A Novel Technique for Assessing Antioxidant Concentration in Retrieved UHMWPE

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Abstract

Background Antioxidants added to UHMWPE to prevent in vivo oxidation are important to the long-term performance of hip and knee arthroplasty. Diffused vitamin E antioxidant polyethylene raised questions about potential in vivo elution that could cause inflammatory reactions in periprosthetic tissues and also potentially leave the implant once again prone to oxidation. Currently, there is no information on the elution, if any, of antioxidants from implant polyethylene materials in vivo.

Questions/purposes (1) Do antioxidants, especially diffused vitamin E, elute from antioxidant polyethylene in vivo? (2) Can extraction of the retrieved antioxidant polyethylene (to remove absorbed species from the in vivo environment near the articular and nonarticular surfaces) improve the identification of antioxidant content? (3) Can actual antioxidant content be estimated from calculated antioxidant indices by accounting for ester content (from

absorbed species) near the articular and nonarticular surfaces?

Methods An institutional review board-approved retrieval laboratory received 39 antioxidant polyethylene hip and knee retrievals at revision from 25 surgeons with in vivo time of 0.02 to 3.6 years (median, 1.3 years). These consecutive antioxidant polyethylene retrievals, received between May 2010 and February 2016, were made from three different antioxidant highly crosslinked polyethylene materials: diffused vitamin E, blended vitamin E, and hindered phenol antioxidant pentaerythritol tetrakis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)] propionate (here and after referred to as PBHP). Retrievals were analyzed using Fourier transform infrared (FTIR) spectroscopy. Absorbed ester index ($1725\text{--}1740\text{ cm}^{-1}$ normalized to $1365\text{--}1371\text{ cm}^{-1}$), and vitamin E index ($1245\text{--}1275\text{ cm}^{-1}$) and PBHP index ($1125\text{--}1150\text{ cm}^{-1}$), normalized to $1850\text{--}1985\text{ cm}^{-1}$, were defined. Microtomed thin sections of PBHP and vitamin E retrievals were hexane-extracted to remove absorbed species from the in vivo environment in an effort to improve identification of antioxidant content. Paired Student's t-tests were used to compare as-retrieved articular antioxidant index with expected antioxidant index (the bulk value for blended antioxidants where constant antioxidant content is expected throughout and the extrapolated original vitamin E concentration at the articular surface based on the as-manufactured vitamin E concentration gradient). Linear regression was used for each of the retrievals to evaluate the correlation of antioxidant index to ester content with the goal of extrapolation to the antioxidant index at zero ester content.

Results On average, vitamin E index at the articular surface (0.04 ± 0.03) was reduced compared with expected vitamin E index (0.09 ± 0.04 ; 95% confidence interval [CI] of the difference, $0.04\text{--}0.07$; $p < 0.001$), and PBHP index at

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the articular surface (0.06 ± 0.02) was elevated compared with the average PBHP index from the bulk (0.03 ± 0.00 ; 95% CI of the difference, 0.03–0.05; $p < 0.001$). Extraction returned the PBHP index at the articular surface (0.03 ± 0.00) to bulk values (95% CI of the difference, -0.001 to 0.004; $p = 0.326$); diffused vitamin E was removed by extraction. Crossplots of vitamin E index and PBHP index with ester index showed significant ($p < 0.001$ for 32 of the 35 retrievals with sufficient data) linear trends ($r \geq 0.89$) that allowed extrapolation of the articular surface antioxidant indices at zero absorbed ester index.

Conclusions Absorbed esters from time in vivo caused erroneous values of antioxidant index to be calculated. However, hexane extraction to remove absorbed species also removed diffused vitamin E. Correlating antioxidant indices with ester content, measured by FTIR in unextracted antioxidant retrievals, provides a nonaltered method for estimating actual articular surface vitamin E index and demonstrates that there was no measurable elution in these short-term retrievals.

Clinical Relevance Assessing antioxidant content in retrieved polyethylene inserts is important to determine how much of the antioxidant remains in place to prevent oxidation of the polyethylene over time in vivo. Retrieval analyses reporting antioxidant content must account for absorbed species to be valid. Because standard hexane extraction removes both absorbed species and vitamin E from diffused vitamin E retrievals, the correlation method presented in this study is the recommended analysis alternative.

Introduction

UHMWPE has been the bearing material of choice in total joint arthroplasty for over 50 years [11]. The characteristics of the polyethylene have changed as manufacturers have improved the quality of the polyethylene bearings over the decades. Highly crosslinked polyethylene (crosslinking UHMWPE using higher doses than that for sterilization followed by thermal treatment to reduce or eliminate the free radicals produced by irradiation) [5, 13, 14] was developed around the turn of the century to reduce wear rates and increase oxidation resistance. Highly crosslinked polyethylene materials have been shown to be effective at reducing wear rates, especially in the hip [6, 8, 12]. However, oxidation resistance remained elusive, as oxidation of highly crosslinked tibial inserts [4, 20, 21] demonstrated.

The most recent change in polyethylene has been the addition of antioxidants to prevent in vivo oxidation. Manufacturers have developed proprietary formulations of

antioxidant polyethylene using alpha tocopherol (here and after referred to as vitamin E) or other hindered phenols such as pentaerythritol tetrakis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)] propionate (here and after referred to as PBHP) as the antioxidant. Vitamin E can be diffused into the polyethylene after crosslinking [7]. Alternately, either vitamin E [17] or other hindered phenols [15] can be blended into the polyethylene resin before consolidation. This second method requires higher irradiation doses to reach the desired level of crosslinking, because the antioxidant inhibits crosslinking as well as oxidation [16].

Developing a method to assess antioxidant concentration in retrieved polyethylene inserts is important to determine how much of the antioxidant remains in place in the implant after in vivo service. Initial concerns with the introduction of diffused vitamin E antioxidant polyethylene were that vitamin E would elute in vivo, raising questions about whether elutable species would cause inflammatory reactions in periprosthetic tissues and whether antioxidant elution would leave the implant once again prone to oxidation. Concerns about elution from diffused vitamin E antioxidant polyethylene were essentially allayed by in vitro testing [19]. In vitro studies have suggested that the antioxidant benefit conveyed by vitamin E remain with UHMWPE, even at severely reduced concentrations [18]. Animal studies have suggested that inflammatory reactions resulting from vitamin E are not expected [3, 10]. However, no in vivo results have been published to date to corroborate the in vitro results.

The questions explored in this study are: (1) Do antioxidants, especially diffused vitamin E, elute from antioxidant polyethylene in vivo? (2) Can extraction of in vivo absorbed species from retrieved antioxidant polyethylene improve the identification of antioxidant content? (3) Can the detected antioxidant content in as-retrieved antioxidant implants be used to accurately determine elution, if any, from antioxidant retrievals? In other words, can actual antioxidant content be estimated from calculated antioxidant indices by accounting for ester content (from absorbed species) near the articular and nonarticular surfaces?

Methods and Materials

An institutional review board-approved retrieval laboratory received 39 antioxidant polyethylene hip and knee retrievals at revision from 25 surgeons with in vivo time of 0.02–3.6 years (median, 1.3 years). All retrievals were contributed directly to our program by the explanting surgeon or their hospital representative and were received between May 2010 and February 2016. Although not expected to impact the analyses reported in this study, the surgeon-

reported reasons for retrieval were infection (10), loosening (eight), instability (six), pain (five), and other (six). One tibial insert was retrieved for osteolysis and one for polyethylene wear. One acetabular liner and one tibial insert fractured in vivo. These consecutive retrievals represented antioxidant polyethylene marketed originally by three manufacturers (Table 1).

The retrievals were cut with a band saw through the medial condyle in the AP plane and sectioned with a Jung sledge microtome (Leica Microsystems, Wetzlar, Germany) to a thickness of approximately 250 μm . The thin sections were analyzed using Fourier transform infrared (FTIR) spectroscopy to assess the presence of absorbed species, antioxidant, and extent of oxidation. Line scans were performed through the thickness of the inserts from the articular surface to the backside using a Thermo-Scientific iN10 FTIR microscope (Madison, WI, USA). Parameters for the measurements were 32 scans per 100- μm depth interval using a wave number interval of 2 cm^{-1} and an aperture of 100 μm^2 . Absorbed ester index was defined as the area under the ester peak from 1725 to 1740 cm^{-1} normalized to the area under the 1368- cm^{-1} peak (1365–1371 cm^{-1}). Ketone oxidation index was defined as the area under the ketone peak (1713–1718 cm^{-1}) normalized to the area under the 1368- cm^{-1} peak (1365–1371 cm^{-1}) [4]. ASTM oxidation index (OI) was defined as the total area of the carbonyl peak absorptions centered near 1720 cm^{-1} normalized to the total area of the peak absorptions centered near 1370 cm^{-1} [1]. Time in vivo resulted in absorption of lipids and other species from the in vivo environment. These in vivo species had characteristic ester absorption in FTIR spectra in both the carbonyl region (centered around 1740 cm^{-1}) [9], known to artificially raise the ASTM OI [1], and in the C-O stretching bands (1050–1300 cm^{-1}) [9]. The location of characteristic peaks in FTIR spectra for vitamin E [19] and PBHP [15] also lie within these C-O stretching bands.

Vitamin E antioxidant index was defined as the area under the peak from 1245 to 1275 cm^{-1} normalized to the polyethylene skeletal absorbance at 1895 cm^{-1} (1850–1985 cm^{-1}) [19] (Fig. 1). PBHP antioxidant index was

defined as the area under the peak from 1125 to 1150 cm^{-1} normalized to the polyethylene skeletal absorbance at 1895 cm^{-1} (1850–1985 cm^{-1}) [15] (Fig. 2). FTIR line scans reported values from the articular surface to the backside of

Fig. 1A–B (A) A portion of an FTIR spectrum is shown to highlight the location of the peak that is characteristic of vitamin E antioxidant. (B) Vitamin E index calculated from an FTIR scan from the articular to nonarticular surface of a never-implanted diffused vitamin E tibial insert illustrates the as-manufactured antioxidant concentration gradient.

Table 1. The median values of in vivo time, mean $\pm$ SD of patient age, and patient BMI for the antioxidant polyethylene retrievals analyzed in this study are summarized

Antioxidant retrievals	Number	Months in vivo		Patient age (years)	Patient BMI (kg/m^2)
		Median	Range		
PBHP-hindered phenol (DePuy-Synthes, Warsaw, IN, USA)	20 knees	18	0–43	62 ± 6	34 ± 9
Diffused vitamin E (Zimmer-Biomet, Warsaw, IN, USA)	9 hips, 7 knees	17	4–40	59 ± 7	30 ± 7
Blended vitamin E (Zimmer-Biomet)	2 hips, 1 knee	3	2–16	70 ± 9	35 ± 6

BMI = body mass index.

Fig. 2 A portion of an FTIR spectrum is shown to highlight the location of the peak that is characteristic of PBHP antioxidant.

the bearing. Calculated indices (ASTM OI, vitamin E index, PBHP index, ketone oxidation index, and ester index) were dimensionless numbers and were reported in arbitrary units (au). Lipid extraction of PBHP retrievals was performed on microtomed thin sections by immersion in refluxing hexane at 65°C for 72 hours. This protocol was required as part of the manufacturer's postmarket surveillance. Lipid extraction of vitamin E retrievals was performed on microtomed thin sections, also by immersion in refluxing hexane, for 16 hours, the shorter extraction time having been shown to be appropriate for vitamin E retrievals [22].

Statistical Analysis

One-way analysis of variance was used to compare patient variables and in vivo time among the three antioxidant materials. Paired Student's *t*-tests were used to compare as-retrieved articular antioxidant index with expected antioxidant index (the bulk value for blended antioxidants where constant antioxidant content is expected throughout and the extrapolated original vitamin E concentration at the articular surface based on the as-manufactured vitamin E concentration gradient). Linear regression was used for each of the retrievals to evaluate the correlation of antioxidant index with ester content with the goal of extrapolation to the antioxidant index at zero ester content. The vitamin E profile in each diffused vitamin E retrieval was evaluated using linear regression. The endpoint of these regressions was used to define the original vitamin E concentration at the articular surface of each diffused vitamin E retrieval. IBM SPSS Statistics for Macintosh, Version 22.0 (Armonk, NY, USA) was used for all statistical analyses of the data.

A

B

Fig. 3A–B (A) Mean $\pm$ SD of maximum ketone oxidation index for retrievals of each of the antioxidant materials is much lower than the critical ketone oxidation that can lead to implant fatigue [2]. Oxidation data from an earlier study of highly crosslinked retrievals [4] that did not contain antioxidants but had similar in vivo time (median, 19 months; range, 1–43 months) to the retrievals in the current study cohort provide context for the oxidation measured in these antioxidant retrievals. (B) ASTM OI and ketone oxidation index versus depth are shown for an as-retrieved, not extracted tibial insert after 3 years in vivo. The location of maximum ketone OI is highlighted. Elevated ASTM OI at the articular and nonarticular surfaces is the result of absorbed species from the in vivo environment and does not indicate polyethylene oxidation.

Results

The differences in patient age ($p = 0.184$), patient body mass index ($p = 0.637$), and in vivo time ($p = 0.357$) among the three antioxidant materials did not reach significance (Table 1). The antioxidant polyethylene retrievals exhibited minimal oxidation that was statistically indistinguishable among the three materials, as demonstrated by their maximum ketone oxidation indices: PBHP 0.09 ± 0.01 , diffused vitamin E 0.09 ± 0.03 , and blended vitamin E 0.06 ± 0.03 ($p = 0.148$). Oxidation data from an

Table 2. Extrapolated articular antioxidant indices from the proposed correlation compared with as-retrieved articular and bulk antioxidant indices for PBHP antioxidant retrievals

Sample name	Component	In vivo months	Maximum ketone oxidation index	As-retrieved articular antioxidant index	Bulk antioxidant index (mean $\pm$ SD)	Extrapolated antioxidant index at ester = 0	p value	Standard error of the constant	Linear correlation coefficient
A1	Tibial knee	18	0.1	0.073	0.027 $\pm$ 0.002	0.025	< 0.001	0.001	0.99
A2	Tibial knee	17	0.1	0.098	0.025 $\pm$ 0.002	0.022	< 0.001	0.001	1.00
A3	Tibial knee	20	0.1	0.058	0.027 $\pm$ 0.001	0.027	< 0.001	0.002	0.98
A4	Tibial knee	5	0.1	0.063	0.025 $\pm$ 0.003	0.019	< 0.001	0.001	0.99
A5	Tibial knee	33	0.1	0.061	0.024 $\pm$ 0.002	0.022	< 0.001	0.005	0.99
A6	Tibial knee	36	0.1	0.053	0.021 $\pm$ 0.002	0.022	< 0.001	0.001	0.98
A7	Tibial knee	1	0.1	0.030	0.027 $\pm$ 0.002	*	*	*	*
A8	Tibial knee	32	0.1	0.099	0.025 $\pm$ 0.002	0.023	< 0.001	0.001	1.00
A9	Tibial knee	15	0.1	0.087	0.022 $\pm$ 0.001	0.021	< 0.001	0.001	1.00
A10	Tibial knee	0	0.1	0.031	0.025 $\pm$ 0.002	*	*	*	*
A11	Tibial knee	20	0.1	0.073	0.023 $\pm$ 0.001	0.019	< 0.001	0.001	1.00
A12	Tibial knee	8	0.1	0.048	0.021 $\pm$ 0.002	0.017	< 0.001	0.001	1.00
A13	Tibial knee	9	0.1	0.040	0.025 $\pm$ 0.002	0.019	< 0.001	0.001	0.99
A14	Tibial knee	28	0.1	0.036	0.029 $\pm$ 0.003	0.023	< 0.001	0.001	1.00
A15	Tibial knee	43	0.1	0.040	0.024 $\pm$ 0.001	0.022	< 0.001	0.001	1.00
A16	Tibial knee	5	0.1	0.060	0.026 $\pm$ 0.001	0.024	0.001	0.002	0.99
A17	Tibial knee	15	0.1	0.067	0.027 $\pm$ 0.002	0.026	< 0.001	0.001	0.99
A18	Tibial knee	6	0.1	0.071	0.028 $\pm$ 0.001	0.023	< 0.001	0.001	1.00
A19	Tibial knee	18	0.1	0.059	0.026 $\pm$ 0.001	0.023	< 0.001	0.001	1.00
A20	Tibial knee	36	0.1	0.077	0.027 $\pm$ 0.001	0.025	< 0.001	0.001	1.00

* Insufficient data to allow correlation of antioxidant index to ester content.

earlier study of highly crosslinked retrievals [4] provide context for the oxidation measured in these antioxidant retrievals (Fig. 3A). These highly crosslinked retrievals did not contain antioxidants, but had similar in vivo time to the retrievals in the current study cohort (median, 19 months; range, 1–43 months). Near the articular and nonarticular (loaded) surfaces of the antioxidant retrievals, absorption of in vivo fluids increased ASTM OI similarly for the three materials (PBHP ASTM OI median 0.4, range 0.2–0.7; diffused vitamin E ASTM OI median 0.5, range 0–0.6; blended vitamin E ASTM OI median 0.4, range 0–0.6; $p = 0.574$; example data from retrieval A6 shown in Fig. 3B).

In PBHP antioxidant retrievals (labeled A1–A20 in order of date of receipt; Table 2), PBHP index at the articular surface of as-received retrievals (0.06 ± 0.02) was elevated compared with the average PBHP index from the bulk (0.03 ± 0.00 ; 95% confidence interval [CI] of the difference, 0.03–0.05; $p < 0.001$). Hexane extraction of PBHP retrievals returned the PBHP index at the articular surface (0.03 ± 0.00) to bulk values (95% CI of the difference, -0.001 to 0.004 ; $p = 0.326$; Fig. 4).

In diffused vitamin E antioxidant retrievals (labeled E1–E16 in order of date of receipt; Table 3), vitamin E index at the articular surface (0.04 ± 0.03) was reduced compared with the expected vitamin E index (0.09 ± 0.04 ; 95% CI of the difference, 0.04–0.07; $p < 0.001$; Fig. 5A). The lowered vitamin E index was seen in both infused and blended/grafted vitamin E polyethylene (labeled V1–V3 in order of date of receipt; Table 4). In diffused vitamin E polyethylene, hexane extraction removed both the absorbed species and the vitamin E (Fig. 5B).

Antioxidant index correlated positively with ester content for PBHP retrievals, as illustrated for one PBHP retrieval (Fig. 6A) and summarized for all PBHP retrievals (Table 2). Antioxidant index correlated negatively with ester content for vitamin E materials, as illustrated for one diffused vitamin E retrieval (Fig. 6B) and summarized for all vitamin E retrievals (Tables 3, 4). Extrapolation of these correlations to zero ester content allowed estimation of actual antioxidant indices at the articular surface in both PBHP and vitamin E antioxidant retrievals (Tables 2, 3, 4).

Discussion

Antioxidant polyethylene was developed to address the problem of free radicals in polyethylene resulting from irradiation used in crosslinking or sterilization with the expectation of improving the long-term survivorship of joint replacements. An objective evaluation of the in vivo performance of antioxidant polyethylenes is important to

Fig. 4 PBHP index versus depth through a retrieved tibial insert, as-received and after hexane extraction shows that only the effect of the absorbed esters on the PBHP index is removed by hexane extraction. The PBHP antioxidant is not removed by hexane extraction.

validate promising in vitro assessments of efficacy. Each manufacturer represented in this retrieval cohort used a different antioxidant or method of incorporating the antioxidant. Despite these differences in approach, all of these antioxidant materials appeared to be effective at minimizing polyethylene oxidation over the in vivo period of this study. Elevated ASTM OI relative to the interior was found near the articular and nonarticular surfaces of as-retrieved, antioxidant retrievals, which is known to be an artifact of using ASTM OI for polyethylene exposed to absorbed species from the in vivo environment. Extraction of absorbed species from retrievals is necessary for the ASTM OI to correctly report actual polyethylene oxidation [1]. These absorbed species, which contain ester groups identifiable by FTIR, also impact the low wave numbers of the FTIR spectra, where characteristic peaks for antioxidants are found. The absorbed species cause paradoxical values of antioxidant indices to be calculated that can lead to misinterpretation of antioxidant polyethylene performance if taken at face value. However, the results of this study suggest that antioxidant content can be estimated from calculated antioxidant indices by correlation with ester content (from absorbed species) near the articular and nonarticular surfaces.

Limitations of this study are twofold. First, retrieval studies are able to report on observed phenomena in retrievals, but one must exercise caution to put those results in the context of all implanted devices. The observations and comparisons within a retrieval data set can show relative frequency of occurrence of an observed phenomenon and whether the phenomenon occurs in multiple designs by a number of manufacturers or is specific to a design or manufacturer. Evaluation of retrievals is necessary to determine if in vitro testing accurately reflects the long-

Table 3. Extrapolated articular antioxidant indices from the proposed correlation compared with as-retrieved articular antioxidant indices and antioxidant indices extrapolated from vitamin E gradient for diffused vitamin E antioxidant retrievals

Sample name	Component	In vivo months	Maximum ketone oxidation index	As-retrieved articular antioxidant index	Extrapolated articular vitamin E from gradient	Vitamin E index versus depth linear correlation coefficient *	Extrapolated antioxidant index at ester = 0	p value	Standard error of the constant	Linear correlation coefficient
E1	Tibial knee	6	0.1	0.105	0.142	0.97	0.143	< 0.001	0.003	0.96
E2	Acetabular	0	0.1	0.031	0.037	0.93	†	†	†	†
E3	Tibial knee	10	0.1	0.102	0.134	0.96	0.124	< 0.001	0.001	1.00
E4	Tibial knee	31	0.1	0.014	0.106	0.96	0.098	< 0.001	0.001	1.00
E5	Tibial knee	23	0.1	0.024	0.085	0.99	0.084	< 0.001	0.001	0.98
E6	Acetabular	7	0.1	0.023	0.057	0.88	0.063	< 0.001	0.001	1.00
E7	Acetabular	11	0.1	0.026	0.051	0.98	0.054	< 0.001	0.001	1.00
E8	Acetabular	4	0.1	0.015	0.097	0.95	0.104	< 0.001	0.001	1.00
E9	Tibial knee	34	0.1	0.020	0.099	0.857 (0.002)	0.112	< 0.001	0.004	0.98
E10	Tibial knee	36	0.1	0.041	0.129	0.95	0.113	< 0.001	0.004	0.97
E11	Acetabular	6	0.0	0.062	0.071	0.99	0.075	0.023	0.003	0.94
E12	Acetabular	5	0.1	0.009	0.063	0.957 (0.004)	0.068	< 0.001	0.003	0.94
E13	Tibial knee	17	0.1	0.040	0.122	0.96	0.114	< 0.001	0.002	0.99
E14	Acetabular	8	0.1	0.040	0.137	0.99	0.125	< 0.001	0.001	1.00
E15	Acetabular	40	0.1	0.021	0.048	0.98	0.052	< 0.001	0.002	0.89
E16	Acetabular	18	0.1	0.017	0.041	0.99	0.052	< 0.001	0.001	0.99

* p < 0.001 for all; standard error 0.001 (unless noted); † insufficient data to allow correlation of antioxidant index to ester content.

Fig. 5A–B (A) A retrieved diffused vitamin E tibial insert (retrieval E4) shows elevated ester indices (absorbed esterified fatty acids and lipids from the in vivo environment) and ASTM OI near the articular and nonarticular surfaces. Vitamin E index is lower where ester index is higher. (B) Vitamin E index, ester index, and ASTM OI versus depth for the same diffused vitamin E retrieved tibial insert after hexane extraction shows that both the effect of the absorbed esters on ASTM OI, ester index, and vitamin E index and the vitamin E antioxidant itself are removed by hexane extraction.

term performance of devices in vivo. The retrievals available for study were in vivo 3.6 years or less. Short in vivo time retrievals can reassure that materials are performing as designed but are limited in their predictive capacity. Only longer term retrievals can suggest how a material or technology will function in the in vivo environment over the lifetime of the patient. However, evaluation of these short in vivo time retrievals was useful in identifying an analysis problem and suggested that the relationships that we found between antioxidant index and absorbed ester index can be used to analyze longer in vivo time retrievals.

Analysis of as-retrieved antioxidant inserts and liners showed antioxidant index to be altered in all retrievals, regardless of antioxidant type. In both diffused and blended vitamin E retrievals, the antioxidant index measured at the articular surface was lower than that expected from the

antioxidant concentration gradient in the bulk of the retrieval. These findings could be interpreted as elution of vitamin E from the polyethylene, erroneously alarming the physician reader or researcher. However, as observed in measured ester index and ASTM OI versus depth for these retrievals, absorbed species from the in vivo environment were present at the articular and nonarticular surfaces. Oral et al. [18] showed interference with the vitamin E peak in FTIR spectra by lipids introduced in vitro from bovine serum used in hip simulator wear testing. This same type of interference was seen in FTIR spectra from vitamin E retrievals in this study, and it affected the vitamin E index calculated for these retrievals. Absorbed species near the articular surface generate a broad absorbance in the FTIR spectral region 1230–1290 cm^{-1} . Because this absorbance extends beyond the absorbance range where vitamin E is identified (1245–1275 cm^{-1}), the relatively low magnitude vitamin E peak is obscured by the absorbed species and is not identifiable.

In PBHP antioxidant retrievals, the antioxidant index at the articular surface was higher than expected based on the average concentration in the bulk of the retrieval, again resulting from absorbed lipids interfering with the hindered phenol peak in the FTIR spectrum. Absorbed species near the articular surface generate an additional FTIR absorbance peak (1130–1150 cm^{-1}) within the absorbance range where PBHP is identified (1125–1150 cm^{-1}), thereby increasing the antioxidant index calculated for PBHP retrievals.

Hexane extraction of the absorbed species proved to be an effective technique for determining the actual antioxidant index at the articular surface for PBHP antioxidant retrievals. Comparison of as-retrieved and after-extraction PBHP index demonstrated lowering of the articular surface PBHP index to that measured in the unaffected bulk of the retrieval. Extraction allowed measurement of the actual antioxidant index rather than the artificially higher index measured in as-retrieved inserts. Because PBHP is a solid antioxidant blended with the UHMWPE resin before the polyethylene is consolidated [15], this finding is consistent with expectations that concentrations will remain constant through the thickness of the implant and over time in vivo. Clinically, the findings of this study support that the antioxidant remains at an appropriate concentration to perform its intended duty and is not being released into the joint space.

In the case of diffused vitamin E polyethylene, hexane extraction removed both the absorbed species and the vitamin E. This problem also has been reported by Oral et al. [18] when attempting to remove absorbed lipids from bovine serum in samples from in vitro wear testing. We thus confirm that hexane extraction of diffused vitamin E is not a viable method to determine actual articular vitamin E

Table 4. Extrapolated articular antioxidant indices from the proposed correlation compared with as-retrieved articular and bulk antioxidant indices for blended vitamin E antioxidant retrievals

Sample name	Component	In vivo months	Maximum ketone oxidation index	As-retrieved articular antioxidant index	Bulk antioxidant index (mean $\pm$ SD)	Extrapolated antioxidant index at ester = 0	p value	Standard error of the constant	Linear correlation coefficient
V1	Tibial knee	2	0.1	0.003	0.007 $\pm$ 0.002	0.009	0.045	0.001	0.99
V2	Acetabular	3	0.0	0.001	0.006 $\pm$ 0.001	*	*	*	*
V3	Acetabular	16	0.1	0.003	0.005 $\pm$ 0.001	0.005	<	0.001	0.001

0.93

* Insufficient data to allow correlation of antioxidant index to ester content.

Fig. 6A–B (A) PBHP index, before extraction, from retrieval A6 shows a strong positive linear correlation with absorbed ester index ($p < 0.001$). This correlation gives an extrapolated PBHP index of 0.02 at ester index = 0 for this retrieval. (B) Vitamin E index from retrieval E13 shows a strong negative linear correlation with absorbed ester index ($p < 0.001$). This correlation gives an extrapolated vitamin E index of 0.114 at ester index = 0 for this retrieval.

index in retrievals. Because many clinical research programs use hexane extraction before analysis of retrieved devices, the clinician is cautioned to carefully understand the potential measurement artifacts induced through this chemical process. Inadvertent changes to the material

under analysis could lead to incorrect interpretations about in vivo behavior and the prognosis for future success.

Analysis of antioxidant retrievals suggested that the antioxidant indices seemed to change as a function of absorbed ester content, increasing the index for PBHP retrievals and decreasing the index for vitamin E retrievals. Correlation of antioxidant indices with absorbed ester content, as determined from unextracted retrievals, showed a strong positive linear correlation for PBHP antioxidant retrievals. Extrapolation of these correlations to zero ester content gave PBHP index values consistent with both bulk and extracted PBHP indices. These results served as a verification of this new method of determining articular antioxidant indices. The correlation of antioxidant indices with absorbed ester content, as determined from unextracted retrievals, showed strong negative linear correlations for both diffused and blended vitamin E antioxidant retrievals. Extrapolation of these correlations to zero ester content gave vitamin E index values consistent with expected vitamin E index, supporting that vitamin E was not eluting in any measurable amount into the in vivo environment.

Assessing antioxidant content in retrieved polyethylene inserts is important to determine how much of the antioxidant remains in place to prevent oxidation of the polyethylene over time in vivo. Retrieval analyses reporting antioxidant content must account for absorbed species to be valid. The FTIR technique introduced in this study correlates retrieval antioxidant indices with ester content to estimate actual articular surface antioxidant index. This technique addresses concerns identified by Oral et al. [18] in trying to measure vitamin E concentration for hip simulator samples exposed to bovine serum. Absorbed lipids (esterified fatty acids) from the bovine serum were reported to have convoluted the vitamin E (α -tocopherol) absorbance peak, making accurate assessment of vitamin E concentration impossible. This technique also avoids the issue identified by Oral et al. [18] of extracting vitamin E when hexane extraction is used to extract absorbed lipids. This correlation method, validated by analyzing extracted

and unextracted PBHP antioxidant retrievals, can be used to monitor antioxidant content in longer time retrievals.

Antioxidant polyethylene is an important option to improve the long-term performance of joint replacements. In this study cohort of antioxidant retrievals with a maximum of 3.6 years in vivo, we see no evidence of oxidation of the polyethylene or elution of the antioxidant. Accurate evaluation of retrieved antioxidant polyethylene to assure that the antioxidant is remaining in place over the life of the implant is necessary to assess past and forecast future performance.

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