 to 60 sec. An imaging system and high-resolution color monitor were incorporated so as to allow selection and Raman analysis of single particles with a spatial resolution of several microns.

Because of the finding of β-carotene on the UHMWPE wear particles, as discussed under Results, efforts were made to dope pristine UHMWPE powder (415 GUR) with β-carotene, and experiments were performed to assess any effects of the tissue digestion process. In addition, the retrieved UHMWPE tibial inserts were examined using micro-Raman spectroscopy. The procedures for the doping of UHMWPE with β-carotene and the control digestion experiments are described below.

The β-carotene used for all experiments was Type I synthetic, crystalline all-trans-β-carotene (Sigma Chemical). The β-carotene-doped UHMWPE particles were prepared as follows: 350 mg of β-carotene were dissolved at room temperature in 80 mL of pure isopropyl alcohol, and 14 g of UHMWPE powder (415 GUR) then were added. The solution was agitated for 6 h and then rinsed repeatedly in isopropyl alcohol. The final rinsed particles were air dried in a petri dish for approximately 24 h. The same procedure was used for the doping of bulk UHMWPE pellets. The β-carotene

TABLE I
Clinical Data for the Synovial Fluid, Tissue, and Retrieved Component Samples

Age/Sex/Weight (Yr/—/Lb)	Prosthesis Type	<i>In Situ</i> (Months)	Reason for Revision
48/F/179	Synatomic	90	Failed patella
63/M/238	M-G I	36	Instability
65/M/235	AMK	40	Osteolysis Polyethylene wear
66/F/168	AMK	81	Failed patella
67/F/159	Synatomic	48	Polyethylene wear
71/M/250	Osteonics uni	89	Osteolysis
76/M/155	PCA	96	Osteolysis

tene-doped particles and pellets were stored in sealed containers and kept in darkness to preclude any photochemical activity.

Bulk UHMWPE pellets (1 cm × 3 mm) were used to assess the effects of the NaOH digestion of the β -carotene signals. Synovial tissue samples were divided in half, then pellets of UHMWPE were added to one half, and β -carotene-doped UHMWPE pellets were added to the second half. The tissue samples then were digested in NaOH, as described above, and the polyethylene pellets were removed, rinsed in ethyl alcohol, and dried.

RESULTS

The focus of the present study was on the identification and analysis of individual UHMWPE wear particles after separation from synovial fluid and tissue samples. Polyethylene is well suited for Raman spectroscopy, and the principal Raman active bands have been well characterized.²⁴ Figure 1 is a Raman spectrum obtained from a single UHMWPE particle (415 GUR). All spectra were recorded from 950–1550 cm^{-1} , which spans the “fingerprint” region associated with polyethylene. Polyethylene features are present at 1065, 1132, 1170, 1295, 1370, 1416, 1440, and 1460 cm^{-1} , with the corresponding vibrational assignments labeled in Figure 1. The dominant bands are the C-C stretching vibrations at 1065 and 1132 cm^{-1} , and the C-H twisting vibration at 1295 cm^{-1} .

The unique Raman spectrum of polyethylene enabled the absolute identification of individual UHMWPE wear particles, as we noted in an earlier study.²³ Individual particles ranging in size from several microns to 100 microns were analyzed using the Raman micro-probe. The Raman spectra of several iso-

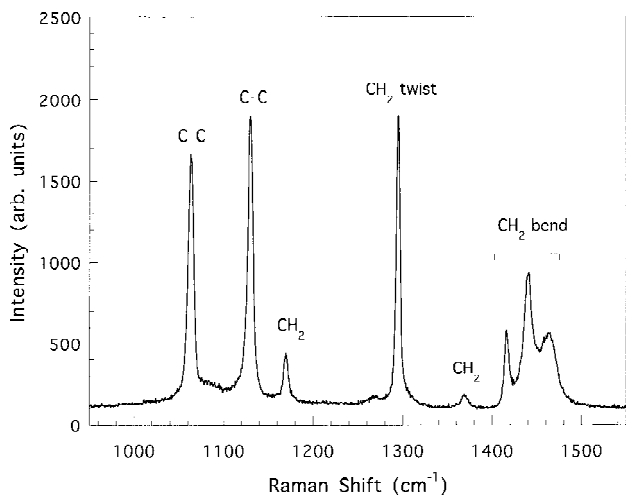


Figure 1. Raman spectrum of reference UHMWPE particle (415 GUR).

lated wear particles are presented in Figure 2. These spectra are all of UHMWPE wear particles, based on the exhibited polyethylene features, and demonstrate the utility of micro-Raman for UHMWPE identification. Using micro-Raman spectroscopy many particles that earlier had been observed to exhibit characteristics often associated with polyethylene, including birefringence, diffuse light scattering, and recognized morphologies, were found not to be polyethylene.

What we found most interesting are the additional peaks (i.e., non-UHMWPE) near 1005, 1155, and 1514 cm^{-1} , observed in the wear particle spectra and shown in Figure 2. These additional bands were observed, with varying strengths, on all of the separated tissue and synovial fluid polyethylene wear particles we examined. As explained below, these peaks are attributed to the presence of β -carotene on the polyethylene surface and are consistent with published literature regarding Raman spectroscopy of β -carotene.^{25,26}

In an effort to duplicate the spectra in Figure 2, we doped pristine UHMWPE particles with β -carotene and recorded the Raman spectra. The Raman spectrum of one of these β -carotene-doped UHMWPE particles is presented in Figure 3 along with the spectrum of a separated UHMWPE wear particle. The agreement between the spectra of the *in vivo*-generated wear particle and the β -carotene-doped particle is excellent. Also included in Figure 3 is the Raman spectrum of the all-trans- β -carotene powder used to dope the polyethylene particles. The primary peaks at 1005, 1155, and 1525 cm^{-1} are observed in the β -carotene reference spectrum along with the secondary bands at 960 and 1190 cm^{-1} . An additional feature in Figure 3 is noted: the β -carotene band at 1525 cm^{-1} , which corre-

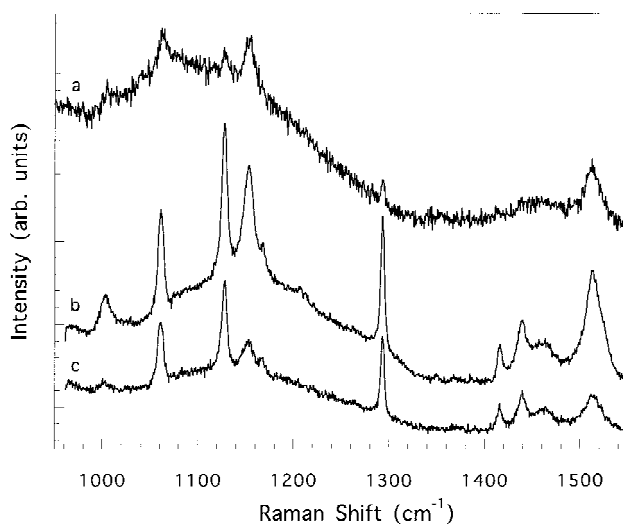


Figure 2. Raman spectra of individual UHMWPE wear particles separated from synovial fluid and tissue samples: (a) synovial fluid particle; (b) synovial tissue particle; (c) synovial tissue particle. The spectra have been shifted vertically for clarity.

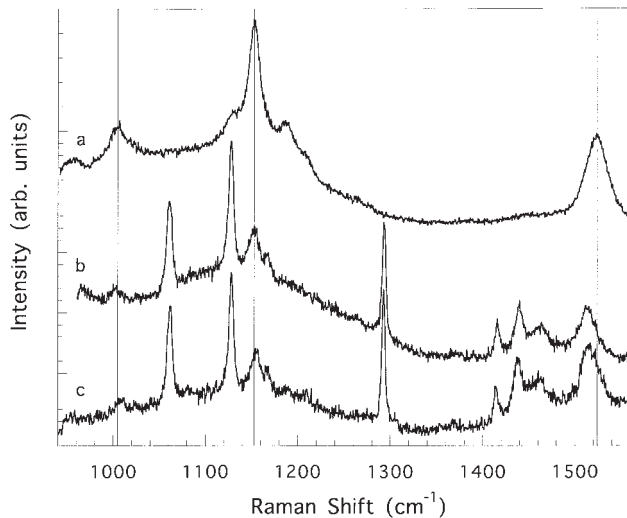


Figure 3. Raman spectra of (a) pure all-trans- β -carotene powder; (b) UHMWPE wear particle separated from synovial tissue; (c) β -carotene-doped UHMWPE particle. The spectra have been shifted vertically for clarity.

sponds to the C = C stretching vibration, is shifted by about 10 cm^{-1} between the β -carotene reference spectrum and the spectra of the polyethylene particles. This shift was noted on the spectra of both separated wear debris and on our β -carotene-doped particles.

Although the focus was on the Raman analysis of polyethylene wear debris, in light of the β -carotene findings, the retrieved UHMWPE tibial inserts were examined also. The Raman spectra recorded from various positions on a single tibial insert are presented in Figure 4. The two upper spectra were both recorded within the primary wear zone and correspond to similar surface morphologies, whereas the lower spectrum

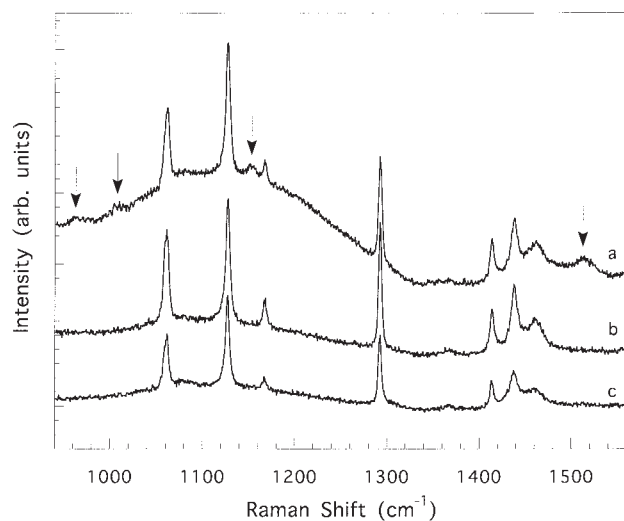


Figure 4. Raman spectra of retrieved UHMWPE tibial insert: (a) β -carotene signal (see arrows) within primary wear region; (b) primary wear region with no β -carotene signal; (c) central surface of implant, no wear. The spectra have been shifted vertically for clarity.

corresponds to the central surface of the implant, an area with no surface wear. The β -carotene signal corresponding to the peaks at 1005 , 1155 , and 1514 cm^{-1} is pronounced in the upper spectrum, whereas no detectable β -carotene bands are present in the two lower spectra. This intermittency was typical of the Raman analysis of retrieved implants, in which β -carotene was detected on several implants but the signals were very localized and, although restricted to the wear zone, revealed no correlation with observed surface morphologies.

Raman spectra were recorded from the control (i.e., undoped) and the β -carotene-doped UHMWPE pellets following NaOH digestion in the tissue sample halves. The β -carotene-doped polyethylene pellets exhibited Raman spectra identical to the spectra of the original, undigested doped pellets. This finding demonstrated that the β -carotene signal on UHMWPE is not affected by the NaOH/tissue digestion process. However, for the control polyethylene pellets (i.e., undoped), 50% of the samples revealed weak but distinct β -carotene signals after the NaOH digestion with tissue from implanted knee joints.

DISCUSSION

We have demonstrated that micro-Raman spectroscopy is of great utility in the identification and analysis of wear particulates such as ultra-high molecular weight polyethylene. This approach holds potential for the analysis of wear debris because, at present, the positive identification of individual polyethylene particles during imaging studies remains qualitative. Significantly, we observed many particles that exhibited characteristics often associated with polyethylene that, based on Raman spectroscopy, were found not to be polyethylene. Micro-Raman analysis for the study of wear debris is attractive both for detailed particle studies and for "training" sessions in the recognition of polyethylene wear particles. Finally, other types of wear particles, such as TiO_2 , have Raman-active vibrational modes and are therefore assessable by the micro-Raman technique.

In addition to using micro-Raman spectroscopy for the identification of UHMWPE wear debris, we have performed a detailed analysis of the chemical nature of these *in vivo*-generated wear particles. The most significant finding was the presence of β -carotene on UHMWPE wear particles separated from both synovial fluid and tissue samples and on retrieved UHMWPE tibial inserts. In a thorough literature search, we found that β -carotene has been reported in normal human femoral bone,²⁷ but never, to our knowledge, has β -carotene been associated with *in vivo*-generated wear debris. Furthermore, the β -caro-

tene bands in this work are not associated with bone fragments due to the absence of the sharp phosphate peak at 960 cm^{-1} , that would indicate bone.²⁸

Although we have attributed the nonpolyethylene spectral features in this work to the presence of β -carotene, it is useful to discuss several of the candidates that were evaluated, namely other carotenoids and unmethylated polyene molecules. Unmethylated polyene chains, including those possibly derived from the polyethylene molecule, may be ruled out based on the observed Raman bands near 1005 and 1155 cm^{-1} .^{29,30} With the absence of the methyl groups from the polyene chain, carotenoids lose the band at 1005 cm^{-1} and exhibit a shift in the 1155 cm^{-1} band to approximately 1135 cm^{-1} ; hence the present spectra are indicative of methylated compounds.³¹ Of the alternative carotenoid candidates, the most viable are equinone and canthaxanthin, which are identical to β -carotene with the exception of an additional oxygen attached to one or both of the β -ionone end groups, respectively. Although the Raman spectra of these two carotenoids and β -carotene are similar, based on the positions of the primary bands (1005 , 1155 , and 1525 cm^{-1}) and the secondary bands at 960 , 1190 , and 1210 cm^{-1} , the observed spectra are most indicative of β -carotene. This conclusion is consistent with a recently published Raman study of carotenoids in bird feathers.³⁰

The above comments support the identification of β -carotene on the UHMWPE wear particles examined in this study. The present finding, however, raises several questions concerning the origin of the β -carotene. First, is there an apparent mechanism that accounts for the observed combination of β -carotene and UHMWPE?

The β -carotene molecule ($\text{C}_{40}\text{H}_{56}$) consists of a long, conjugated carbon chain with four attached methyl groups and a β -ionone ring structure attached to both ends. The ring structure is similar to the structure of terpenes. Notably, the sorption of terpenes, especially limonene, into low-density polyethylene can be considerable and has been reported in the food packaging industry.^{32,33} The similarity between the molecular structure of terpenes and β -carotene, the long carbon chain of β -carotene, and the extreme nonpolarity recently were cited as being indicative of β -carotene's susceptibility to sorption into polyethylene.³⁴ The authors confirmed the sorption of β -carotene into low-density polyethylene and noted that the sorption was three times higher at 25°C than at 4°C . It is apparent from these previous studies that the sorption of β -carotene into polyethylene is a favorable process and one that would be expected to proceed at human physiological temperatures.

The above conclusions also are consistent with our control digestion experiments. The finding of weak β -carotene signals on the undoped UHMWPE pellets

following digestion with tissue is not surprising given the apparent susceptibility of β -carotene sorption into polyethylene. It is not known whether the absorbed β -carotene originated from the tissue sample, or if it migrated from the surface of concomitant UHMWPE wear debris. Clearly, however, in view of the β -carotene recorded on retrieved tibial inserts and on wear particles from digested and undigested synovial fluid samples, the presence of significant β -carotene signals on UHMWPE particles derived from tissue is not an artifact of NaOH digestion pursuant to particle separation.

The susceptibility of β -carotene sorption into polyethylene provides a mechanism for the physical coupling of β -carotene and polyethylene, but provides no explanation for the origin of β -carotene within the synovium of implanted joints. Clearly, further research is required to document the presence of β -carotene in both normal and implanted joints. We find it notable that among the large volume of β -carotene literature, one research group predicted that β -carotene would tend to be concentrated in the human body in the particular membranes and organelles that are exposed to the lowest partial pressures of oxygen.³⁵ This observation is consistent with the present finding of β -carotene within implanted joints, which is a region of relatively low oxygenation.

Additional information about the molecular nature of β -carotene on UHMWPE wear debris can be gained from the relative position of the β -carotene $\text{C}=\text{C}$ stretching band near 1520 cm^{-1} . The $\text{C}=\text{C}$ stretching frequencies in the polyethylene-bound β -carotene spectra were from 5 to 10 cm^{-1} lower than those in the pure β -carotene reference spectrum. This can be seen in Figure 3, with the shift being present in the spectra of both the isolated wear particle and in the β -carotene-doped polyethylene particle. Saito et al. reported a similar difference of 10 cm^{-1} in the Raman spectra of all-trans- β -carotene when measured in the solid state and in a cyclohexane solution.²⁵ The larger wavenumber corresponded to the β -carotene in solution, with the difference attributed to a slight distortion from the planar structure in the conformation of the conjugated double bonds. Accordingly, the observed $\text{C}=\text{C}$ frequency shift in the present study is suggestive of some molecular interaction between the β -carotene and UHMWPE rather than a simple superposition of the molecules.

We also address the possible implications of the presence of β -carotene on *in vivo*-generated polyethylene wear debris. β -carotene is a vitamin A precursor and is an anti-oxidant capable of quenching singlet oxygen and scavenging free-radical species.³⁵⁻³⁷ This raises several intriguing questions. First, is there a role of β -carotene in the mitigation of *in vivo* oxidation and degradation of UHMWPE components? Such an effect would be consistent with the reported anti-oxidant

characteristics of β -carotene. Furthermore, is the presence of β -carotene within the implanted joints coincidental, or does it function in the resulting histiocytic response to polyethylene wear debris? Clearly β -carotene is important in the biochemical processes of the human body; therefore, the presence of β -carotene in regions with significant wear debris may indicate an active role in lessening any adverse response. These points suggest that further research is needed, especially with the increasing interest in the role of polyethylene wear debris in the pathogenesis of osteolysis. Further insight into these issues may lead to a better understanding of the host response to polyethylene orthopedic components.

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References

- H. Ohashi, A. Kobayashi, K. Yoshida, Y. Yutani, Y. Yamano, H. Oonishi, and H. Iwaki, "Histological evaluation of bone-cement interface affected by polyethylene particles in rabbit knee," *J. Mater. Sci. Mater. Med.*, **5**, 610-612 (1994).
- D. R. Graham, "Polyethylene wear: A cause of failure of the variable-axis total knee prosthesis," *J. Bone Jt. Surg.*, **70A**, 942-943 (1988).
- F. Garcia and D. R. Mitrovic, "Joint reaction to polyethylene implantation: A method for inducing osteoarthritic change and osteophyte formation in the rabbit knee joint," *J. Orthop. Res.*, **4**, 420-426 (1986).
- I. C. Clarke, P. Campbell, and N. Kossovsky, "Debris-mediated osteolysis—A cascade phenomenon involving motion, wear, particulates, macrophage induction, and bone lysis," in *Particulate Debris from Medical Implants: Mechanisms of Formation and Biological Consequences*, K. R. S. John (ed.), American Society for Testing and Materials, Philadelphia, 1992, pp. 7-26.
- M. Jasty, "Clinical reviews: Particulate debris and failure of total hip replacements," *J. Appl. Biomater.*, **4**, 273-276 (1993).
- W. J. Maloney, R. L. Smith, D. Huene, and H. Rubash, "Characterization of *in vivo* wear particles isolated from membranes around failed cementless total hip replacements," *Implant Retrieval Symposium, Trans. Soc. Biomater.*, **15**, 28 (1992).
- T. P. Schmalzried, M. Jasty, and W. H. Harris, "Periprosthetic bone loss in total hip arthroplasty: Polyethylene wear debris and the concept of the effective joint space," *J. Bone Jt. Surg.*, **74A**, 849-863 (1992).
- A. S. Shanbhag, J. J. Jacobs, T. T. Glant, J. L. Gilbert, J. Black, and J. O. Galante, "Composition and morphology of wear debris in failed uncemented total hip replacement," *J. Bone Jt. Surg.*, **76B**, 60-67 (1994).
- J. A. Savio, L. M. Overcamp, and J. Black, "Size and shape of biomaterial wear debris," *Clin. Mater.*, **15**, 101-119 (1994).
- P. G. Bullough, E. F. DiCarlo, K. K. Hansraj, and M. C. Neves, "Pathologic studies of total joint replacement," *Orthop. Clin. N. Am.*, **19**, 611-625 (1988).
- D. W. Howie, B. Manthey, S. Hay, and B. Vernon-Roberts, "The synovial response to intraarticular injection in rats of polyethylene wear particles," *Clin. Orthop.*, **292**, 352-357 (1993).
- R. S. Moore, A. Tsai, D. G. Baker, P. Ducheyne, and J. M. Cuckler, "A quantitative analysis of inflammatory response to polyethylene particulates *in vivo*," *Orthop. Trans.*, **18**, 626 (1994).
- M. Horikoshi, W. Macaulay, R. E. Booth, L. S. Crossett, and H. E. Rubash, "Comparison of interface membranes obtained from failed cemented and cementless hip and knee prostheses," *Clin. Orthop. Rel. Res.*, **309**, 69-87 (1994).
- J. J. Jacobs, A. Shanbhag, T. T. Glant, J. Black, and J. O. Galante, "Wear debris in total joint replacements," *J. Am. Acad. Orthop. Surg.*, **2**, 212-220 (1994).
- J. Bosco, J. Benjamin, and D. Wallace, "Quantitative and qualitative analysis of polyethylene wear particles in synovial fluid of patients with total knee arthroplasty," *Clin. Orthop. Rel. Res.*, **309**, 11-19 (1994).
- P. Campbell, S. Ma, B. Yeom, H. McKellop, T. P. Schmalzried, and H. C. Amstutz, "Isolation of predominantly submicron-sized UHMWPE wear particles from periprosthetic tissues," *J. Biomed. Mater. Res.*, **29**, 127-131 (1995).
- P. Eyerer and Y. C. Ke, "Property changes of UHMW-polyethylene hip cup endoprostheses during implantation," *J. Biomed. Mater. Res.*, **18**, 1137-1151 (1984).
- E. V. Nagy and S. Li, "Analysis of retrieved knee components via Fourier transform infrared spectroscopy," *16th Annual Meeting of the Society for Biomaterials*, Charleston, South Carolina, May, 1990, p. 274.
- S. Li, E. V. Nagy, and B. A. Wood, "Chemical degradation of polyethylene in hip and knee replacements," *Orthop. Trans.*, **16**, 397 (1992).
- L. C. Sutula, K. A. Saum, J. P. Collier, and B. H. Currier, "Time dependent oxidation and damage in retrieved and never implanted UHMWPE components," *41st Annual Meeting of the Orthopaedic Research Society*, Orlando, Florida, February, 1995, p. 118.
- M. S. Jahan, C. Wang, G. Schwartz, and J. A. Davidson, "Combined chemical and mechanical effects on free radicals in UHMWPE joints during implantation," *J. Biomed. Res. Soc.*, **25**, 1005-1017 (1991).
- P. Campbell, H. McKellop, B. Yeom, P. Grigoris, R. Salovey, and H. C. Amstutz, "Isolation and characterization of UHMWPE particles from periprosthetic tissues," *20th Annual Meeting of the Society for Biomaterials*, Boston, April, 1994, p. 391.
- D. L. Wolfarth, D. W. Hahn, G. Bushar, and N. Parks, "Separation and characterization of polyethylene wear debris from synovial fluid and tissue samples of revised knee replacements," *J. Biomed. Mater. Res.*, **34**, 57-61 (1997).
- P. C. Painter, M. M. Coleman, and J. L. Koenig, *The theory of Vibrational Spectroscopy and Its Application to Polymeric Materials*, Wiley-Interscience, New York, 1982.
- S. Saito, M. Tasumi, and C. H. Eugster, "Resonance Raman spectra ($5800-40\text{ cm}^{-1}$) of all-trans and 15-cis isomers of beta-carotene in the solid state and in solution," *J. Raman Spect.*, **14**, 299-309 (1983).
- S. Saito and M. Tasumi, "Normal-coordinate analysis of beta-carotene isomers and assignments of the Raman and infrared bands," *J. Raman Spect.*, **14**, 310-321 (1983).
- A. Bertoluzza, C. Fagnano, A. Tinti, S. Mancini, R.

- Caramazza, P. G. Marchetti, and G. Maggi, "Raman spectroscopy in the study of normal and pathological tissue structure and of prosthetic material biocompatibility," *Society of Photo-Optical Instrumentation Engineers*, **1922**, 172-183 (1992).
28. R. Smith and I. Rehman, "Fourier transform Raman spectroscopic studies of human bone," *J. Mater. Sci. Mater. Med.*, **5**, 775-778 (1995).
 29. S. Saito and M. Tasumi, "Normal-coordinate analysis of retinal isomers and assignments of Raman and infrared bands," *J. Raman Spect.*, **14**, 236-245 (1983).
 30. M. Veronelli, G. Zerbi, and R. Stradi, "In situ resonance Raman spectra of carotenoids in bird's feathers," *J. Raman Spect.*, **26**, 683-692 (1995).
 31. H. Okamoto, S. Saito, H. Hamaguchi, M. Tasumi, and C. H. Eugster, "Resonance Raman spectra and excitation profiles of tetrademethyl-beta-carotene," *J. Raman Spect.*, **15**, 331-335 (1984).
 32. K. Hirose, B. R. Harte, J. R. Giacini, J. Miltz, and C. Stine, "Sorption of d-limonene by sealant films and effect on mechanical properties," in *Food and Packaging Interactions*, ACS Symposium Series no. 365, J. H. Hotchkiss (ed.), American Chemical Society, Washington, D.C., 1988, pp. 28-41.
 33. T. Imai, B. R. Harte, and J. R. Giacini, "Partition distribution of aroma volatiles from orange juice into selected polymeric sealant films," *J. Food Sci.*, **55**, 158-161 (1990).
 34. T. J. Nielsen and G. E. Olafsson, "Sorption of beta-carotene from solutions of a food colorant powder into low-density polyethylene and its effect on the adhesion between layers in laminated packaging material," *Food Chem.*, **54**, 255-260 (1995).
 35. G. W. Burton and K. U. Ingold, "Beta-carotene: An unusual type of lipid antioxidant," *Science*, **224**, 569-573 (1984).
 36. P. F. Conn, C. Lambert, E. J. Land, W. Schalch, and T. G. Truscott, "Carotene-oxygen radical interactions," *Free Rad. Res. Comms.*, **16**, 401-408 (1992).
 37. N. I. Krinsky, "Antioxidant functions of carotenoids," *Free Rad. Biol. Med.*, **7**, 617-635 (1989).

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