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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1596789> since 2016-09-23T10:16:15Z

Published version:

DOI:10.1016/j.jmbbm.2016.08.003

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(Article begins on next page)

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Wear performance of neat and vitamin E blended highly Cross-Linked PE under severe conditions: the combined effect of accelerated ageing and third body particles during wear test.

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Running title: Effects of third-body particles on PE wear

Journal: JMBBM

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ABSTRACT

The objective of this study is to evaluate the effects of third-body particles on the *in vitro* wear behaviour of three different sets of polyethylene acetabular cups after prolonged testing in a hip simulator and accelerated ageing. Vitamin E-blended, cross-linked polyethylene (XLPE_VE), cross-linked polyethylene (XLPE) and conventional polyethylene (STD_PE) acetabular cups were simulator tested for two million cycles under severe conditions (i.e. by adding third-body particles to the bovine calf serum lubricant). Micro-Fourier Transform Infrared and micro-Raman spectroscopic analyses, differential scanning calorimetry, and crosslink density measurements were used to characterize the samples at a molecular level.

The STD_PE cups had almost twice as much wear as the XLPE_VE components and four times as much wear as the XLPE samples; statistically significant differences were found between the mass losses of the three sets of cups. The observed wear trend was justified on the basis of the differences in cross-link density among the samples (XLPE > XLPE_VE > STD_PE).

FTIR crystallinity profiles, bulk DSC crystallinity and surface micro-Raman crystallinity seemed to have a similar behaviour upon testing: all of them (as well as the all-trans and ortho-trans contents) revealed the most significant changes in XLPE and XLPE_VE samples. From a general point of view, the more severe third-body wear testing conditions determined more noticeable changes in all spectroscopic markers with respect to previous tests. Unexpectedly, traces of bulk oxidation were found in both STD_PE (unirradiated) and XLPE (remelting-stabilized), which were expected to be stable to oxidation; on the contrary, XLPE_VE demonstrated a high oxidative stability in the present, highly demanding conditions.

Keywords: Accelerated ageing; Third-body particles; Oxidation; Vitamin E; Hip simulator; micro-Raman spectroscopy.

1. INTRODUCTION

Although ultra-high molecular weight polyethylene (UHMWPE) has been the material of choice for acetabular liners in total hip arthroplasty (THA) for more than 50 years (Brach del Prever et al., 2009; Kurtz et al., 2008), oxidative degradation of UHMWPE and biological response to debris particles, released by orthopaedic implants, are still key factors in periprosthetic osteolysis and subsequent implant loosening (Bracco and Oral, 2011; Brach del Prever et al., 2009; Grupp et al., 2014; Ingham and Fisher, 2000; James et al., 2009). Many efforts have been aimed at improving UHMWPE wear performances in order to reduce wear problems. In particular, highly cross-linked polyethylenes (XLPEs), developed in the last 15 years, have shown a markedly increased wear resistance (Bistolfi and Bellare, 2011; Muratoglu, 2009). In order to warrant the oxidation resistance, thermal treatments (annealing or remelting) were applied to the first and second generation of radiation crosslinked polyethylenes (Dumbleton et al., 2006; McKellop et al., 1999; Muratoglu et al., 2001). In recent years, a different approach to stabilization has been attempted and suitable additives (i.e. vitamin E), capable to interrupt the oxidation cycle by decreasing the reactivity of the radical species, are being added to UHMWPE, giving origin to a third generation of XLPEs (Bracco and Oral, 2011; Kurtz et al., 2009a; Lurf et al., 2010).

Currently, it is important to characterize the orthopaedic components from a tribological point of view under severe conditions: i.e. to expose these components to an accelerated ageing in order to compare their behaviour with that observed following long-term exposure to natural ageing during shelf storage and/or *in vivo* exposure in human patients (Kurtz et al., 2009b, 1999). For this purpose, a number of ageing protocols have been developed over time (Affatato et al., 2016; Kurtz et al., 2001; Rocha et al., 2009). A procedure of accelerated ageing would hopefully simultaneously reproduce the chemical changes in materials, as well as the depth profiles of these changes, including oxidation (Rocha et al., 2009).

The main aim of this work was to evaluate if the addition of vitamin E was effective in improving the oxidation resistance and the wear behaviour of polyethylene, under third-body wear conditions. Since it has been observed that abrasive wear, in the presence of third-body particles, can accelerate polyethylene wear (Bragdon et al., 2003), the adding of third-body particles on PE

acetabular cups would recreate a *worst-case scenario*. In our experiment, third body particles were added to different polyethylene configurations, previously tested after accelerated ageing (Affatato et al., 2016), and our investigation was also aimed to establish a correlation, if any, between the observed wear behaviour and the chemical and morphological changes occurred in the PE as a result of the test under these severe conditions.

The three sets of acetabular cups were comparatively characterized by micro-Raman spectroscopy to gain insights into the wear mechanism, i.e. the possible changes in surface crystallinity, phases distribution and chain orientation upon wear. In addition, each component was analysed by Micro-FTIR spectroscopy to monitor the crystallinity profiles and oxidation level and by differential scanning calorimetry (DSC) to gain information on possible variations of the bulk crystallinity, following accelerated ageing and simulator testing. Gravimetric measurements of the cross-link density were also performed and compared to that of the original samples.

2. Materials and methods

In vitro wear simulation was performed using three different batches of polyethylene acetabular cups (28-mm inner × 44-mm outer dimensions; 4 specimens for each batch) coupled with 28-mm cobalt-chromium-molybdenum (CoCrMo) femoral heads. In particular, conventional UHMWPE acetabular cups (STD_PE) were machined from polymer bars made of GUR 1020 (Orthoplastics Ltd, Lancashire, UK). XLPE acetabular cups were obtained from a cylindrical bar, firstly electron beam-irradiated to 70 kGy, then thermally treated at 135°C for 12 hours, in order to remove free radicals formed during irradiation. After these treatments, the cups were machined to their final shape. Similarly, XLPE_VE acetabular cups were machined from a Vitamin E-blended UHMWPE bar (0.1% w/w; Orthoplastics Ltd, Lancashire, UK), after electron beam irradiation to 70 kGy followed by a thermal treatment at 160°C under nitrogen for 12 hours. All the cups were then subjected to ethylene oxide sterilization (EtO).

2.2 Wear test details

Wear test was performed using a 12-station hip joint simulator (IORSynthe, Bologna, Italy). The lubricant used was 25% (v/v) new born calf serum balanced with distilled water, with 0.2% sodium azide in order to retard bacterial growth, 20 mM EDTA (ethylenediaminetetracetic acid) to minimize precipitation of calcium phosphate. The final protein mass concentration value of the lubricant was 28 mg/mL, which is in agreement with that recommended by international guidelines (Organization, 2012). In addition, in order to reproduce severe test conditions (*a worst-case scenario*) debris particles were added to the lubricant. The debris particles used were Polymethyl-methacrylate (PMMA) containing 90% of PMMA and 10% of barium sulphate (BaSO₄) as radiopaque material. The particles mean size was between 70 and 200 µm. These particles were added to the lubricant at each wear-stop (i.e. every 0.4 million cycles) with a concentration of 5 mg/L. In addition, a concentration of 1 mg/L was added at the head-cup coupling interface at each wear stop using a pipette. The components were rotated across the stations after each wear-stop to minimize the effect of inter-station kinematic variability. In this test, for each station an axial load up to 2600 N was applied, as recommended by the ISO 14242-3 international guideline, according to the rotation test frequency. Flexion/Extension and Adduction/Abduction translations are programmed using a biaxial rocking motion common to all channels. Typically, in an *in vivo* situation, the surgeon fixes the acetabular cup with an abduction of about 45° and this scenario is reproduced *in vitro* by considering an inclination of 23° with respect to a horizontal plane as observed in a previous study (Taddei et al., 2006). The acetabular cup orientation was set in up-side-down (inverted anatomical) position with the aim of reproducing worst-case scenarios, i.e. the entrapment of polyethylene wear debris, generated during the test, at the head-cup interface.

The mass loss of the cups was determined every 0.4 million cycles using a new microbalance (Sartorius Cubis MSE 225, Göttingen, Germany) with a sensitivity of 0.01 mg and an uncertainty of 0.01 mg. Each mass measurement was repeated three times and the average mass was used for calculations. Wear trend was determined from the mass loss of each acetabular cup, corrected by the acetabular soak control; the wear rates, calculated from the steady-state slopes of the mass loss versus number of cycles lines, were obtained using least squares linear regression. A non-parametric

Kruskall-Wallis (K-W) test was used for the statistical significance. Statistical significance was set at $P < 0.05$.

2.3 Roughness measurements

The surface roughness of all CoCrMo femoral heads was measured using a contact profilometer Hommel Tester T8000 (Hommel Werke, Koeln, Germany) equipped with a diamond stylus tip (radius 0.020 mm). Scanning operations were performed on ten points for each femoral head: one point on the pole and three random points on three different planes identified according to a previously standardized protocol (Affatato et al., 2016, 2006). Immediately before measurement, the specimens were wiped with acetone. Sampling lengths were taken using a cut-off of 0.08 mm (tracing length 0.48 mm). To characterize the roughness of the femoral head profile, five main indicators were considered: Ra, Rq, Rt, Rsk, and Rku. In particular, Ra represents the arithmetical average height of the roughness irregularities (i.e. peaks and valleys) from the mean line. Rq is the current term for what was formerly called root-mean-square (RMS); it is more sensitive to occasional highs and lows, making it a valuable complement to Ra. The Rt parameter is defined as the height difference between the highest peak and lowest valley in the tracing length for the whole analysed surface area. Rz is calculated by measuring the vertical distance from the highest peak to the lowest valley within five sampling lengths, then averaging these distances. Finally, Rsk and Rku are the Skewness and Kurtosis indexes of the 3D surface texture, respectively. Ssk represents the degree of symmetry of the surface heights about the mean plane; the best surface has Rsk equal to 0. Rku is equivalent to the peak density of the profile (representing the indentation density), and the best surface has Rku equal to 3. Major details are available in the international literature (Affatato et al., 2016, 2006; Dong et al., 1993; Que and Topoleski, 1999; Que et al., 2000)

2.4 Micro-Raman analyses

The PE components were non-destructively analysed by micro-Raman spectroscopy before and after the third-body wear test. Micro-Raman spectra were obtained using a Jasco NRS-2000C instrument ($\lambda_{exc} = 532$ nm) with a microscope of 50 \times magnification and a confocal pinhole with an aperture diameter of 200 μ m, under the conditions previously reported (Affatato et al., 2016). All the

spectra were recorded in back-scattering conditions with 5 cm^{-1} spectral resolution using the 532 nm Green Diode Pumped Solid State (DPSS) Laser Driver (RgBLase LLC, USA) with a power of ca. 20 mW. A 160 K cooled digital CCD (Spec-10: 100B, Roper Scientific Inc.) was used as detector. The worn components were analysed near the centre of the articulating surface, which appeared the most damaged area according to previously reported data [21]; at least twelve spectra were recorded. At a first approximation, PE can be considered a composite of three different phases (Strobl and Hagedorn, 1978): a crystalline phase, an isotropic amorphous phase and an intermediate anisotropic disordered phase (i.e. a “third phase” with a prevailing trans conformation of the chains, which have lost their lateral order). To evaluate the effects of wear testing on the PE structure, several spectroscopic markers were calculated according to the literature (Lagaron et al., 1999; Strobl and Hagedorn, 1978).

The fractions of orthorhombic (α_o), amorphous (α_a) and intermediate (α_b) phases were calculated from the relative intensities of selected Raman bands, according to Strobl and Hagedorn (Strobl and Hagedorn, 1978), as reported in our previous study (Affatato et al., 2016). The fractions of all-trans sequences and all-trans sequences in an orthorhombic environment (namely ortho-trans) were evaluated according to Lagaron *et al.* (Lagaron et al., 1999), as reported in our previous study (Affatato et al., 2016). The occurrence of orientation upon wear tests was assessed by evaluating the A_{1130}/A_{1060} area ratio, as previously reported (Affatato et al., 2016). Statistical analyses were performed using the non-parametric Wilcoxon test.

2.5 Micro-FTIR spectroscopy

Fourier Transformed Infrared (FTIR) measurements were performed by means of a Spectrum Spotlight 300 Microscope (Perkin-Elmer, Shelton, Connecticut, USA). The cups were sectioned in two halves and $180\text{ }\mu\text{m}$ thick slices were microtomed from the cross section (PolyCuts Microtome, Reichert-Jung, NuBlock, Germany). Line scan spectra (resolution 4 cm^{-1} , 16 scans per spectrum) were collected on a $100\times 100\text{ }\mu\text{m}^2$ area, every $300\text{ }\mu\text{m}$, from the bearing surface to the backside, so as to include the whole cup thickness. Line scans were collected from two different regions of each sample, namely at the rim and at the bearing surface, respectively. All spectra were normalized at 2020 cm^{-1} at absorption of 0.05, corresponding to a film thickness of ca. $100\text{ }\mu\text{m}$. When applicable, a maximum

oxidation index (OI) was calculated per ASTM F2102 [ASTM F2102-13, Standard Guide for Evaluating the Extent of Oxidation in Polyethylene Fabricated Forms Intended for Surgical Implants, ASTM International, West Conshohocken, PA, 2013], as the ratio of the area under the carbonyl peak centred near 1720 cm⁻¹ and the area of the absorption peak centred near 1370 cm⁻¹. FTIR crystallinity profiles were obtained from the line-scan spectra, by correlating the absorption of the crystalline band at 1896 cm⁻¹ with that of the amorphous band at 1305 cm⁻¹ for each spectrum. The crystallinity percentage was calculated by the relation (Costa et al., 2001):

$$\%crystallinity = \frac{\frac{A_{1896}}{A_{1305}}}{\frac{A_{1896}}{A_{1305}} + 0.25} \times 100$$

2.6 Determination of cross-link density

The cross-link density was determined gravimetrically. Approximately 15 mg of each material were taken from the core of the cups and swollen in 25 mL of xylene at 135°C for 3 hours. The initial weight and xylene uptake were used to calculate the swell ratio and the cross-link density, as previously reported (Affatato et al., 2012).

2.7 Differential scanning calorimetry (DSC)

In order to determine their bulk crystallinity, DSC measurements were carried out on all samples (n=3), using a Perkin Elmer Pyris 6 instrument (Perkin-Elmer, Waltham, Massachusetts, USA). Specimens weighing approximately 5 mg were taken from the core of each sample and heated from 40°C to 180°C at a heating rate of 10°C/min. Percentage of the crystallinity was calculated by normalizing the heat of fusion of the sample, calculated as the integral from 60 to 160°C of the heating curve, to the heat of fusion of a pure polyethylene crystal (293 J/g) (Wunderlich and Dole, 1957).

3. Results

As shown in **Figure 1** and **Table 1**, during the whole third-body wear test, the STD_PE cups had almost twice as much wear as the XLPE_VE components and four times as much wear as the XLPE samples; statistically significant differences were found between the mass losses of the three sets of cups after the third-body wear test ($p = 0.039$, **Table 1**). The mass loss of the XLPE acetabular cups was significantly lower than for the STD_PE ones ($p = 0.034$, **Table 1**).

Visual examination of the femoral heads after the test showed some apparent smearing effects on the surrounding area. The surface analysis of the metallic femoral heads, after the third-body wear test, showed a small increase of the roughness parameters (**Table 2**), but no statistically significant differences were observed between the values of each of them measured before and after the test for the three sets of samples ($p = 0.700$, Mann-Whitney Test U). Moreover, no significant differences in the roughness parameters were observed between the three sets of femoral heads at the end of the test.

As an example, **Figure 2** shows the average micro-Raman spectra recorded on the STD_PE 3 acetabular cup before and after the third-body wear test. As can be easily seen, the spectrum recorded after testing showed with a significantly lower intensity the band at 1418 cm^{-1} , assigned to the orthorhombic crystalline phase; at the same time, the components at about 1080 and 1305 cm^{-1} , assigned to the amorphous phase, strengthened. Changes in relative intensities were observed also for the bands at about 1130 and 1060 cm^{-1} . These qualitative trends were confirmed by the average values of α_o , α_a and α_b (**Figure 3**), all-trans, ortho-trans and A_{1130}/A_{1060} (**Figure 4**) spectroscopic markers calculated from the micro-Raman spectra recorded on the cups before and after the third-body wear test. From a general point of view, it must be noted that the standard deviation associated with the spectroscopic values often increased upon the test. As can be easily seen from **Figure 2**, the α_o orthorhombic content underwent a general decrease upon testing, while a parallel increase in the α_a and α_b (to a lower extent) contents occurred. The XLPE samples underwent more significant changes in the α_o contents than the STD_PE specimens. The same behaviour was observed for the all-trans and ortho-trans contents reported in **Figure 4**.

Two distinct absorptions, at 1718 and 1740 cm^{-1} respectively, were observed in the carbonyl area of the FTIR spectra. **Figure 5** shows the line scan of the most worn samples of each group, taken from

the rim (right) and articulating (left) regions, respectively. Although with very different intensities, all samples showed a detectable absorption at 1740 cm^{-1} , indicating the presence of lipids diffused from the lubricant to the polymer matrix. The absorption was maximum at the bearing surface of XLPE (**Figure 5c**), followed by the rim area of the same sample (**Figure 5d**) and by both regions of XLPE_VE (**Figure 5e and f**). In addition, scattered absorption peaks at 1718 cm^{-1} , attributed to oxidation products (ketones) (Bracco et al., 2006a, 2006b), were also evident in the bulk of STD_PE, both at the rim (**Figure 5a**, maximum OI = 0.07) and at the articulating region (**Figure 6b**, maximum OI = 0.4), and at the articulating region of XLPE (**Figure 6d**, maximum OI = 0.5). In all cases the maximum OI was recorded in the bulk of the liner, while no measurable oxidation was detected at the bearing surface. No traces of oxidation products were observed in the FTIR spectra of any region of XLPE_VE.

The cross-link density (v_d) was not significantly different from that measured on the original samples (Affatato et al., 2012), being $0.165\pm 0.010\text{ mol/dm}^3$ for XLPE, $0.110\pm 0.008\text{ mol/dm}^3$ for XLPE_VE and $< 0.050\text{ mol/dm}^3$ (below significance) for STD_PE. Bulk DSC crystallinity was $50\pm 5\%$ for STD_PE, $43\pm 5\%$ for XLPE and $46\pm 3\%$ for XLPE_VE, thus slightly lower, if compared to that of the original samples (Affatato et al., 2012). However, a quite high standard deviation also indicates some inhomogeneity of the samples, following the prolonged test. **Figure 6** shows the crystallinity depth profiles as calculated from the FTIR line scan spectra. All samples showed significantly lower crystallinity on the surface than in the bulk. The difference was higher for XLPE, followed by XLPE_VE, while it was less significant for STD_PE. The bulk crystallinity confirmed the trend evidenced by DSC (STD_PE > XLPE_VE > XLPE). Interestingly, peaks in crystallinity were observed at those locations where the maximum oxidation was found.

4. Discussion

Polyethylene is today the most commonly used bearing biomaterial in acetabular components for total hip arthroplasty (MDDI, n.d.; Sobieraj and Rimnac, 2009). Unfortunately, the ideal material does not exist and PE fails during hip implantation; the main concern of this failure is the biological response to particulate PE debris generated by either conventional metal on polyethylene or ceramic

on polyethylene bearing surfaces, even if clinical performance has highlighted the evident benefits of XLPEs in total hip arthroplasty (Kumar et al., 2014; Kurtz et al., 2008). In this study we wondered if the addition of vitamin E was effective in improving the oxidation resistance and thus the wear behaviour of polyethylene, under third-body wear conditions. Moreover, our study was also aimed at clarifying if the observed wear behaviour can be correlated to chemical and morphological changes occurred in the PE liners.

The results of this investigation clearly showed less wear for the XLPE than for the other configurations. After a prolonged accelerated ageing (Affatato et al., 2016) and by the addition of third-body cement particles, the mass loss was found to decrease along the series $STD_PE > XLPE_VE > XLPE$, in agreement with that expected on the basis of the cross-link density of the tested samples, which showed the inverse trend. However, statistically significant differences were found only between the mass losses of STD_PE and $XLPE$ ($p = 0.034$, **Table 1**). No statistical differences were found between $XLPE_VE$ and $XLPE$ samples ($p = 0.890$, **Table 1**), in agreement with Oral et al. (Oral et al., 2013). Moreover, also other authors have reported that the wear rates of UHMWPE with or without vitamin E were not significantly different (Bladen et al., 2013).

Several studies have investigated the effects of the addition of debris particles during a wear test. Affatato et al. (Affatato et al., 2002) have reported that CoCrMo femoral heads tested in the presence of PMMA particles showed evidence of macroscopic damage with a PE mass loss about four times greater than without PMMA. Bragdon and co-workers (Bragdon et al., 2003) have shown that by adding two kinds of debris (aluminum oxide and PMMA) to conventional UHMWPE *versus* XLPE in a hip wear simulator, the mass loss of the former was always greater than that of the latter. In contrast, the incremental wear rate of each material remained essentially unchanged in the presence of PMMA third-body particles. Kubo et al. (Kubo et al., 2009) have comparatively tested conventional UHMWPE and XLPE cups in a hip joint simulator by adding to the lubricant a commercial PMMA bone cement powder (used as the “abrasive” medium). These authors have found that the XLPE components maintained a superior finish with respect to the conventional UHMWPE samples. Wang and Essner (Wang and Essner, 2001) have reported that the UHMWPE cups showed a strong dependence of wear rate on the concentration of the PMMA particles in the lubricant. These authors

have found that “higher concentrations of PMMA particles led to an accelerated wear of the acetabular cups and mild scratching damage was observed on the CoCr heads after testing”.

A recent paper by Grupp *et al.* (Grupp *et al.*, 2014) has compared the *in vitro* wear behaviour of accelerated aged XLPE and vitamin E-added XLPE acetabular cups coupled to CoCrMo heads under third-body wear and “clean” conditions; going from the latter conditions to the former, the gravimetric wear rate of vitamin E-added XLPE acetabular cups increased by 6.9 times (i.e. from 3.4 ± 0.5 mg/Mc to 23.5 ± 15.5 mg/Mc) and even more for XLPE samples. Our XLPE_VE components behaved surprisingly well, since we have observed the same wear rate under “clean” and third body wear conditions, i.e. 52/mg/Mc (**Figure 1**). It must be pointed out though, that such wear rate was unexpectedly high under “clean” conditions (Affatato *et al.*, 2016).

Concerning the roughness measurements, several studies were performed on the effect of surface roughness on polyethylene wear and debris particle generation (Affatato *et al.*, 2006; Elpers *et al.*, 2014; Williams *et al.*, 2007); all these studies showed that with roughened or damaged surfaces, the morphology of the PE debris generated by wear can be altered. Nevertheless, to the authors knowledge, no study has been done to analyse the inverse effect: the effect of third-body particles on the surface roughness. In this study it was emphasize that no statistically significant changes in the measured parameters were observed upon the test. From our results we can state that the addition of PMMA particles did not significantly alter the surface roughness of CoCr femoral heads. This fact can be explained by considering that the material which is more affected by the influence of third-body wear is the polyethylene, since it is the weakest material of the analysed coupling.

On the other hand, the presence of the PMMA debris definitely altered the morphology of the acetabular cups on a molecular scale. From a general point of view, it must be noted that the standard deviation associated with the values of the micro-Raman spectroscopic markers often increased upon the test; this behaviour suggests that the surface of the specimens underwent an increase in inhomogeneity, consistently with the presence of added debris.

The spectroscopic data showed that the testing conditions caused a decrease in crystallinity, as revealed by the weakening of the Raman marker band of the orthorhombic phase at 1418 cm^{-1} (**Figure 2**) and the corresponding decrease of the α_0 content (**Figure 4**), as well as by the FTIR crystallinity

obtained from the surface of the samples, which was found to be lower than in their bulk (**Figure 6**). These results are in agreement with those reported by Galetz and Glatzel (Galetz and Glatzel, 2010) who have ascribed the destruction of the order in the PE crystalline structure to an adhesive interaction between the polymer and the metal counterface. An analogous decrease in crystallinity was observed also in previous tests carried out on PE components tested under severe conditions (Abdel Jaber et al., 2014; Tozzi et al., 2014). According to the literature (Kanaga Karuppiah et al., 2008; Simis et al., 2006), a decrease in crystallinity represents a worsening of the wear properties, resulting in decreases in elastic modulus, yield strength, scratch resistance and micro-hardness, as well as in increases in friction force, wear depth and width. On the other hand, a higher amorphous content may be related to a higher capacity of shape recovery after removal of the applied load, but a lower resistance to creep deformation (Takahashi et al., 2014); at the same time, Kang et al. demonstrated that in XLPE components a decrease in crystallinity resulted in lower tensile strength with higher creep deflection, but also in a lower wear rate (Kang and Nho, 2001).

Most micro-Raman markers (**Figures 3 and 4**) had trends analogous to those observed in the previous test (Affatato et al., 2016); however, as expectable on the basis of the more severe wear conditions of the present test, more significant variations were observed in all the markers. The most significant changes were observed for the XLPE and XLPE_VE samples (**Figures 3 and 4**). These findings may be related to the DSC results, which showed higher crystallinity changes with respect to the original samples (Affatato et al., 2012) for the XLPE and XLPE_VE acetabular cups. Actually, going from the starting specimens to those tested for 9 million cycles (i.e. after the third-body wear test), the DSC crystallinity changed from $52\pm 2\%$, $49\pm 2\%$ and $52\pm 2\%$ for STD_PE, XLPE and XLPE_VE, respectively (Affatato et al., 2012), to $50\pm 5\%$, $43\pm 5\%$ and $46\pm 3\%$. The FTIR crystallinity results were in agreement with the micro-Raman and DSC trends: the depth profiles reported in **Figure 6** show that the crystallinity decrease is even more pronounced on the surface than in the bulk. Nonetheless, surface and bulk crystallinity values had a similar trend. Not surprisingly, peaks of crystallinity were observed in correspondence of the oxidized spots, as it is known that chain scissions caused by oxidative degradation can result in easier folding of the polymer chains and in a subsequent increase in crystallinity (Goldman et al., 1996). The FTIR surface crystallinity decreased along the

series STD_PE > XLPE_VE > XLPE, in agreement with the trend of the average crystallinity values obtained by micro-Raman spectroscopy on the surface (i.e. the first tens of micrometres) of the three sets of acetabular cups, at the end of the third-body wear test (**Figure 3**); the differences in the absolute crystallinity values obtained through the two techniques may be ascribed to their different sampling volumes.

It is interesting to stress that the FTIR crystallinity differences between the bulk and the surface areas were decreasing along the series XLPE > XLPE_VE > STD_PE (**Figure 6**), in agreement with the micro-Raman data, which showed the most significant changes in α_o and all-trans contents as well as chain orientation (i.e. A_{1130}/A_{1060} ratio) for the XLPE and XLPE_VE samples (**Figures 3 and 4**). These findings could appear unexpected by considering that the mass loss decreased according to the inverse trend. Actually, **Figure 7** shows that the α_o and all-trans contents as well as A_{1130}/A_{1060} ratio well correlated with the sample mass loss; in other words, crystallinity and morphological changes were link to bulk material properties (gravimetric loss, i.e. wear). The graphs reported in **Figure 7** demonstrate that the least macroscopically worn samples (i.e. those which lost the lowest mass) were the most degraded samples on a molecular scale (i.e. underwent the most significant changes in α_o and all-trans contents as well as chain orientation) and *vice versa*. At this purpose, it must be recalled that micro-Raman spectroscopy was used to analyse the skin of the worn samples. If the surface of the sample is worn away during the test, the underlying (i.e. less degraded) layer is analysed. In this light, it is not surprising that the most worn samples, i.e. STD_PE acetabular cups, showed the least significant differences in crystallinity between the surface and the bulk. If compared with the previous test (Affatato et al., 2016), the only micro-Raman spectroscopic markers that showed a different behaviour after the third-body wear test were the ortho-trans content and the A_{1130}/A_{1060} ratio. The former underwent a general increase in all the samples, the latter a general decrease, while different behaviours among the three sets of samples were observed upon the second test (Affatato et al., 2016). These trends revealed that under the more severe third-body wear testing conditions, the three sets of samples behaved similarly with regards to the distribution of the all-trans sequences within the orthorhombic phase (i.e. ortho-trans content) and polymeric chains orientation

(i.e. A_{1130}/A_{1060} ratio). At this purpose, it must be recalled that if the molecules are oriented in a preferred direction, the 1130 cm^{-1} band has been reported to become stronger with respect to the 1060 cm^{-1} band, which results in a A_{1130}/A_{1060} increase (Pigeon et al., 1991). Therefore, it is not surprising that, under third-body wear conditions, polymeric chains underwent a loss of orientation, as revealed by the A_{1130}/A_{1060} decrease.

As in our previous test, diffusion of lipids was observed in all samples, although to a different extent (**Figure 5**): the evidence of absorbed lipids was maximum in XLPE, while it was almost insignificant in STD_PE. We have previously warned against misinterpretations of the wear results caused by lipids absorption (Affatato et al., 2012). However, in the present case, the layer of diffused lipids was much thinner than that observed in previous tests, so that their weight is likely not significant compared to the wear mass loss. This occurred because, in the highly demanding conditions of the present test, the amount of wear was much higher than in conventional tests. In particular, a minimum amount of lipids was observed at the articulating surface of STD_PE, which conversely experienced the maximum wear, likely indicating that the polyethylene layer containing the diffused lipids has been worn away during the test. On the other hand, the maximum amount of lipids was found in XLPE, which also showed the minimum wear.

Our results confirmed that, as already observed (Affatato et al., 2012), the radical scavenger effect of vitamin E reduces the cross-linking efficiency, thereby limiting the improvement in wear resistance provided by radiation cross-linking at a given radiation dose. However, the present study also showed that, while XLPE_VE did not show any chemical degradation, both additive-free STD_PE and XLPE cups evidenced traces of oxidation. In both cases, the maximum oxidation was significant but limited to few spots in the deep bulk of the articulating region; in addition, a lower but more spread oxidation was also observed at the rim of STD_PE. Since these samples did not show any oxidation either at the beginning of the tests, or after the first 2 million cycles (Affatato et al., 2012), we should assume that the observed degradation had developed during the subsequent tests. Not surprisingly, there was no correlation between the observed oxidation and the wear behaviour, since the amount of oxidation is limited and mainly confined to the bulk of the cups. Moreover, the absence of significant differences in the cross-linking densities of all samples before and after the test

demonstrated that this level of degradation has not had an impact on the overall material properties. However, this finding has a much interesting potential: standard, not-irradiated and post-irradiation melted, cross-linked polyethylenes do not contain detectable free radicals at the time of implantation (Muratoglu et al., 2001) and thus were expected to be resistant against oxidation. Nevertheless, analyses of clinical retrievals of both tibial and acetabular polyethylene inserts showed that, in some cases, oxidation had developed during *in vivo* use (Muratoglu et al., 2010; Regis et al., 2014; Reinitz et al., 2015). The occurrence of oxidation was attributed to mechanisms correlated with lipids absorption and/or with the cyclic stress acting during *in vivo* service, even if its exact origin is still under debate. With this in mind, we speculated that the mild oxidation observed here, following a combination of cyclic stress and accelerated ageing, might have the same origin of that observed in the retrievals. If this is the case, in our knowledge, this is one of the first studies in which such phenomenon has been reproduced *in vitro*; moreover, the present results demonstrate that vitamin E is effective in stabilizing UHMWPE also against these relatively new and unexpected mechanisms of oxidation. Although the oxidation observed here was limited to the bulk of the cups and did not have an apparent effect on the wear behaviour in our test, its evolution *in vivo*, under real time ageing conditions, is unpredictable. Overall, our findings suggest that the use of an efficient antioxidant should be recommended to increase the longevity of the UHMWPE implants.

5. Conclusions

This study was approached to acquire further knowledge about the effects that third-body cement particles could have on the wear behaviour of different PE acetabular cups upon prolonged testing and ageing. Under these conditions, mass loss was found to increase along the series XLPE < XLPE_VE < STD_PE, although only the differences between XLPE and STD_PE appeared significant. On the contrary, although not relevant to the actual wear behaviour, traces of bulk oxidation were found in both STD_PE and XLPE. Even though the origin of such unexpected oxidation is still under debate, it must be noted that, interestingly, XLPE_VE demonstrated a high oxidative stability also in the present, highly demanding conditions. Roughness of CoCrMo femoral

heads did not significantly increase upon the third-body wear test and the three sets of femoral heads coupled to the three different sets of acetabular cups did not show significant differences in roughness parameters.

Bulk DSC crystallinity, surface micro-Raman crystallinity and FTIR crystallinity seemed to have a similar behaviour upon testing; actually, all of them revealed the most significant changes in XLPE and XLPE_VE samples. From a general point of view, the more severe third-body wear testing conditions determined more noticeable changes in all spectroscopic markers with respect to the previous tests.

Acknowledgements

The authors wish to thank Tomaso Villa (Politecnico di Milano, Dept. of Structural Engineering, Milano-Italy) for providing the components and Barbara Bordini (Rizzoli Orthopaedic Institute) for her help with the statistical analyses.

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Tables & Figures Captions

Table 1 - Cumulative mass loss (mean \pm standard deviation) for the three sets of PE acetabular cups tested. Values and Statistical Analysis Performed using a Kruskal-Wallis Non parametric Test.

Table 2 - Mean roughness (\pm Standard Deviation) for the CoCrMo femoral heads coupled with the three different sets of acetabular cups before and after the third test.

Figure 1 – Wear behaviour and Regression coefficient for the three sets of PE acetabular cups tested.

Figure 2 – Average micro-Raman spectra of the STD_PE 3 acetabular cup before and after the third-body wear test in the CH₂ bending (δ), wagging (ω), twisting (τ) and rocking (ρ), and C-C stretching (ν ; s = symmetric; a = anti-symmetric) regions; the assignments to the amorphous (A), crystalline (C) and third (A_{trans}) phases have been given according to the literature.

Figure 3 – Values (average \pm standard deviation) of α_o , α_a and α_b contents as obtained from the micro-Raman spectra recorded before and after the third-body wear test on the STD_PE, XLPE_PE and XLPE acetabular cups. Only significant differences have been indicated ($p < 0.05$). The numbers reported on the arrows are the p values.

Figure 4 – Values (average \pm standard deviation) of all-trans and ortho-trans contents, and A_{1130}/A_{1060} ratios as obtained from the micro-Raman spectra recorded before and after the third-body wear test on the STD_PE, XLPE_VE and XLPE acetabular cups. Only significant differences have been indicated ($p < 0.05$). The numbers reported on the arrows are the p values.

Figure 5 – FTIR line-scan of: a) STD_PE, rim, b) STD_PE articulating region, c) XLPE, rim, d) XLPE articulating region, e) XLPE_VE, rim, f) XLPE_VE articulating region. Green arrows indicate the 1740 cm⁻¹ absorption (esters - lipids); red arrows indicate the 1718 cm⁻¹ absorption (ketones – oxidation products).

Figure 6 – FTIR crystallinity profiles at the articulating region of the most worn sample of each group.

Figure 7 – Trend of the % changes in the α_o and all-trans contents as well as A_{1130}/A_{1060} ratio, determined by micro-Raman spectroscopy upon the test, as a function of the mass loss of each sample.

Table 1 - Cumulative mass loss [mean (mg) ± standard deviation] for the three sets of PE acetabular cups tested. Values and Statistical Analysis Performed using a Kruskal-Wallis Nonparametric Test.

Cycles [Mc]	Mean [mg] ±Standard deviation			K-W test (p-value)	LSD Post Hoc test (p-value)		
	STD_PE	XLPE_VE	XLPE		STD_PE vs. XLPE_VE	STD_PE vs. XLPE	XLPE_VE vs. XLPE
0.4	57.4 ±35.3	27.5 ±8.8	7.3 ±5.9	0.061	0.051	0.056	0.052
0.8	111.2 ±49.6	42.3 ±11.4	15.1 ±4.6	0.039	0.890	0.034	0.408
1.2	154.8 ±47.6	65.8 ±11.7	23.1 ±4.5	0.027	0.539	0.022	0.539
1.6	204.2 ±54.5	85.1 ±19.1	39.7 ±19.6	0.027	0.539	0.022	0.539
2	272.9 ±59.1	117.5 ±42.6	52.7 ±21.3	0.039	0.408	0.034	0.890

Not significant = p value > 0.05; K-W = Kruskal-Wallis; Mc = millions cycles.

Table 2 - Mean roughness (\pm Standard Deviation) for the CoCrMo femoral heads coupled with the three different sets of acetabular cups before and after the third test.

	<i>Before the third-body wear test</i> [19]			<i>After the third-body wear test</i>		
	<i>STD_PE</i>	<i>XLPE</i>	<i>XLPE_VE</i>	<i>STD_PE</i>	<i>XLPE</i>	<i>XLPE_VE</i>
<i>Ra</i> <i>[μm]</i>	<i>0.04\pm0.02</i>	<i>0.03 \pm0.01</i>	<i>0.04 \pm0.02</i>	<i>0.05\pm0.02</i>	<i>0.04 \pm0.01</i>	<i>0.05 \pm0.02</i>
<i>Rt</i> <i>[μm]</i>	<i>0.41 \pm0.07</i>	<i>0.38 \pm0.17</i>	<i>0.37 \pm0.05</i>	<i>0.61 \pm0.07</i>	<i>0.58 \pm0.17</i>	<i>0.47 \pm0.05</i>
<i>Rq</i> <i>[μm]</i>	<i>0,09 \pm0,06</i>	<i>0,05 \pm0,02</i>	<i>0,05\pm0,01</i>	<i>0,07\pm0,05</i>	<i>0,04\pm0,01</i>	<i>0,04\pm0,01</i>
<i>Rz</i> <i>[μm]</i>	<i>0,23\pm0,14</i>	<i>0,15\pm0,02</i>	<i>0,15\pm0,03</i>	<i>0,19\pm0,14</i>	<i>0,13\pm0,03</i>	<i>0,13\pm0,04</i>
<i>Rsk</i>	<i>-0.35\pm0.96</i>	<i>-0.36 \pm0.49</i>	<i>-0.46 \pm0.29</i>	<i>-0.55\pm0.96</i>	<i>-0.66 \pm0.49</i>	<i>-0.45 \pm0.29</i>
<i>Rku</i>	<i>10,76\pm13,62</i>	<i>8,95\pm2,55</i>	<i>6,52\pm1,66</i>	<i>10,08\pm13,5</i>	<i>6,03\pm2,02</i>	<i>4,69\pm0,66</i>

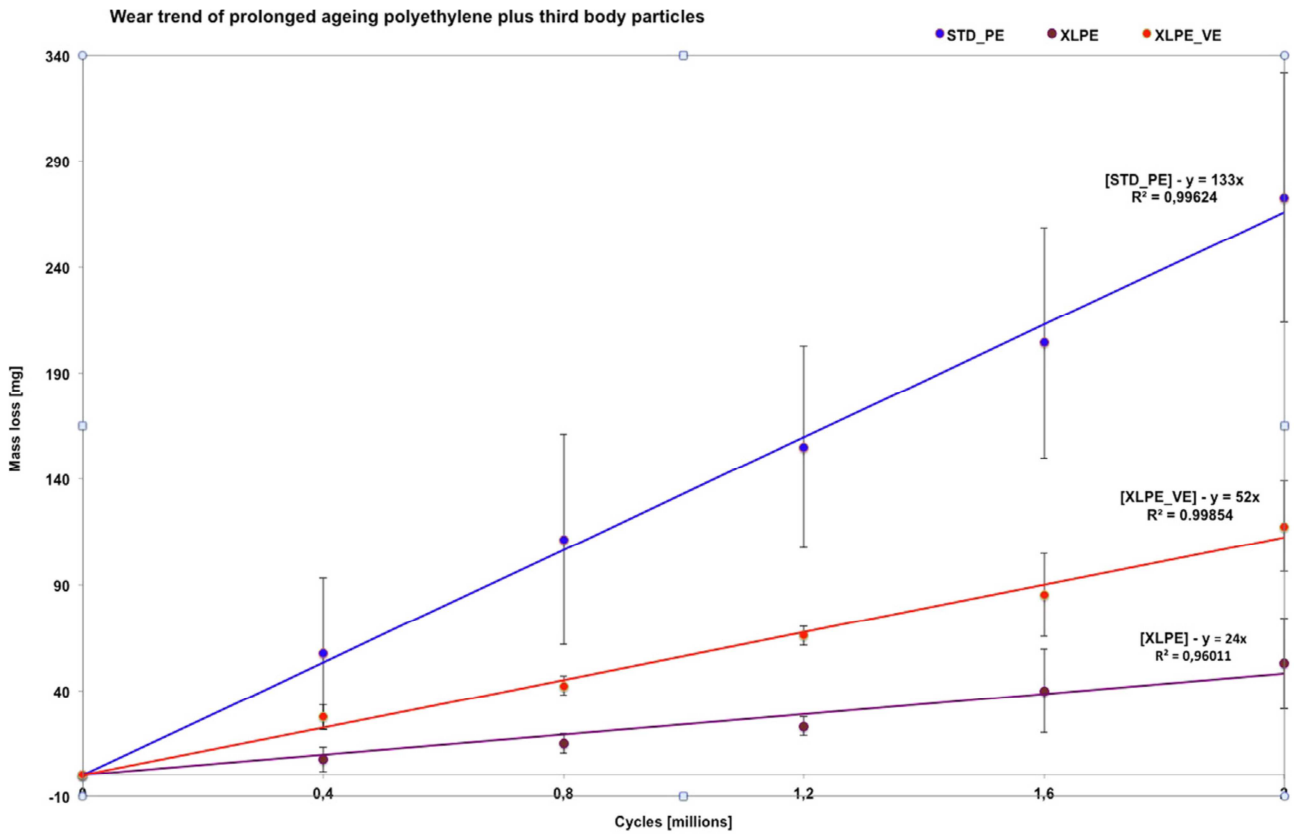


Figure 1

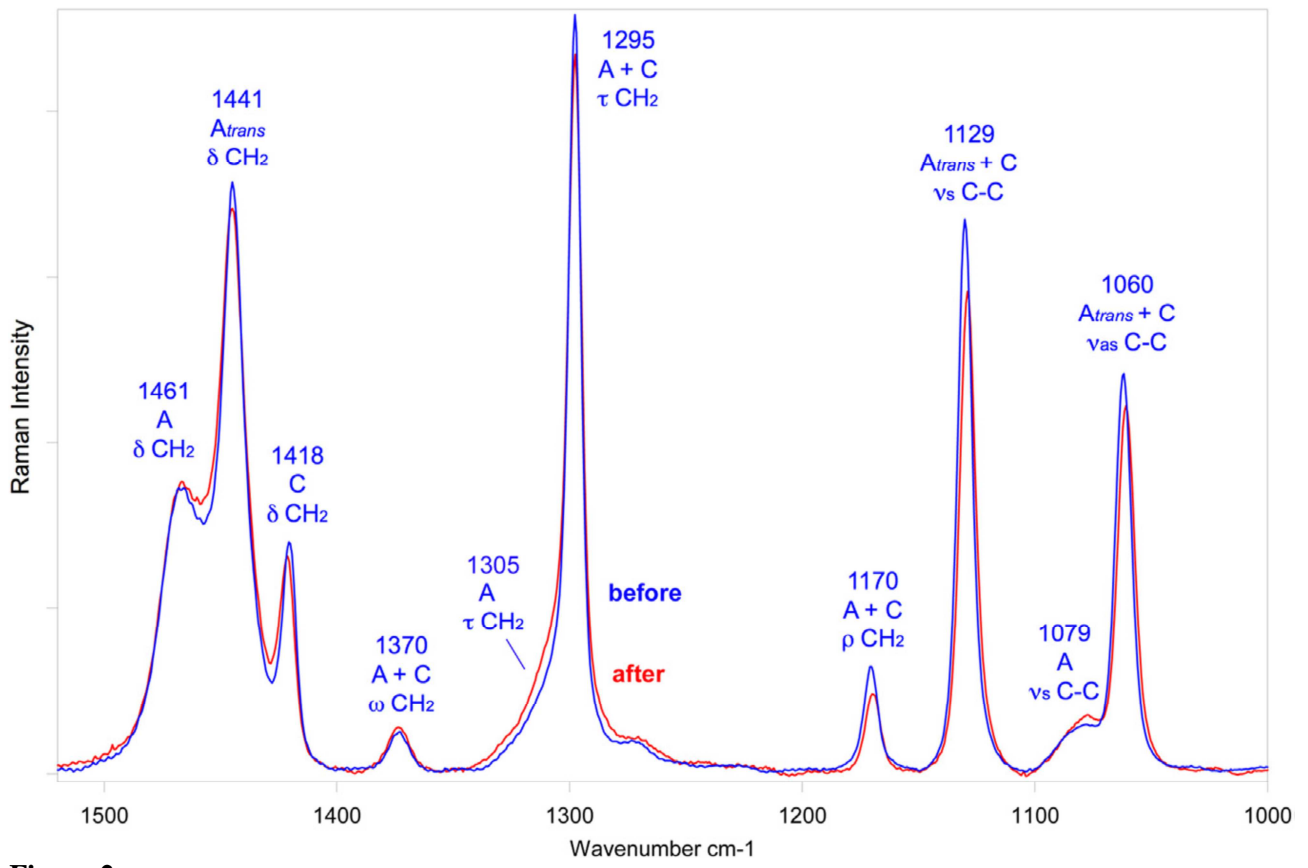


Figure 2

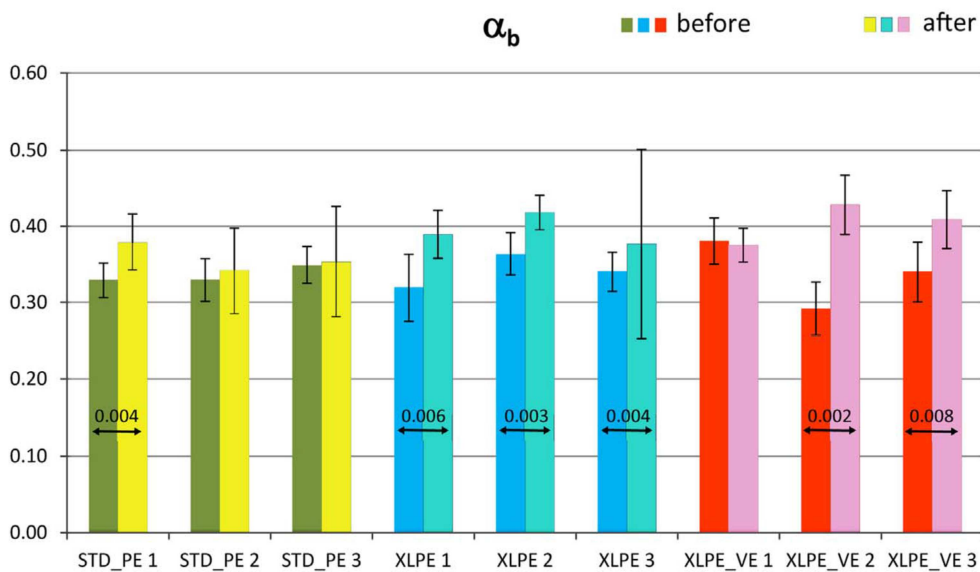
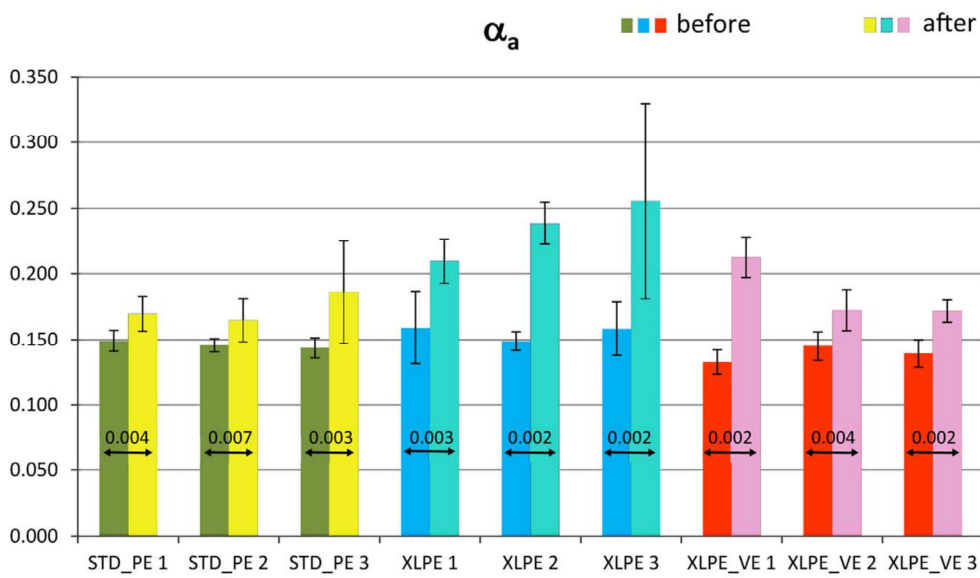
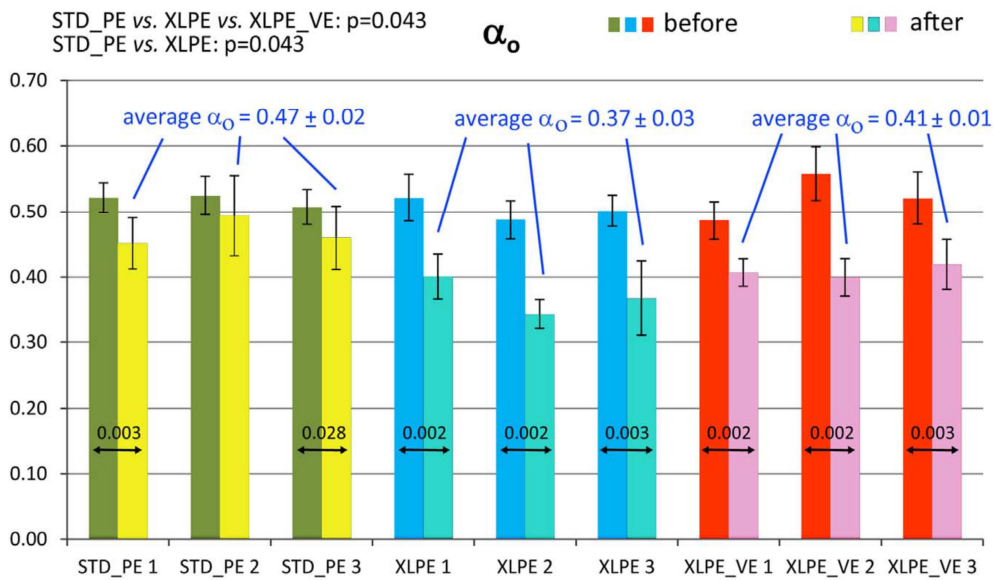


Figure 3

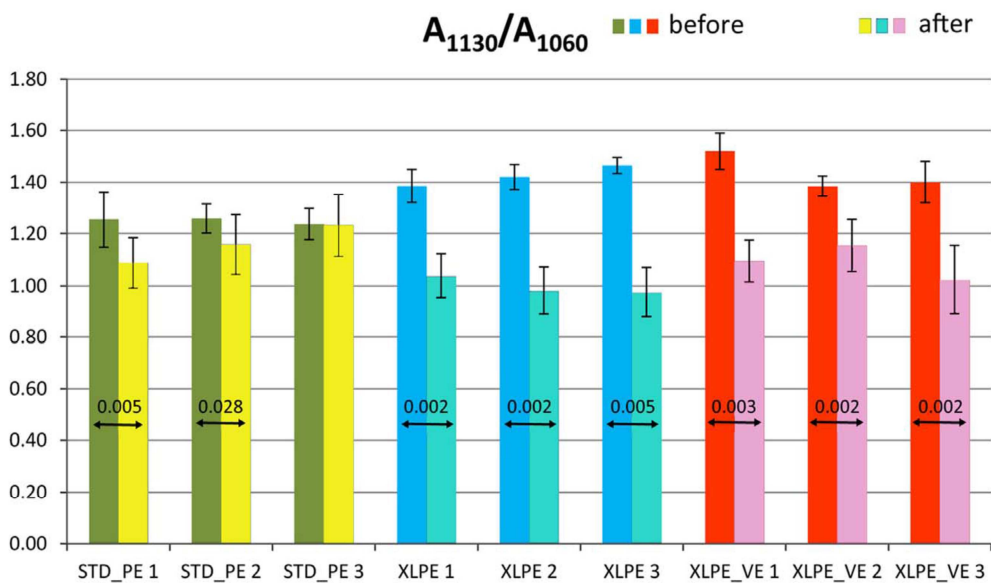
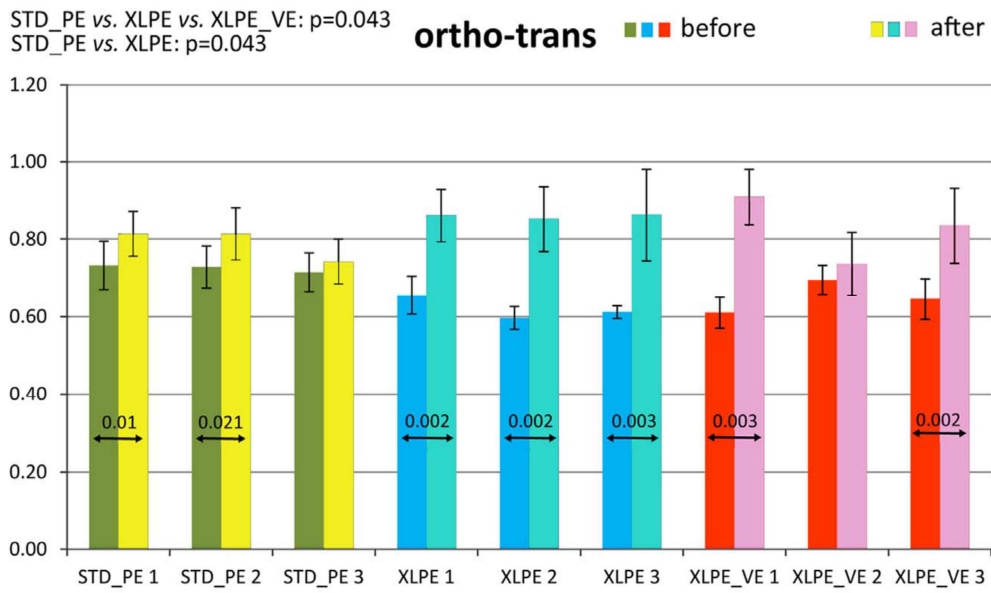
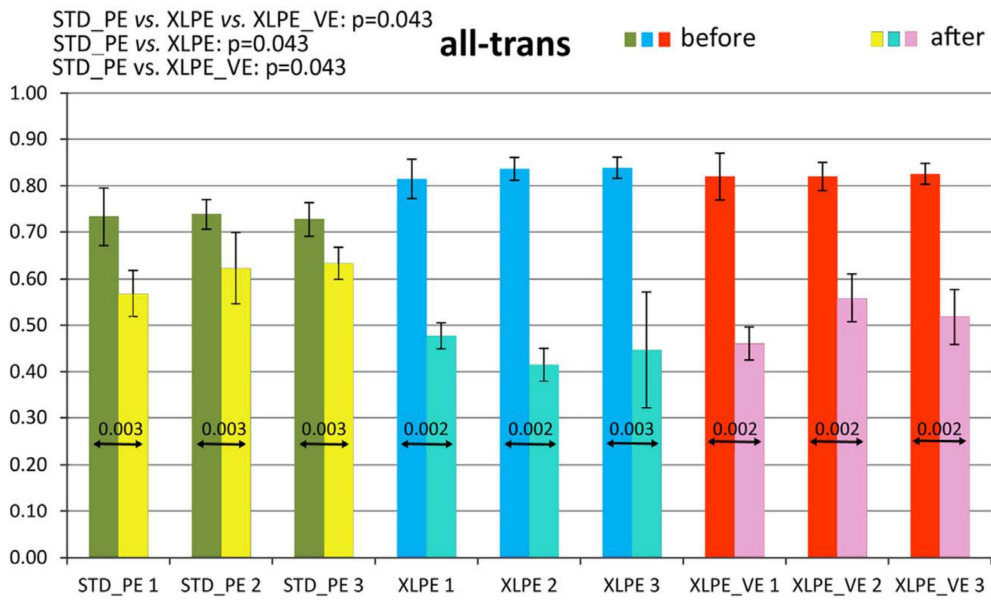


Figure 4

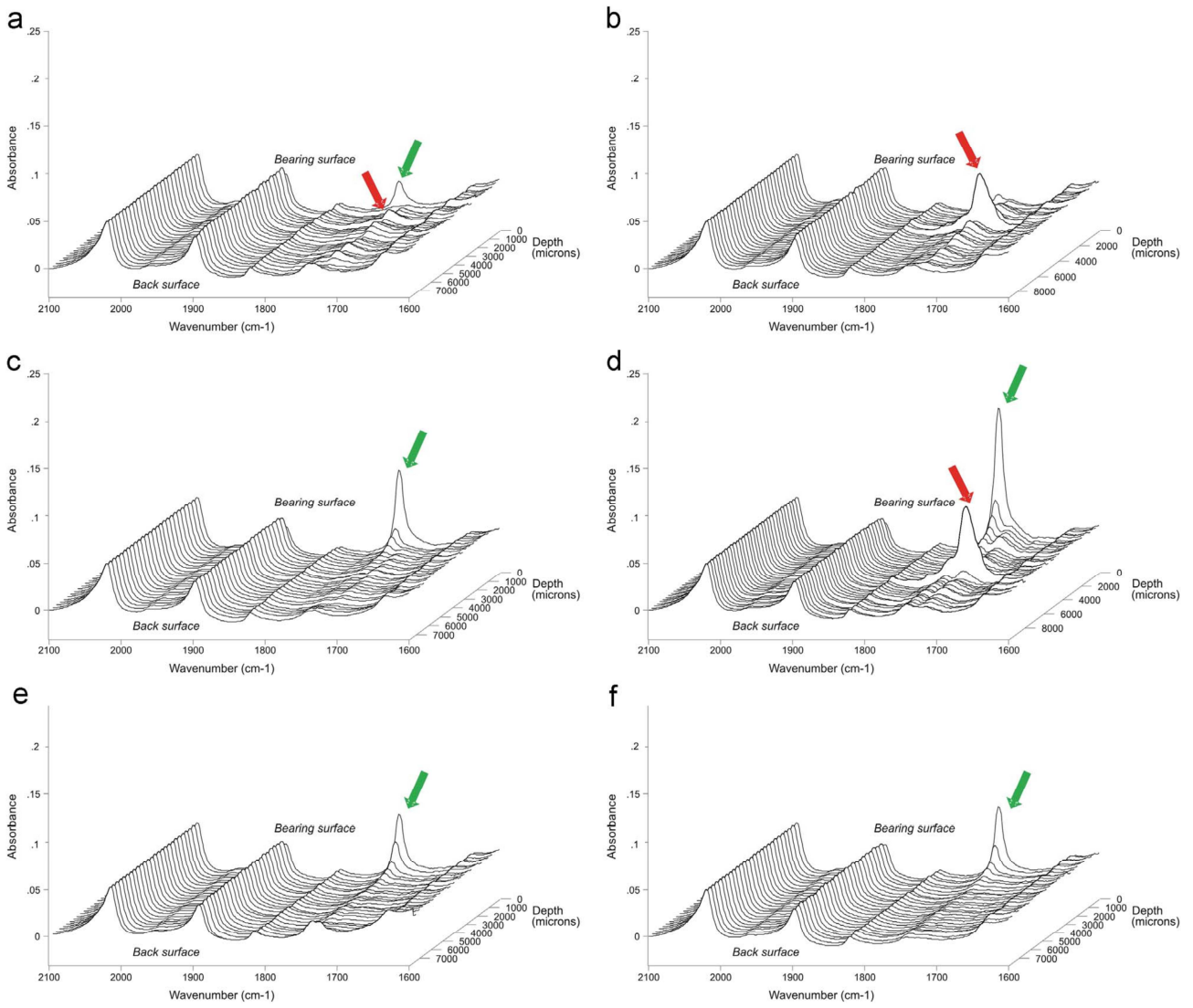


Figure 5

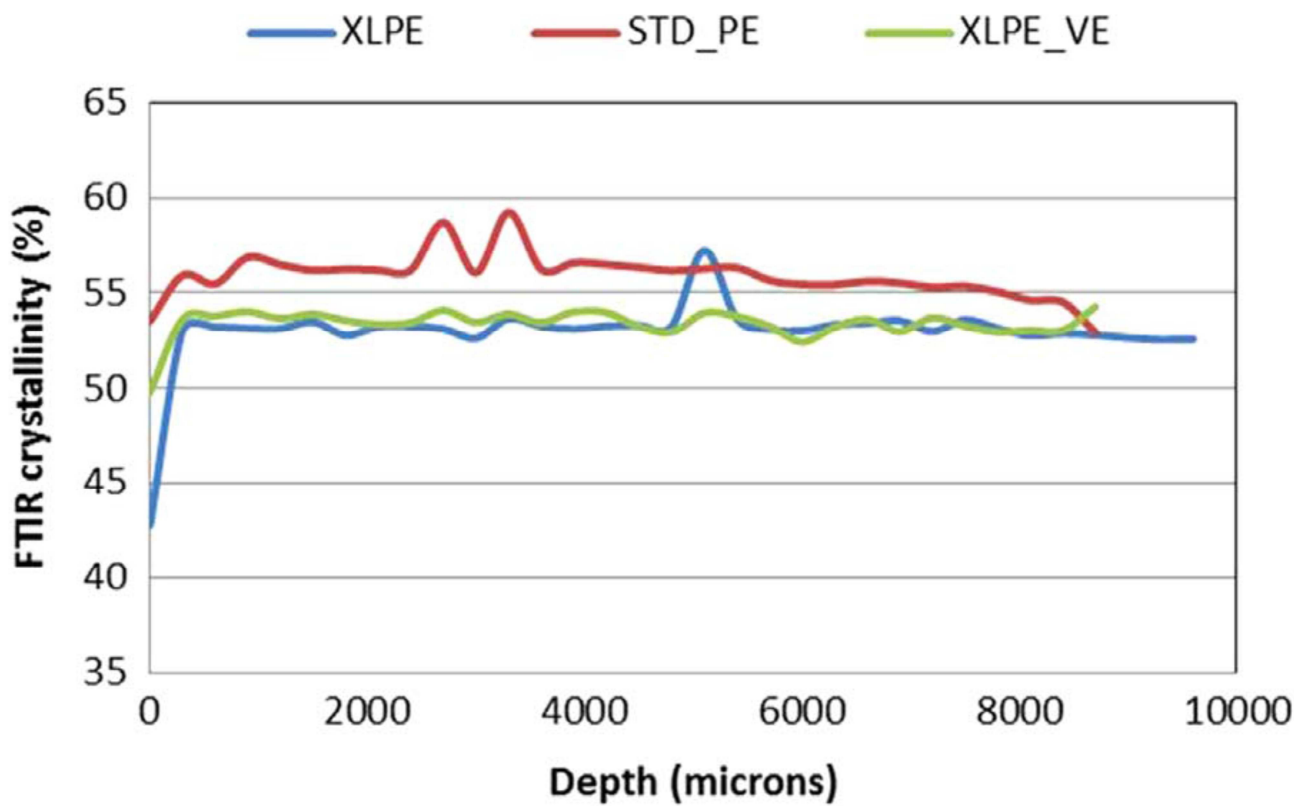


Figure 6

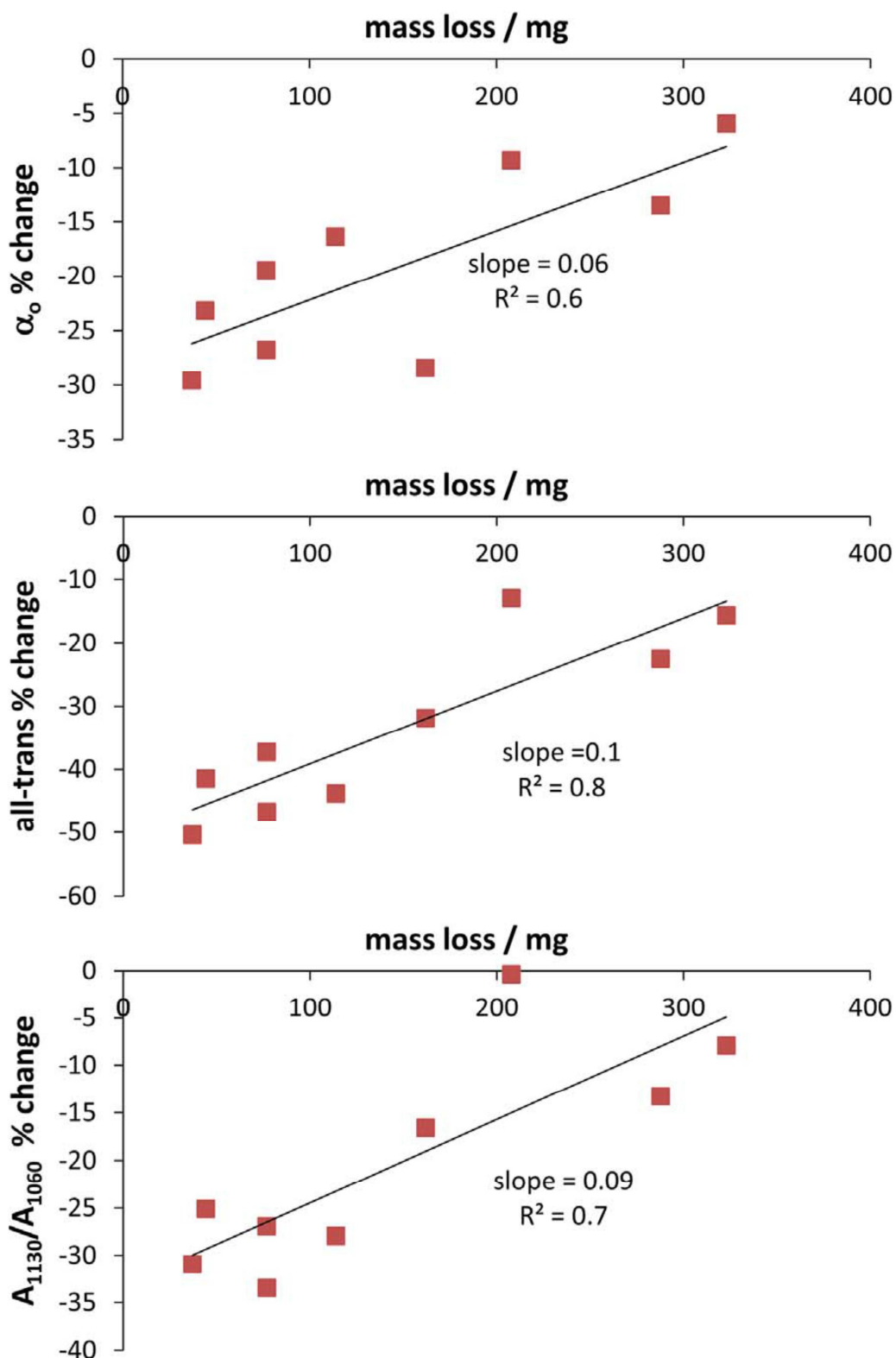


Figure 7