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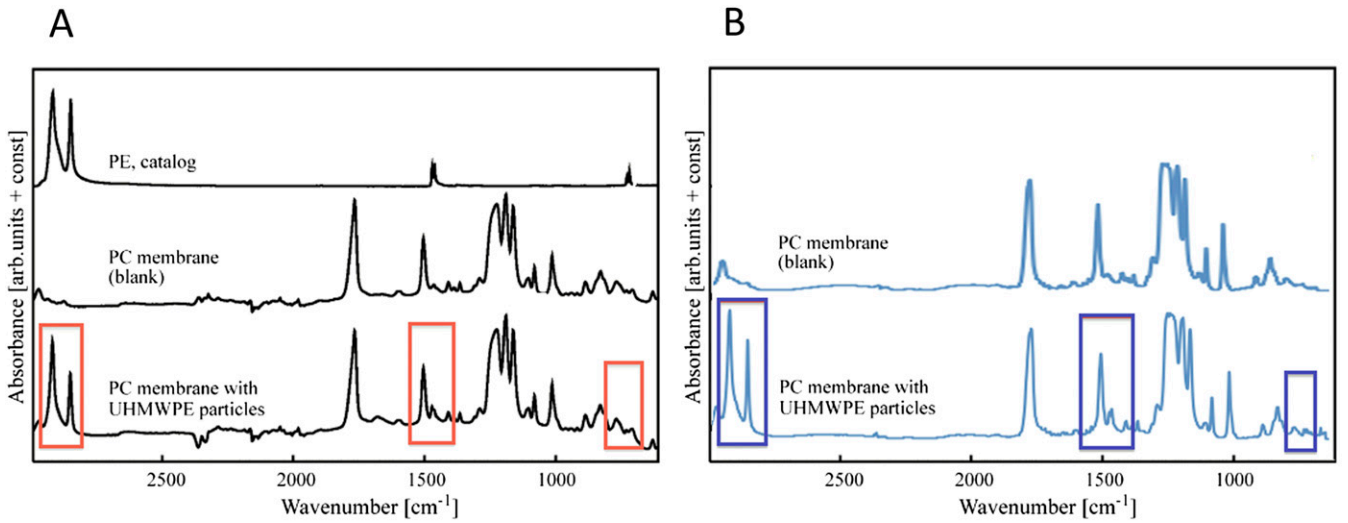

Fig. E-1

To validate the purity of polyethylene (UHMWPE) particles, polycarbonate (PC) membranes were scanned with use of Fourier transform infrared spectroscopy in transmission. Multiple regions of the membranes from two patient tissue digests were scanned and compared with blank membranes. A comparison of the current Fourier transform infrared spectroscopy findings with previous spectra (**Fig. E-1A**) showed agreement in the vibrational peaks for polyethylene (**Fig. E-1B**)[54].

TABLE E-1 Implant Oxidation

		Dome*		Rim*	
	Impingement Classification	Max. OI	Max. HI	Max. OI	Max. HI
Case					
1	Severe	0.70	0.30	2.22	0.78
2†	Severe	1.00	0.55	1.69	0.92
3	Severe	0.57	0.55	3.84	0.62
4	Severe	0.39	0.36	1.62	0.41
5	Severe	1.16	0.45	5.69	0.63
6	Severe	0.72	0.39	4.65	0.58
7	Severe	2.87	0.38	7.19	0.47
8	Mild	0.21	0.34	0.88	0.46
9	Mild	0.49	0.53	3.61	0.66
10	Mild	0.42	0.41	3.30	0.51
11	Mild	0.40	0.38	2.35	0.77
P value†					
Severe vs. mild		0.05	0.89	0.30	0.77
11 vs. all 48§		0.87	0.23	0.59	0.79

*OI = oxidation index, and HI = hydroperoxide index. †The only patient who underwent revision because of osteolysis. ‡Significance was evaluated with use of independent t tests and set at $p < 0.05$. §Subset of eleven cases in which periprosthetic tissue samples were retrieved versus all forty-eight total disc replacements.

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TABLE E-2 Linear Penetration

		Dome		Rim	
	Impingement Classification	Linear Penetration *(mm)*	% of Combined Penetration	Linear Penetration *(mm)*	% of Combined Penetration
Case					
1	Severe	0.11	0.21	0.42	0.79
2*	Severe	0.92	0.53	0.81	0.47
3	Severe	0.34	0.13	2.38	0.87
4	Severe	0.33	0.85	0.06	0.15
5	Severe	0.21	0.25	0.63	0.75
6	Severe	0.36	0.78	0.10	0.22
7	Severe	0.58	0.42	0.81	0.58
8	Mild	0.23	0.89	0.028	0.11
9	Mild	0.37	0.95	0.019	0.05
10	Mild	0.31	0.78	0.089	0.22
11	Mild	0.54	0.86	0.085	0.14
P value†					
Severe vs. mild		0.77	0.02‡	0.03§	0.02§
11 vs. all 48#		0.65	—	0.68	—

*The only patient who underwent revision because of osteolysis. †Significance was evaluated with use of independent t tests and set at $p < 0.05$. ‡Significantly higher in the mild-impingement group. §Significantly higher in the severe-impingement group. #Subset of eleven cases in which periprosthetic tissue samples were retrieved versus all forty-eight total disc replacements.

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TABLE E-3 Equations and Definitions of Wear Particle Characteristics and Biological Activity Indices*

Equation No.	Parameter	Equation	Definition
1	ECD (μm)	$\sqrt{\frac{4 \cdot A_P}{\pi}}$	Diameter of a circle with an area equivalent to the particle area (A_P)[63]
2	AR (unitless)	$\frac{L_P}{W_P}$	Ratio of particle length to particle breadth[63]
3	R (unitless)	$\frac{4 \cdot A_P}{\pi \cdot (L_P)^2}$	Length-based measure of circularity ranging from zero to one (perfect circle)[63]
4	FF (unitless)	$\frac{4 \cdot \pi \cdot A_P}{(\text{perimeter})^2}$	Perimeter-based measure of circularity ranging from zero to one (perfect circle)[63]
5	B(r)	$\left\{\begin{matrix}\text{Scalar}\\1.00\\0.20\\0.04\end{matrix}\right\}\left\{\begin{matrix}\text{Particle Size Range}\\\text{ECD} < 1\ \mu\text{m}\\1\ \mu\text{m} < \text{ECD} < 10\ \mu\text{m}\\\text{ECD} > 10\ \mu\text{m}\end{matrix}\right\}$	Biological activity scalars for individual size ranges
6	V_P	$^1/_{18} \cdot \pi \cdot \text{ECD}^3$	Individual particle volume
7	V(r)	$\left\{\begin{matrix}\Sigma V_{P(\text{ECD}<1\ \mu\text{m})}\\\Sigma V_{P(1\ \mu\text{m}<\text{ECD}<10\ \mu\text{m})}\\\Sigma V_{P(\text{ECD}>10\ \mu\text{m})}\end{matrix}\right\} \cdot \frac{1}{\Sigma V_{P(\text{Total})}}$	Volume fraction distribution
8	SBA	$\sum V(r) \cdot B(r)$	Specific biological activity (SBA) per unit volume of wear debris[46]
9	FBA	$\text{SBA} \cdot V_{\text{Total}}$	Functional biological activity (FBA), SBA scaled by V_{Total}[46]

*ECD = equivalent circular diameter, A_P = particle area, AR = aspect ratio, L_P = particle length, W_P = particle width, R = roundness, FF = form factor, B(r) = biological activity scalars by size range, V_P = particle volume, V(r) = volume fraction distribution, SBA = specific biological activity, FBA = functional biological activity, and V_{Total} = particle volume (mm^3/g of tissue).

TABLE E-4 Particle Size, Shape, and Number*

	Impingement Classification	ECD† (μm)	AR† (unitless)	R† (unitless)	FF† (unitless)	Particle Number ($\times 10^9$/g)	Macrophage/ Giant-Cell Scores‡ (range, 0-3+)	FBA
Case								
1	Severe	0.58 ± 1.35	2.07 ± 0.80	0.54 ± 0.15	0.52 ± 0.26	1.80	2/1	3.44
2§	Severe	0.61 ± 0.60	1.84 ± 0.56	0.58 ± 0.14	0.32 ± 0.20	2.59	3/2	3.50
3	Severe	1.37 ± 2.04	2.11 ± 0.99	0.55 ± 0.19	0.58 ± 0.24	2.60	3/2	4.08
4	Severe	0.52 ± 1.21	1.81 ± 0.64	0.60 ± 0.15	0.55 ± 0.23	1.20	1/0	1.10
5	Severe	1.01 ± 1.41	1.93 ± 0.74	0.58 ± 0.17	0.65 ± 0.21	1.88	3/3	2.94
6	Severe	0.89 ± 1.32	1.85 ± 0.64	0.59 ± 0.15	0.57 ± 0.25	1.54	2/3	2.98
7	Severe	0.69 ± 1.13	2.03 ± 0.91	0.56 ± 0.18	0.60 ± 0.24	2.36	3/3	1.52
8	Mild	0.65 ± 1.09	2.08 ± 0.89	0.55 ± 0.18	0.63 ± 0.21	0.98	3/0	0.79
9	Mild	0.40 ± 0.67	1.85 ± 0.65	0.59 ± 0.15	0.47 ± 0.23	1.99	2/1	0.82
10	Mild	0.58 ± 0.70	2.10 ± 1.02	0.55 ± 0.19	0.58 ± 0.24	1.19	1/0	0.56
11	Mild	0.59 ± 0.78	1.86 ± 0.61	0.58 ± 0.15	0.61 ± 0.26	1.49	3/0	1.46
P value: severe vs. mild#		0.15	0.77	0.78	0.58	0.10	0.75/0.04	0.012
Spearman rho correlation#,**								
Macrophage score vs. FBA							$\rho = 0.40$, $p = 0.22$	
Giant-cell score vs. FBA							$\rho = 0.64$; $p = 0.04$	
Combined score vs. FBA							$\rho = 0.62$; $p = 0.04$	

*ECD = equivalent circular diameter, AR = aspect ratio, R = roundness, FF = form factor, and FBA = functional biological activity. †The values are given as the mean and standard deviation. ‡1+ = one to nine macrophages per high power field (500×) and one or two giant cells per medium-power field (250×); 2+ = ten to forty-nine macrophages per high-power field and three to eight giant cells per medium-power field; 3+ = fifty or more macrophages per high-power field and nine or more giant cells per medium-power field. Obtained, in part, from the study by Punt et al.[6]. §The only patient who underwent revision because of osteolysis. #Significance was evaluated with Mann-Whitney U tests and set at $p < 0.05$. **Spearman rho correlation was used to determine correlations between the unseparated mild and severe inflammatory responses and FBA. ''Combined'' means the overall chronic inflammatory response (combined macrophage and giant-cell scores).

Appendix: Methodology

Wear Particle Isolation

Tissue samples were fixed in 10% phosphate buffered formalin, decalcified in a 3.5% sodium formate and 25% formic acid solution, and then embedded in paraffin6. Wear particle isolation was performed with modifications of the methods used by Margevicius et al.[48,84]. Tissues were deparaffinized in xylene overnight and then washed twice in xylene and twice in 100% ethanol. After drying for two hours, 0.02 to 0.03 g of tissue were digested in 5 mL of 65% HNO_3. To account for regional particle differences, samples from all retrieved tissues were combined for analysis. After the first twenty-four hours, the tissues were agitated and then subsequently were digested for an additional twenty-four hours. In a previous study, we evaluated wear particle morphology before and after nitric acid digestion and found no effect on particle morphology or size[85].

Following digestion, the tubes were vortexed for three thirty-second intervals, and placed in an ultrasonic bath (Cole-Parmer, Vernon Hills, Illinois) for two minutes to achieve uniform dispersion. Subsequently, the sample was vacuum-filtered through a 1.0-μm-pore polycarbonate membrane (GE Healthcare, Piscataway, New Jersey), and the filtrate was collected. After filtration, the membrane was washed with 10 mL of 65% HNO_3, and the wash was pulled through by a vacuum. This washing process was repeated with methanol. To prevent particle aggregation, the filtrate was diluted with 15 mL of methanol containing 2% Nonidet P-40 (AppliChem, Darmstadt, Germany). The solution was vortexed for three thirty-second intervals, then placed in an ultrasonic bath for two minutes, and immediately filtered through a 0.05-μm membrane. The second membrane was sequentially washed with 65% HNO_3 followed by methanol. On the basis of the thorough digestion, centrifugation steps were not employed during wear particle isolation. Each membrane was dried for two hours at room temperature and was prepared for scanning electron microscopy.

Imaging

Polycarbonate membranes with isolated polyethylene wear debris were fixed onto aluminum stubs with double-sided carbon tape and sputter-coated with a 5-nm-thick layer of platinum/palladium with use of a 208 HR (high-resolution) sputter coater (Cressington, Watford, England) to minimize sample drift and/or damage by the electron beam. Polyethylene wear debris was visualized with use of an XL30 environmental scanning electron microscope (FEI/Philips, Hillsboro, Oregon) equipped with a Schottky field-emission gun in the Drexel University Centralized Research Facility. Imaging was performed at a working distance of 12 mm and a beam intensity of 5 kV.

Primary membranes (1.0-μm pore size) were imaged at magnifications of 500× and 1000×; five and ten images were collected, respectively. Secondary membranes (0.05-μm pore size) were imaged at a magnification of 12000×; ten images were collected from three separate regions, which did not include the edge or center of the membrane to account for flow gradient effects during filtration. A minimum of 1000 particles was analyzed for each patient.

Wear Particle Analysis

Analysis of environmental scanning electron microscope images was initially performed with use of a gray-scale threshold to isolate the polyethylene particles, which have higher gray-scale values. Subsequently, the individual particle areas and dimensions were determined with use of NIH ImageJ (Bethesda, Maryland). The resulting areas and dimensions were used to characterize particle number, size (equivalent circular diameter [Table E-3, Equation 1]), shape (aspect ratio [Equation 2]), roundness (Equation 3), and form factor (Equation 4) according to ASTM F1877[86].

To characterize particle biological activity, we used equations originally developed by Fisher et al.[46,47]. The biological activity function, B(r), which separately ranks the biological relevance of particles in <0.1 to 1, 1 to 10, and >10-μm size ranges, was assumed to be the same as the function that was previously published for biological activity (Table E-3, Equation 5)[51]. To determine particle volume, V_P (Equation 6), calculations were based on the work of Scott et al.[48]. Subsequently, the volume fraction distribution, V(r) (Equation 7), was determined for the <1, 1 to 10, and >10-μm size ranges[51]. To determine the specific biological activity per unit volume (Equation 8), the product of B(r) and V(r) was determined. Finally, to determine functional biological activity (Equation 9), specific biological activity was normalized by total particle volume (mm^3/g of tissue). The use of particle volume scaled per gram of tissue weight for functional biological activity differs from the original method of Fisher et al., who used component wear volume ($mm^3/10^6$ cycles) to calculate functional biological activity[46,47].

Particle Validation

To validate the purity of polyethylene particles, multiple regions of polycarbonate membranes from two patient tissue digests were scanned with use of Fourier transform infrared spectroscopy (Thermo 6700 FTIR Spectrometer with continuum microscope attachment; ThermoFisher Scientific, Billerica, Massachusetts) in transmission and compared with blank membranes. A comparison of the current findings with previous spectra (Fig. E-1A) showed agreement with peaks for polyethylene (Fig. E-1B)[50].

Inflammatory Response Evaluation

Fixed tissues were embedded in paraffin, and 6-μm sections were mounted on Fisher Superfrost/Plus slides (ThermoFisher Scientific), dewaxed, rehydrated, and stained with hematoxylin and eosin to evaluate tissue morphology and the presence of inflammation. Brightfield images (Olympus BX50 microscope; Olympus, Melville, New York) were scored for the presence of macrophages/histiocytes and giant cells (Table E-4) with use of a modified Mirra classification[87]. Two trained histologists independently scored each tissue section, and their findings were in agreement 95% of the time.

Penetration Analysis

Penetration was assessed at the rim and dome of each component by direct measurement with use of a calibrated micrometer (accuracy, 0.001 mm). The full details of this penetration analysis can be obtained in the original publication[43].

Oxidation Analysis

Thin sections were taken from the central axis of the cores to assess oxidation and oxidation potential[43]. Lipids were extracted from the polyethylene by boiling the slices in heptane for six hours. Thin sections from the dome and rim were analyzed with use of Fourier transform infrared spectroscopy, and the oxidation index was calculated in accordance with ASTM F2102-06[88]. To investigate the hydroperoxide content, sections were exposed to nitric oxide, thereby converting the hydroperoxides to nitrates, which were measured with Fourier transform infrared spectroscopy[89,90]. A hydroperoxide index was calculated by normalizing the area under the peak located at 1630 cm^{-1} by the peak centered at 1368 cm^{-1}, as previously described[43]. ■