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Determination of Crystallinity and Crystal Structure of Hylamer™ Polyethylene after *in vivo* Wear

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ABSTRACT: Hylamer™ polyethylene is a crystalline form of polyethylene of 70% crystallinity whereas conventional polyethylene (PE) has 50% crystallinity. Crystallinity is the percentage by weight of the crystalline phase present in the whole polymer, which comprises both amorphous and crystalline phases.

Clinical experience has shown that Hylamer™ components used in joint prostheses, if sterilized by gamma rays in the presence of oxygen, are easily affected by wear, which leads to osteolysis. The authors have analyzed the

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crystallinity of polyethylene liners removed from seven patients who had received HylamerTM polyethylene implants sterilized by gamma rays in air and had suffered prosthetic loosening, using Raman spectroscopy coupled with partial least squares (PLS) analysis. The results have been compared to those of two controls who had received HylamerTM polyethylene implants sterilized by gamma irradiation in a nitrogen atmosphere. The crystal structure of wear particles released into the tissues from the HylamerTM liners sterilized by gamma rays in air is also studied. The materials undergoing two different types of sterilization methods show different crystallinity values (71.50 vs. 69.43), but the crystallinity do not change according to wear (worn and unworn liner region). Both monoclinic and orthorhombic phases are present in the liner, while in wear debris prevalently monoclinic crystals are found in both types of sterilized liners. Different crystallinity rates can explain different wear rates observed *in vivo*.

KEY WORDS: crystallinity, polyethylene, wear, HylamerTM, Raman spectroscopy.

INTRODUCTION

In 1990 Depuy Dupont Orthopaedics introduced a new acetabular liner manufactured from an enhanced UHMWPE known as HylamerTM enhanced bearing polymer [1]. HylamerTM is a crystalline form of polyethylene (PE) on the order of 70% crystallinity compared with the 50% crystallinity of conventional polyethylene. Increased temperature and extremely high pressure are used to reach these values without sacrificing the molecular weight of the polyethylene.

In laboratory studies performed in the early 1990s, by both pin-on-disk testing and hip simulation wear-testing devices, on non-sterilized samples of HylamerTM, it appeared to be superior in strength, creep, wear, and impact resistance [2] and was expected to decrease the amount of wear debris.

HylamerTM was sterilized by gamma irradiation in air environment from 1990 until 1993, then from 1993 to 1995 by gamma irradiation in a nitrogen environment followed by vacuum packaging; finally from 1995 to 1998, the implants were sterilized with the use of a gas plasma process without irradiation.

Subsequent clinical studies showed that HylamerTM, which was sterilized by gamma irradiation in an air environment, underwent increased wear, delamination, and fracturing. These phenomena were attributed to the oxidation of the material [3], to the decrease in molecular weight, and consequent increase in density and crystallinity [4–6].

The oxidation of the UHMWPE has been investigated [7] and the mechanism of polyethylene oxidation due to radiations in the presence

of air is well documented [8]. The oxidative process initiated by radiation is always present, before the implant and during *in vivo* service.

Premnath et al. [9] described the way in which gamma radiation interacts with UHMWPE, and the rationale for the frequently reported incidence of subsurface oxidation. On exposure of UHMWPE to gamma radiation, energy is transferred from the gamma photon to the material. This transfer leads to the emission of one or more electrons from an atom into the medium. Through excitation, ionization, and breakage of bonds, the kinetic energy of these electrons is transferred to the medium, and ultimately the electrons recombine with the ions in the medium, forming free radicals. As a result of these factors, maximum oxidation is found at a certain distance (typically 1–2 mm) below the surface of the UHMPWE and this subsurface band is correlated with cracking and delamination in retrieved acetabular liners [10].

The degree of oxidation reached by a material sterilized by gamma rays also depends on the material starting value of crystallinity. Highly crystalline materials can be more easily oxidized. With regard to this, Goldman et al. [11] proposed two theories. The first is based on the presumption that a higher degree of crystallinity implies more lamellae, which are connected to each other by tie molecules. These tie molecules are prone to chain scission, and as there are more of them in a more crystalline material, the more crystalline material is more prone to oxidation.

The second theory was that free radicals within crystalline regions are less mobile than those located in amorphous regions. The more mobile free radicals are able to recombine or cross link before being oxidized to form peroxy radicals; conversely, the less mobile free radicals in the more crystalline UHMWPE are more likely to lead to peroxy radical formation. The study of crystallinity may provide important information because the crystallinity itself and the correlated density of the UHMWPE samples are related to the oxidation rate.

The aim of this work is, therefore, to analyze by micro-Raman spectroscopy coupled with partial least squares (PLS), retrieved HylamerTM acetabular liners sterilized by means of gamma rays either in air or in nitrogen environment to:

- establish whether the different sterilization processes, besides producing different oxidative phenomena, also induce variations in crystallinity;
- determine whether mechanical friction *in vivo* can bring about a change in surface crystallinity of the liners;
- compare the structure of wear debris with that of the liner from which they have been detached.

MATERIALS AND METHODS

Materials

The polyethylene liners were obtained from seven patients with Hylamer™ liners gamma sterilized in air (Duraloc liner, Depuy Orthopedics, Indiana, USA). The patients were undergoing hip prosthesis revision due to aseptic loosening (Group 1) related to high polyethylene wear. For comparison, two liners were obtained from patients with Hylamer™ liners gamma sterilized in nitrogen atmosphere (Group 2). Tissue samples from joint capsules were obtained from five patients out of seven patients of Group 1. The diagnosis of loosening was made on the basis of pain and radiographic findings. The preoperative radiographs were analyzed for evidence of migration of the implant, the presence of radiolucent lines at the bone-implant interface and erosion, defined as localized resorption of bone, around the implant. Written informed consent was obtained from each patient for the procurement and analysis of the tissue.

The retrieved polyethylene liners were fixed in 10% neutral buffered formalin, then air dried and tested within a few days.

Details of patients and of implants are provided in Table 1. The maximum wear values were calculated by radiographic evaluation according to standardized procedures described elsewhere [12].

Table 1. Details of the seven patients with Hylamer™ liner gamma sterilized in air (Group 1) and two patients with Hylamer™ liner gamma sterilized in nitrogen atmosphere (Group 2).

Group 1	Sex/age	Side	Pathology	Fixation of the prosthesis	Shelf time (years)	Implant duration (months)	Maximum wear of the liner (mm)
1	M/59	R	Postrauma arthrosis	Uncemented	0.6	103	1.11
2	M/63	L	Hip dysplasia	Hybrid	0.6	103	0.65
3	F/63	R	Hip dysplasia	Hybrid	5.4	72.3	2.64
4	M/71	R	Coxarthrosis	Hybrid	1.9	88	3.68
5	F/54	L	Coxarthrosis	Hybrid	4.0	63	3.59
6	F/67	R	Coxarthrosis	Hybrid	3.2	89	2.80
7	F/51	R	Hip dysplasia	Uncemented	3.5	77	3.40
Group 2							
8	F/64	L	Revision	Hybrid	3.5	42	0.50
9	M/68	L	Revision	Hybrid	3.0	36.6	0.43

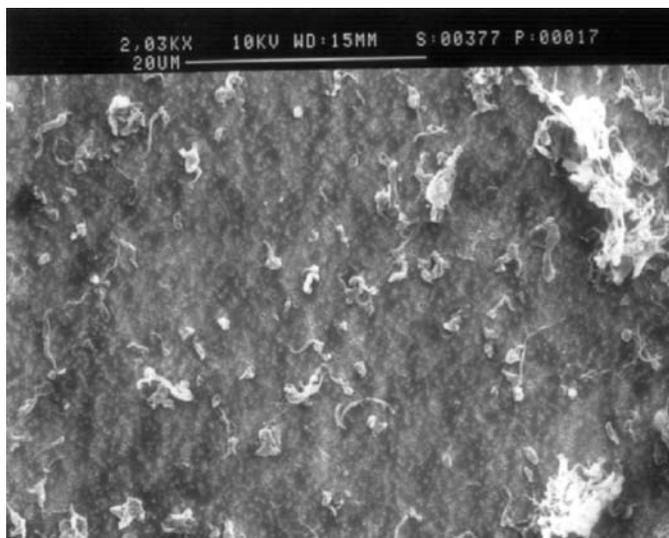


Figure 1. SEM image of polyethylene particles isolated from a joint capsule (2000 \times). Wear debris appear as white or light gray bodies lying on the filter where holes can be appreciated.

Isolation of Wear Debris

Biopsy was immediately fixed in 10% neutral buffered formalin and routinely embedded in paraffin. Polyethylene particles were isolated following the method already described [13]. Briefly, 20- μm -thick slices were obtained from the block of paraffin. These slices were carefully released from the surrounding excess paraffin and placed directly onto a polycarbonate filter (25 mm diameter) with a pore size of 0.2 μm (Millipore, IsoporeTM, membrane filters, Ireland). The filter was then placed onto a polypropylene holder (Swinnex, 25 mm, Millipore), xylol was added to dewax, and slices were then re-hydrated. Finally, digestion took place with sodium hypochlorite. After abundant water flush, filters were dried in a dust-free environment. A part of the filter was sliced and observed in EM by SEM (Figure 1).

Determination of Crystallinity and Crystal Structure by Micro-Raman Spectroscopy

Micro-Raman spectroscopy was used to study the morphology and crystallinity changes of polyethylene liners and the crystal structure of polyethylene debris from the joint capsules.

All the Raman spectra were recorded in back scattering conditions with 5 cm^{-1} spectral resolution in the polyethylene fingerprint region using a Jasco NRS-2000C spectrograph with a microscope at $100\times$ magnification for the debris study and at $20\times$ for polyethylene liner characterization. The laser power was ca. 20 mW using the 488 nm line (Innova Coherent 70) and the detector was a nitrogen frozen CCD from Princeton Instrument Inc. Working at $100\times$ magnification, the mean spot of the laser was ca. $1\ \mu\text{m}$.

For each sample, three spectra in the worn internal surface in the maximum wear area of the polyethylene liners, three spectra on the unworn external surface of the polyethylene liners, and the spectra of ten polyethylene debris particles isolated from the joint capsules were recorded. All the spectra were baseline corrected, normalized to unit area, and then subjected to PLS analyses. The polyethylene debris was observed on the polycarbonate filters used to collect the particles by using an optical microscope and then analyzed by Raman spectroscopy.

Polyethylene powder with crystallinity ranging from 63 to 77% were utilized for the calibration. The crystallinity of the samples was obtained by DSC measurements using a Mettler Toledo TA-STAR Model 821^e calorimeter. The software used for the PLS-Type I analyses was the commercial Grams 386 with the Add-On PLSplus/IQ V.3.0, by Galactic Ind. The calibration was fully cross-validated using the F -test significant minimization of the predicted residual error sum of the squares (PRESS), as a function of the number of factors. The r^2 of the calibration curve was 0.925 [14] and the standard error of prediction (SEP) for one factor was 1.1%.

Crystallinity was determined only on the liners where no band shift between reference and observed Raman spectra could be appreciated. PLS was not applied to wear debris, as some shifts were observed.

Statistical Analysis

Comparisons between the means of crystallinity were made by Student's t -test whenever possible. If the sample size was insufficient, a nonparametric test was applied.

RESULTS

Figure 2 shows the Raman spectra of HylamerTM acetabular liners gamma sterilized in air (Group 1) or in an inert environment (Group 2) in which the spectra are superimposed to evidence their differences.

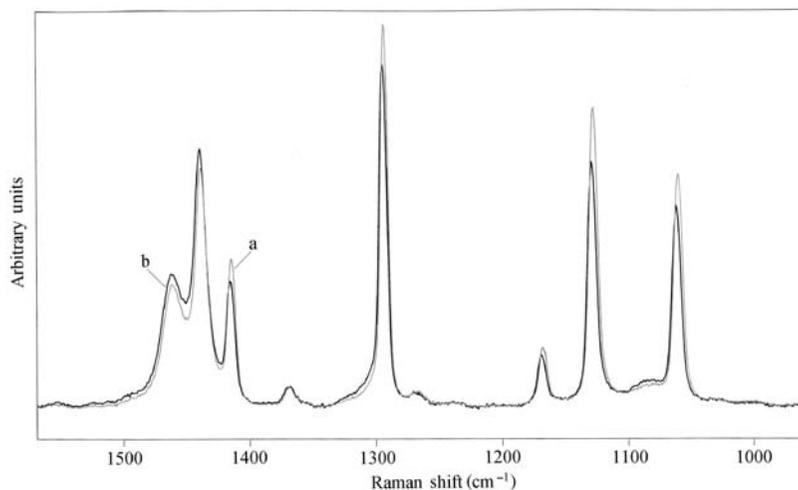


Figure 2. Raman spectra of HylamerTM acetabular liners gamma-sterilized in air (Group 1 = a) and liners gamma sterilized in a nitrogen environment (Group 2 = b).

Table 2. Mean crystallinity rate measured on the surfaces of liners.

	% crystallinity (mean \pm SD)
Group 1	71.5 \pm 1.42
Group 2	69.43 \pm 1.85
<i>p</i> -value <i>t</i> -test	<0.001 S

From a qualitative point of view, the air sterilized liner appears to be more crystalline: the bands prevalently due to the crystalline phase are stronger (in particular those at 1416, 1295, 1131, and 1068 cm^{-1}), while the bands proper of the amorphous phase are weaker (in particular at 1460 cm^{-1} , 1440 cm^{-1} , and the broad bands at 1305 and 1080 cm^{-1}).

PLS analyses confirm these findings (Table 2): the mean crystallinity value for the acetabular liner gamma sterilized in air is 71.50% versus 69.43% for the acetabular liners gamma sterilized in a nitrogen atmosphere. To calculate the two mean values, each liner was tested in six different positions, three in the internal and three in the external surface (internal refers to the surface facing the metal head, i.e., the worn one).

If each single liner is considered, no difference can be observed in the Raman spectra of the worn and unworn surfaces. In Figure 3, one example is reported.

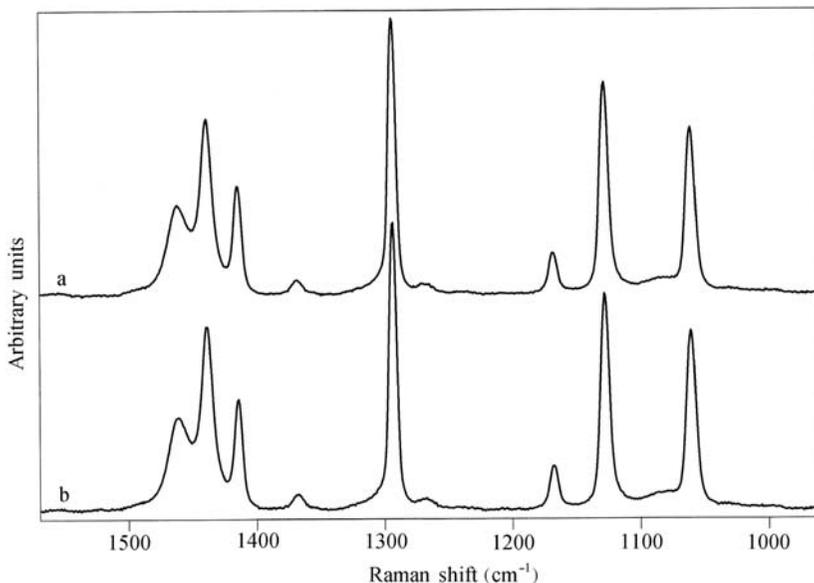


Figure 3. Raman spectra of the worn (Figure 3a) and unworn surfaces (Figure 3b) of one HylamerTM acetabular liners gamma sterilized in air.

Again qualitative methods confirm these findings: no crystallinity differences were found between the worn and the unworn surfaces of each liner, being it either of Group 1 or 2 (Table 3).

For what concerning wear debris, preliminary morphological analysis with SEM showed that the size of debris trapped on the filter ranged between 2 and 100 μ ; bigger particles originate from the delamination of the material.

The analysis of debris by Raman spectra confirms that all the debris isolated were clean polyethylene particles with no signs of impurities. In fact, only the bands proper of polyethylene are present in all of the spectra recorded (Figure 4).

No correlation between the shelf time and the intensity of wear can be ascertained.

In Table 4, the wavenumber of the polyethylene Raman bands in the 1600–1000 cm^{-1} range (PE fingerprint region) with their assignment [15] and the corresponding phases [16] are reported.

Figure 5 shows the Raman comparison between the spectrum of the worn surface of an air-sterilized HylamerTM acetabular liner and the Raman spectrum of the corresponding debris retrieval from the synovial tissue.

Table 3. Crystallinity values found in the unworn and worn areas of each liner. Worn surfaces are those facing metal head of femoral component. Each value is the mean of three measurements.

	Unworn external surface (mean ± SD)		Worn internal surface (mean ± SD)
Group 1			
1	71.9 ± 0.78		70.44 ± 0.83
2	72.16 ± 0.74		72.34 ± 0.46
3	73.48 ± 1.08		70.81 ± 0.41
4	70.50 ± 0.34		69.69 ± 0.59
5	72.85 ± 0.27		71.83 ± 2.27
6	69.35 ± 0.34		72.02 ± 1.01
7	73.43 ± 1.04		71.52 ± 0.69
Mean	71.96 ± 1.58		71.18 ± 1.24
p-value		0.095* NS	
Group 2			
8	70.28 ± 0.80		71.43 ± 1.06
9	68.02 ± 1.04		67.81 ± 0.94
Mean	69.15 ± 1.50		69.62 ± 2.17
p-value		0.952** NS	

*p-value Student's t-test.

**p-value Kolmogorov-Smirnov test.

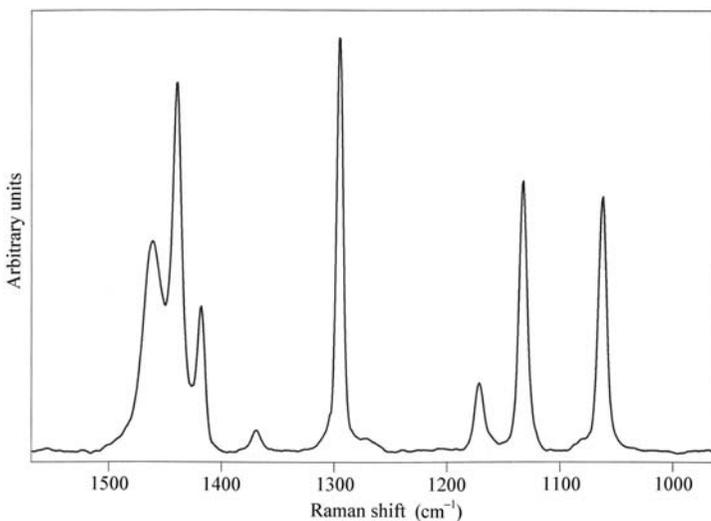


Figure 4. Raman spectra of polyethylene particles with no signs of impurities.

Table 4. Wavenumber of PE Raman bands, assignments, and corresponding phases of reference PE.

Raman band (cm ⁻¹)	Mode	Phase
1460	CH ₂ bending	Amorphous
1440	CH ₂ bending	Amorphous
1416	CH ₂ bending	Crystalline orthorhombic
1370	CH ₂ wagging	Crystalline + amorphous
1305	C-H twisting	Amorphous
1295	C-H twisting	Crystalline
1170	CH ₂ rocking	Crystalline
1131	C-C stretching	Crystalline
1080	C-C stretching	Amorphous
1068	C-C stretching	Crystalline

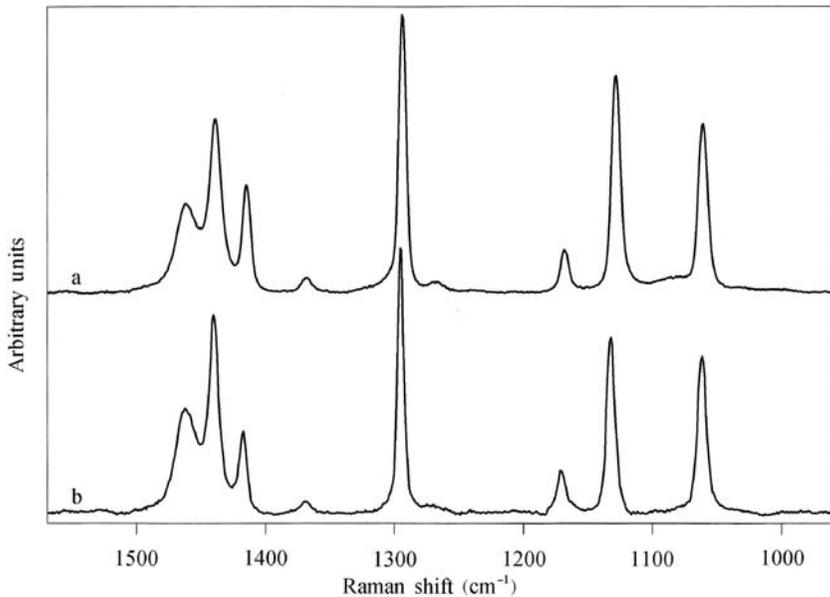


Figure 5. Raman spectra of the worn surface of an air-sterilized Hylamer™ acetabular liner (a) and the debris retrieval from the synovial tissue (b).

The spectra show a great difference in the polyethylene bending region. In particular, the Raman band at 1416 cm⁻¹ loses intensity relative to the band at 1440 cm⁻¹. In the Raman spectrum of the liner, the band at 1416 cm⁻¹ is stronger than that at 1460 cm⁻¹, this intensity ratio is reversed in the debris spectrum.

The intensity of both skeletal vibrations (bands at 1060 and 1130 cm^{-1}) relative to the intensity of the band at 1295 stay practically unchanged. Additionally, the debris spectrum shows shift in the frequency of the CH_2 bending bands from 1440 to 1442 cm^{-1} and from 1416 to 1418 cm^{-1} . The band at 1068 stays unchanged, while the band at 1131 exhibits pronounced shift to higher frequencies.

These shifts can reflect a change in the crystalline phase, from orthorhombic to monocline. The same analysis conducted on standard polyethylene cups and debris did not show this behavior.

DISCUSSION

There are several methods for determining crystallinity in polyethylene such as X-ray diffractometry, differential scanning calorimetry, infrared spectroscopy; for our study Raman spectroscopy was chosen. It is a nondestructive method that does not need any sample preparation, which has been used for the analysis of polyethylene samples [10], since it reflects polyethylene crystals morphology [16].

Chemometric or multivariate methods have been used to correlate Raman data with crystallinity in oriented polymers, where molecular orientation can make univariate calibrations inadequate [17]. Williams et al. [18] obtained a good correlation between Raman spectra of polyethylene and its density by coupling micro-Raman spectroscopy and PLS analysis. Bertoluzza et al. [14] used Raman spectroscopy and PLS regression for the evaluation of the morphology of UHMWPE acetabular liners before and after *in vitro* degradation tests in a hip joint simulator, obtaining a nondestructive Raman method for the evaluation of the crystallinity of this kind of materials without sacrificing the sample. In the same way, Affatato et al. [19] studied the crystallinity of both UHMWPE acetabular liners and debris obtained by *in vitro* test finding that wear caused a general increase in crystallinity on both the cups and in the debris and that the gamma ray sterilized cups showed the higher crystallinity values.

Besides, Raman spectroscopy can be also used to study the crystal structure and phase transition of UHMWPE samples [20]. Relative changes in the skeletal (1000–1200 cm^{-1}) and the bending (1400–1500) regions can be used to define the phase structure.

The Raman analysis we utilized in the present study shows a difference in the degree of crystallinity of the HylamerTM acetabular liners sterilized in air and the same type of liners sterilized in the

absence of oxygen. The former appears to be more crystalline than the latter hence confirming the role of the sterilization environment on the morphology of UHMWPE samples. Oxygen can react with the free radicals generated from the chain scission, thus promoting other chain scissions, while in an inert environment, intermolecular cross-linking can protect the material from degradation [21].

Chenery used Raman spectroscopy to study the oxidation products in UHMWPE samples [22]. He identified some marker bands of oxidation products in the polyethylene fingerprint region at: 1794 cm^{-1} (acyl peroxy), 1770 cm^{-1} (peroxy acid), 1151 cm^{-1} (alcohol), 935 cm^{-1} (epoxy), and at 870 cm^{-1} (peroxy). None of these bands were observed in any of the Raman spectra recorded. This result does not exclude the presence of oxidation species like ketones, alcohols, acids, etc. In fact, many researchers have found that the maximum concentration of these products is several millimeters below the surface [21].

In our measures, we were able to measure worn surfaces at 3.5 mm below the theoretical profile (in the most worn areas). Despite this, there was no trace of oxidation products, probably because we exceeded the area of maximum concentration of these products.

No crystallinity difference was found for the worn and unworn surfaces of the liners and this is true in both groups. This finding suggests that the poor wear resistance of the Hylamer liners is not prevalently caused by the mechanical degradation, but by other reasons as polyethylene phase transformation during the manufacturing or sterilization method.

Only in a few samples, where rim was not damaged by the surgeon during revision, was the rim itself tested. It resulted that Raman spectra was not significantly different from unworn back; on the contrary, the oxidation index was calculated in these few samples, the rim was more severely oxidized than the bearing surface, which in turn was more oxidized than the backside of the liner, according to the observations of Kurz et al. [23].

Lack of correlation between intensity of wear and shelf time could indicate that steady state wear is a function of tribological features not altered by oxidative degradation and mechanical weakening of the polyethylene itself.

One of the limitations of our study is that we could not test experimentally the conditions of an unused liner gamma sterilized in oxygen, because it was no longer available commercially. The inability of controlling the pre-implantation condition is linked to the intrinsic nature of this retrospective study. But even if it had been, it would not

have excluded the presence of oxidative processes during its shelf time. Therefore, we chose to use the external surface of the cup as non-worn sample reference.

When wear debris isolated from tissue were analyzed, all the Raman spectra recorded confirmed that they were polyethylene particles with no sign of organic impurities. This finding supports the ability of the isolation and purification method adopted here to clean the debris avoiding the risk of any change in the morphology of the samples. No other debris products were found.

The differences between the spectra of the HylamerTM acetabular cups and the polyethylene wear debris could mean that overall crystallinity is constant (1000–1200 spectral region practically unaffected), while the changes in the bending region can be attributed to a change in crystal structure. The differences in the spectra of the acetabular cups and the debris could be due to phase transformations. Some of the changes in all the debris spectra were only observed in the Raman spectrum of monoclinic UHMWPE [20], in particular the shift of the bands at 1440 and 1130 cm^{-1} and the lower intensity of the band at 1416 cm^{-1} (orthorhombic crystal structure marker band) that indicate a decrease in the concentration of the orthorhombic phase in the debris in favor of the monoclinic phase.

Both monoclinic and orthorhombic phases are present in the liner, the latter being quantitatively more represented. Wear debris may be monoclinic either due to a phase change occurred during shear between cup and head, either due to a selective wear of regions having a monoclinic phase. At this stage, both hypotheses could be acceptable even if it seems more possible that a phase change occurred on the debris, due to pressure.

Kuralic et al. [20] studied the phase transformation of UHMWPE powder using Raman spectroscopy, and found that shear and high pressure could cause phase transition from orthorhombic to monoclinic phase. The study by Jerosch et al. [24] on HylamerTM acetabular cups using wide-angle X-ray scattering and differential scanning calorimetry analysis showed how this particular type of UHMWPE material contains both orthorhombic and monoclinic phases in the crystalline structure. The changes in the crystal structure of the polyethylene in the HylamerTM cups could be caused by the application of high pressure (235 MPa) during the recrystallization processing of GUR 415 (synthetic substance of HylamerTM).

The prevalent presence of monoclinic phase that we found in the debris could mean that the degradation occurs in this phase.

CONCLUSIONS

1. The crystallinity of HylamerTM liners is increased by gamma sterilization in oxygen when compared to gamma sterilization in the absence of oxygen.
2. *In vivo* mechanical wear does not produce crystallinity changes in liners, for both sterilization methods.
3. Debris detached from both types of sterilized liners show phase changes of the crystalline phase when compared to their source.

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