Nuclear Magnetic Resonance Spectroscopy Spectroscopic Investigation of the Aging Mechanism of Polyethylene Terephthalate Vascular Prostheses

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Received 28 April 2008; accepted 21 January 2009
DOI 10.1002/app.30089
Published online 1 May 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This article attempts to develop and prove a technique to determine the degradation of polyethylene terephthalate (PET) for vascular prostheses. The implicit goal is to be able to quantify the amount of degradation to study the effect of *in vivo* aging. Nuclear magnetic resonance spectroscopy (^{1}H-NMR) provides a comprehensive view of chemical macromolecular structures. Examination of a series of PET vascular prostheses showed significant chemical differences between the virgin prostheses and the explants collected after aging, especially for diethylene glycol and cyclic oligomers groups. Aging was investigated in terms of chemical scission of ester and ether linkages caused by hydrolytic reaction during the *in vivo* stay. Besides, we extended this ^{1}H-NMR technique to determine hydroxyl end-group concentrations and therefore the average number of macromolecular weight. To validate ^{1}H-NMR results, complementary techniques, the chemical titration method and the classical viscosimetric method, were used. The results showed an increase of hydroxyl end-group concentration and a decrease in the macromolecular weight for the explants. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2813–2825, 2009

Key words: polyethylene terephthalate; aging; macromolecular weight; ^{1}H-NMR spectroscopy; vascular prosthesis

INTRODUCTION

Arteries are blood vessels that carry oxygen and nutrients from the heart to the rest of the body. Healthy arteries are flexible, strong, and elastic. Over time, too much pressure in the arteries can make the walls thick and stiff, sometimes restricting blood flow to the organs and tissues [Fig. 1(a,b)].

Cardiovascular diseases are often resolved by replacing the dysfunctioning blood vessel with vascular prosthesis. Fabric materials were first used as vascular prostheses in the 1950s, when Voorhees et al.[1] implanted a polymeric vascular graft manufactured from vinyl chloride and acrylonitrile. Since then, a number of polymers have been used to fabricate vascular prostheses. Ideally, the prostheses should have the same properties as the healthy arteries, not to tire the heart. Polyethylene terephthalate (PET) remains a primary choice for vascular prostheses because it is mechanically compatible with the host artery. Despite this success, the lifespan of the textile prostheses is limited in time and many complications can occur following *in vivo* stay due to an age-related effect [Fig. 1(c–e)]. Failures may be related to the lesions during the implantation and *in vivo* physicochemical degradation of materials.[2–14]

Therefore, it is important to understand in detail what happens throughout the textile structural deformation to improve the quality of the *in vivo* behavior of arterial prostheses and to avoid ruptures in the future. Previous work investigated the nature of morphological changes induced by aging on PET fibers extracted from explanted prostheses.[2–14] For example, the studies realized by Chakfe et al.[2–7] have demonstrated a change in melting behavior between the explants and the virgin prosthesis by using differential scanning calorimetry and X-ray diffraction techniques. That was allotted to the appearance of small crystallites in the amorphous zones of semicrystalline polymer, which causes an increase in crystallinity for the explanted prostheses and a decrease of the average size of crystallites. Other studies[2–4,11] showed a change in the glass transition temperature of the fibers detected by dynamic mechanical analysis. Although these results

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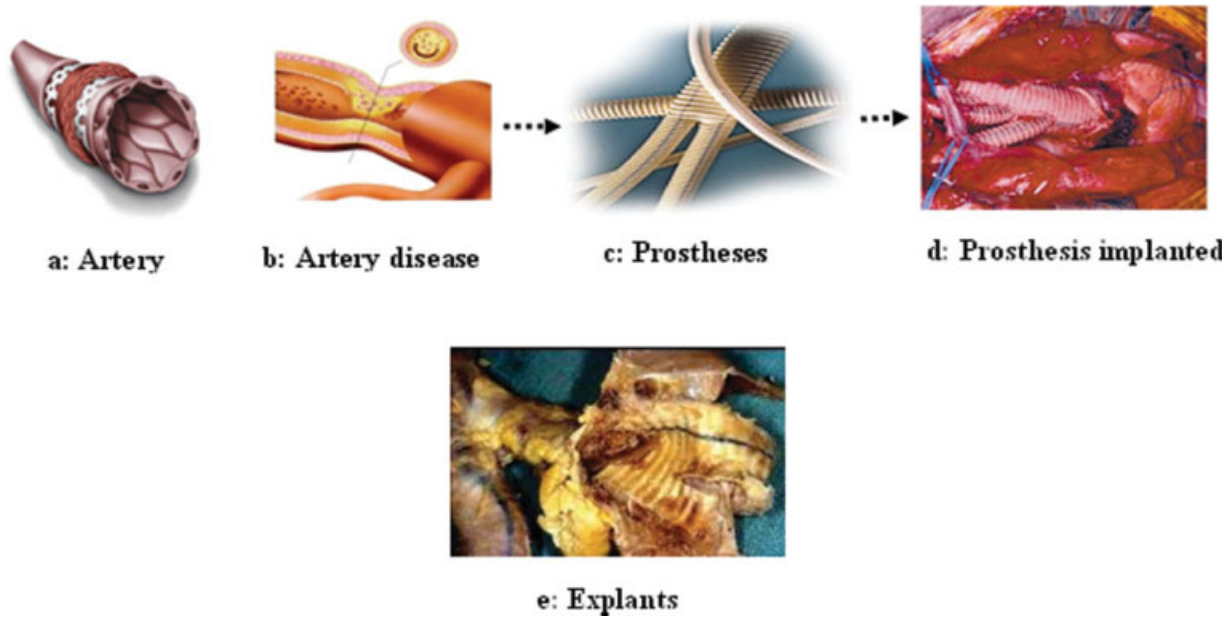

Figure 1 Dysfunctions of arteries and prostheses. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

found in the literature are very interesting,[2–14] the other modifications, such as chemical aging, depend directly on the stay in the biological environment and have not been assessed.[10,12,13] Therefore, these previous studies are considered insufficient and unable to give a total description of aging. These chemical degradations are translated by a modification of macromolecular weight.[15,16] Complete physicochemical studies of explants should be done to know the type of modification that could arise in the polymer.

Although there are only a few publications in the literature that discuss the chemical aging of vascular prostheses (explants are difficult to obtain and only a few laboratories have the possibility to do so), the chemical degradation of classical PET has been studied extensively.[15–19] A systematic study of the chemical degradation of PET, realized by Pohl in 1954,[16] revealed that the hydrolytic scission of polyester chains can be investigated by end-group concentrations by using classical titration. Further to this, several techniques to obtain chemical and structural information about molecules have been described. In addition to chemical methods for end-group analyses such as titration and viscometry method,[20–22] various physical methods have been employed. These include optical spectroscopy, Fourier transform infrared,[23] and UV methods.[24,25]

In the case of PET yarns extracted from different prostheses, all of the methods described to date suffer from some limitations with respect to sensitivity and/or versatility. Some of the associated problems are as follows:

- Large sample size requirement (for both methods: viscosimetric and titration). The sample size is a major problem for us, because of availability of the prostheses is insufficient. Typically, we should be able to work with a mass <5 mg to have the conclusive results.
- In the case of the infrared method, the quantitative analyses are very difficult. For example, the PET end-group bands are broad and poorly integrated.[23,26,27]

Need for development of another technique has been emphasized. NMR-based techniques can offer the advantage of small sample requirement and enable quick (we find all information about end groups and other PET groups in the same spectrum) and reliable study of the chemical aging.[28,29]

MATERIALS AND METHODS

Sample preparation

Cooley double velour prostheses [virgin and explanted prostheses (Fig. 2)], fabricated by Meadox Medical, Oakland, NJ, were tested in this study. Explanted specimens were collected from 1992 to 1999 through the collaboration of several hospitals in Europe. We had the largest stock of various prosthetic explants in Europe but due to their *in vivo* degradation, the quantity of the obtained explants was not sufficient.

For these prostheses, three different zones are distinguished. The first one corresponds to the columns

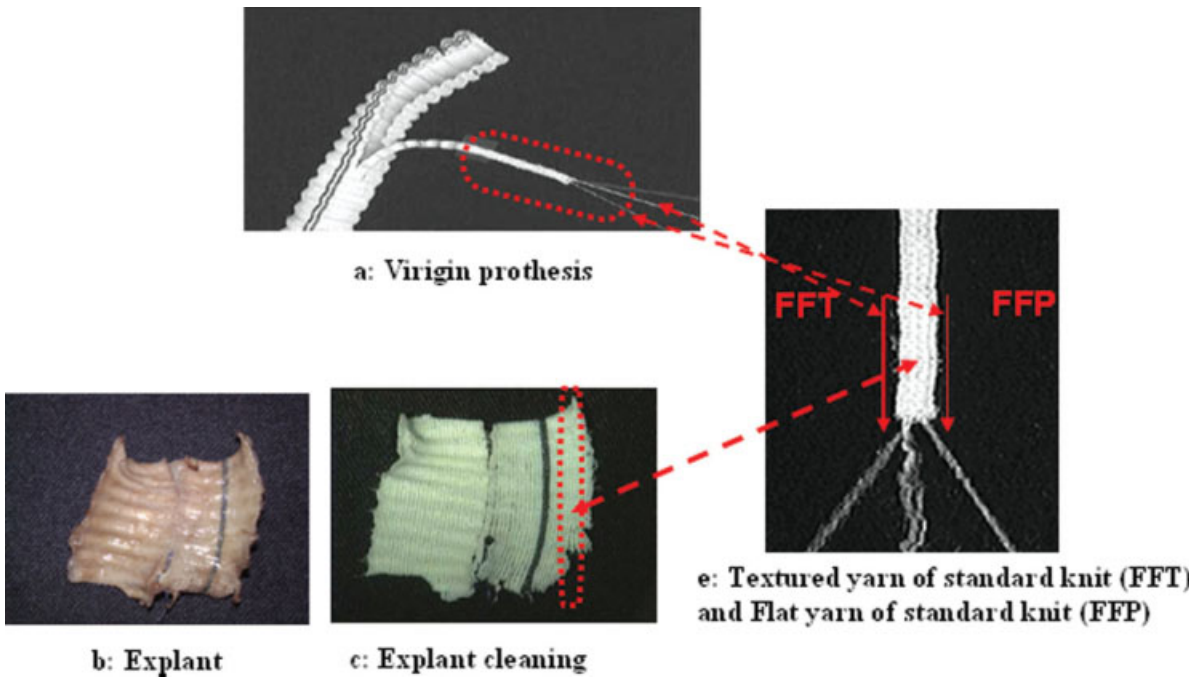

Figure 2 Sample preparation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

made of black yarns, used by the surgeon to correctly place prostheses. These columns are named guide lines. The second zone corresponds to remeshing lines made of yarns forming the junction between the two parts of the basic knitting. The rest of the prosthesis forms the standard knit. For guide lines and standard knit, there are two types of yarns: flat (FFP) and textured (FFT) yarns. Explanted grafts had *in vivo* residency times ranging from 156 to 240 months. The different prostheses used in this study are listed in Table I.

The explanted prostheses were cleaned by using a nondeleterious specific treatment for PET fibers to undertake chemical investigations [Fig. 2(c)]. For this treatment, the explants were immersed in a 10% sodium hypochlorite solution agitating softly for about 3 h, followed by rinsing with distilled water. Hypochlorite remnants were neutralized by using a 0.5% hydrogen peroxide solution. These prostheses were rinsed again with distilled water, dried, and stored.

After washing, the macroscopic examination of explanted prosthesis showed no abnormal deposits, no holes, and no tears on the surface. This examination was realized by using a scanning electron microscopy (SEM; Hitachi S-2360N, Elexience, Verrières le Buisson, France), without metallization, under partial vacuum conditions (0.01–0.2 Torr).

Virgin prosthesis was used as a reference. So, to study only the effect of the chemical aging on the PET properties, this virgin prosthesis was sterilized and then was cleaned with the same treatment as for the explants.

In this study, flat and texturized filaments of standard knit (FFP and FFT, respectively, [Fig. 2(e)]) were analyzed. The filaments were taken by deknitting the prostheses.

TABLE I
Lifetime and Localization of Different Prostheses

No.	Reference	Implantation date	End of implantation	Lifetime (month)	Location of implantation
1	A	1982	1999	204	Aorto bifemorale
2	B	1978	1998	240	Aorto bifemorale
3	C	1981	1995	168	Axillo bifemorale
4	D	1978	1998	240	Aorto bifemorale
5	E	1980	1993	156	Axillo bifemorale
6	F	1979	1997	204	Axillo bifemorale
7	G	1979	1992	156	Axillo bifemorale

The different prostheses are Cooley double velours. The cause of degradation is mainly asymptomatic false aneurysm.

Nuclear magnetic resonance spectroscopy

NMR experiments were recorded at 400 MHz on a Bruker Avance Spectrometer equipped with a QNP Z-gradient probe. All the ^{1}H-NMR spectra were acquired with a 30° pulse corresponding to a pulse width of 3.1 μs. The delay time between each pulse was 8 s. ^{1}H-NMR spectra were recorded for 4000 scans (number of acquired transients) for adequate signal-to-noise ratio. The total acquisition time was ~ 14 h.

Solutions were prepared by dissolving the PET samples in tetrachloroethane-d_2 (deuterated tetrachloroethane 99.9% atom D) at 140°C (typically 2–4 mg) directly in an NMR tube and waiting about 90 s to ensure a complete dissolution of the polymer; then the mixture was cooled to ambient temperature. The experiments were run at room temperature. For these analyses, Topspin 1.3 software was used to treat the spectra and ^{1}D-NMR processor 11.0 (ACD Labs) software was used to determine the peak integrals. Prior to signal integration, a linear baseline correction was applied between 2 and 12 ppm. We respected the same construction of the baseline for all spectra, so the choice of the baseline did not influence the obtained ^{1}H-NMR results.

Method of end-groups titration

Concentrations of carboxyl end groups

For the rapid determination of carboxyl end-groups in PET we used the method described by Pohl.[16] This method entails dissolving the polymer in benzyl alcohol (0.5 g/5 mL) rapidly at a high temperature (180°C), then quickly mixing the solution with chloroform, and titrating with the aid of a sodium hydroxide and phenol red indicator.

The found values must be corrected for the amount of carboxyl groups that are formed during the dissolution at this high temperature. The solvent used, benzyl alcohol, must be of high purity and free of water, lest hydrolysis at the high temperature during dissolution should take place.[16–19] Then, the concentration of carboxyl end-groups [*COOH*] was calculated by the following formula[16]:

$$[\mathrm{COOH}] = \frac{(V_t - V_b) \times 10^6 \times C_{\mathrm{tir}}}{m} - [\mathrm{COOH}]_{\mathrm{CORR}} \quad (1)$$

where [COOH] is the concentration of carboxyl end-groups (mequiv/kg), V_b is the titer value for a blank of the heated benzyl alcohol with chloroform (μL), V_t is the total titer value for the sample titration (μL), $[\mathrm{COOH}]_{\mathrm{CORR}}$ is the correction factor, C_{tir} is the molar concentration of reagent blank, and m is the sample weight (g).

Concentrations of hydroxyl end-groups

Kern et al.[30] reported a detailed study of the conversion of hydroxyl end-groups into carboxyl end-groups by reacting aliphatic polyesters after acylation with succinic anhydride. This offers the possibility of determining the total number of carboxyl end-groups of polyethylene terephthalate by Pohl's titration methods.[15,16]

This method entails dissolving the polymer (0.1 g) in a 2 mL α-methyl naphthalene at 240°C. Temperature is then reduced to 175°C and a quantity of 0.1 g succinic anhydride is added. Heating is maintained for 4 h at 175°C to ensure the total conversion of hydroxyl end-groups into carboxyl end-groups. Subsequently the mixture is poured into an excess of ethanol.

Determination of carboxyl end-groups (by Formula 1) before and after reaction with succinic anhydride identifies the hydroxyl end-groups.[15,30]

Fourier transform infrared spectroscopy

A Bruker Fourier transform infrared spectrophotometer equipped with Bruker Optik GMBH software was used for these studies. The infrared microspectrophotometry in a transmission spectrum of PET fibers is recorded directly in the range of 2000–4000 cm^{-1}. Each spectrum was made from an average of 200 scans at a resolution of 2 cm^{-1}. The diaphragm opening is 0.9, which corresponds to an object diameter of 63 μm and the microscope magnification is ×15. Before analyzing by infrared spectroscopy, each sample was dried overnight in a vacuum oven at 50°C.

Viscosimetric method

In this method, 200 mg of PET sample was dissolved in 20 mL of *m*-cresol at 100°C for 30 min. Then, the sample was cooled to ambient temperature. Afterwards, this solution was diluted ($C_1 = 10^{-2}$ g/mol) with pure solvent (*m*-cresol) to obtain different concentrations ($C_2 = 2 \times 10^{-3}$ g/mol, $C_3 = 4 \times 10^{-3}$ g/mol, and $C_4 = 6 \times 10^{-3}$ g/mol).

If we plot the reduced viscosity on the *y*-axis and concentration on the *x*-axis, we obtain the plot for the different samples. Intrinsic viscosity [η] (the intrinsic viscosity is the hypothetical viscosity at "zero concentration") was determined to calculate macromolecular weight with a simple equation (Mark–Houwink equation):

$$[\eta] = K \times (M_v)^a$$

where (M_v) is the viscosity average macromolecular weight and K and a are the Mark–Houwink constants. There is a specific set of Mark–Houwink

constants for every polymer–solvent combination. Therefore, we had to know these for our polymer–solvent combination to obtain an accurate measurement of macromolecular weight.

For used polymer–solvent combination,[31]

$$K = 0.77 \times 10^{-3}\,\mathrm{mL/g}, \quad a = 0.95$$

therefore,

$$[\eta] = 0.77 \times 10^{-3} \times (M_v)^{0.95} \quad \text{and}$$

$$M_v = \left(\frac{[\eta]}{0.77} \times 10^3\right)^{1/0.95} \quad (2)$$

RESULTS AND DISCUSSION

Qualitative analyses using infrared spectroscopy

At first, the homogeneity properties of the virgin and explanted prosthesis samples were tested to have an overview about the effect of chemical aging. Infrared technique is used to analyze both virgin and explanted prosthesis. In this analysis, 30 fibers are extracted from various zones of each prosthesis (i.e., 30 spectra from various zones are realized for each prosthesis) (Fig. 3).

Figure 3(a) shows no variation of the peak absorbances from different spectra along the virgin sample (the different peak absorbances were represented by various colors). For example, there is no variation of the hydroxyl end-group absorbances along the virgin prosthesis [Fig. 3(b)]. However, a variation of the peak absorbances of the aged sample is observed [Fig. 3(c)]. This behavior may be attributed to the nonhomogeneity of the properties for the explants. Accordingly, a remarkable variation of the hydroxyl end-group absorbances along the explanted prostheses is observed [Fig. 3(d)]. These tests prove that the application of chemical constraints during *in vivo* stay modifies the structure of the polymer and creates a nonhomogeneous degradation for the explants. SEM macroscopic examination (Fig. 4) confirms infrared microscopic examination.

The SEM technique is used to locate the textile lesions to analyze only the most deteriorated part by ^{1}H-NMR technique for quantitative analysis [Fig. 4(b)].

The choice of the ^{1}H-NMR method is very important. This method provides a convenient means to quantify the effect of chemical aging for various explants. On the other hand, this method requires only small amounts of vascular prostheses (2–4 mg) (i.e., using ^{1}H-NMR the most deteriorated part from FFP and FFT can be quantified separately for each explanted prosthesis).

Quantitative analysis using ^{1}H-NMR method

Many types of information can be obtained from a ^{1}H-NMR spectrum, especially quantification of the number and type of chemical entities in a macromolecule. Typical NMR spectrum of PET at room temperature is shown in Figure 5.

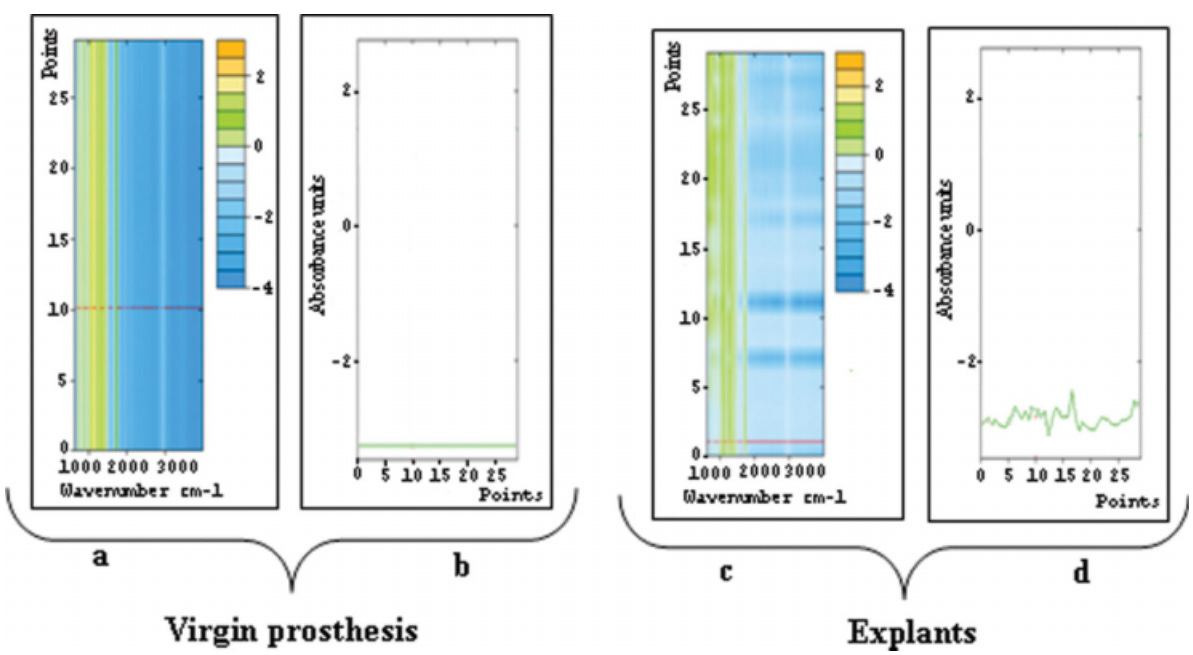

Figure 3 Qualitative analyses using infrared spectroscopy. *x*-Axis represents the wave number and *y*-axis represents the spectrum number. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

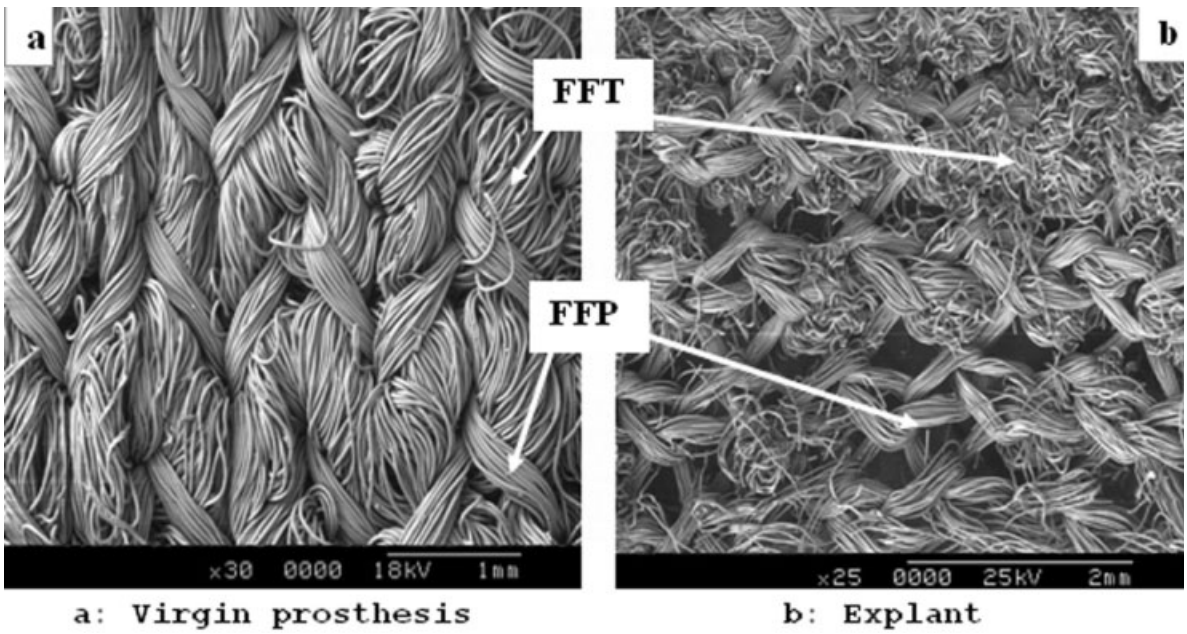

Figure 4 Scanning electron microscopy.

Several main signals of ^{1}H-NMR spectrum are detected (Fig. 5). The chemical shifts of PET spectra were 8.13 ppm (H_1) assigned to aromatic protons of terephthalate units and 4.69 ppm (H_2) assigned to methylene protons. The signals at 3.98 ppm (H_3) and 4.47 ppm (H_4) correspond, to the protons in α- and β-position of hydroxyl end-groups, respectively.[28,29] The H_6 and H_5 signals at 3.88 and 4.5 ppm, respectively (CH_2 in α- and β-position of ether function, respectively), are attributed to the diethylene glycol (DEG) insertion into the PET chain, as shown by Kenwright et al.[32] Also, the ^{1}H-NMR spectrum

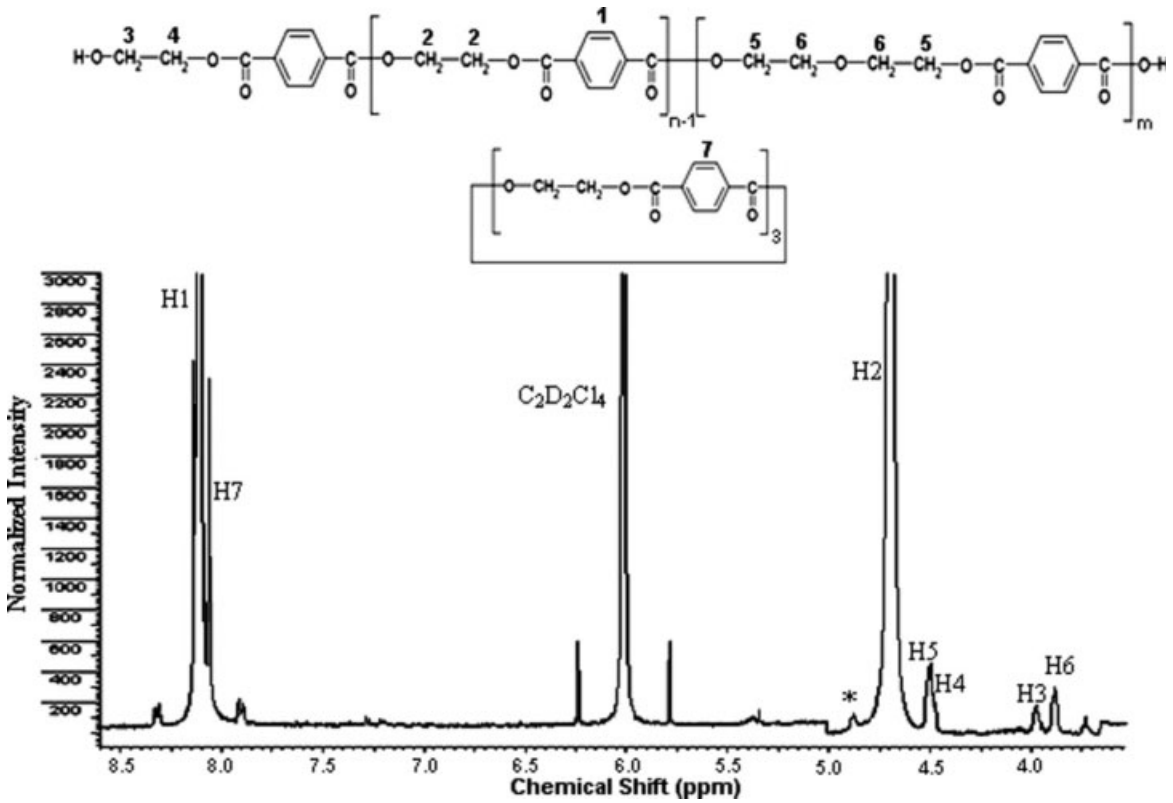

Figure 5 ^{1}H-NMR spectrum of PET [400 MHz, $(CDCl_2)_2$, reference $(CDCl_2)_2$, δ $(CHCl_2)_2$ = 6 ppm]. *^{13}C satellite peak.

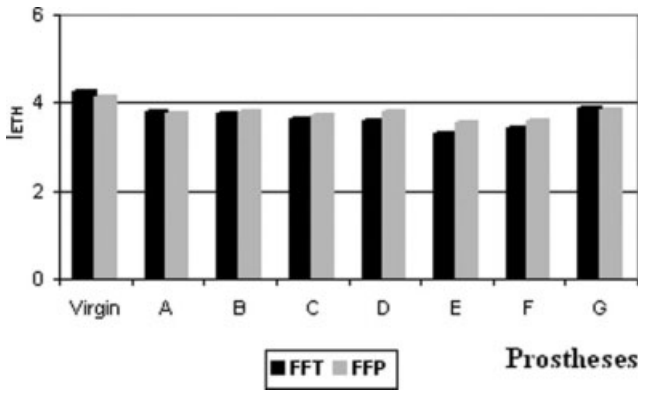

Figure 6 Variation of the ethylene groups for different prostheses. IETH is the integral value of the ethylene group peak.

shows a very fine peak at 8.01 ppm (H_7) corresponding to PET cyclic trimer (i.e., three repeating units), which is present in significant quantity in the PET.[28]

To monitor the changes that occur in the PET polymer during *in vivo* stay, the signal at 8.35 ppm corresponding to ^{13}C satellite of aromatic protons (H_1) was used to normalize all spectra.

Ethylene groups

Ethylene repeating unit comprising the PET macromolecular chain is generally the first and most important attribute of a PET polymer. To compare the ethylene groups, the signal at 4.87 ppm corresponding to ^{13}C satellite of these groups (H_2) was used.

Figure 6 shows a slight reduction of the ethylene quantity for all explants compared to virgin prosthesis. This reduction can be explained by the rupture of macromolecular chains in ethylene repeating unit during an *in vivo* stay. Much work on the degradation of PET has been carried out and it has been considered that the degradation of PET proceeds a random scission of ester linkages.[33,34]

Structural anomalies

The study of polymers starts by understanding the methods used to synthesize the materials. Polymer synthesis is a complex procedure and can take place in a variety of ways.

Certain secondary reactions can occur during the synthesis of the PET, leading to nonconforming groups such as the formation of DEG and PET cyclic oligomer.

Diethylene glycol

The DEG groups are formed by secondary reaction during PET synthesis (Fig. 7). DEG is known to be a weak point in the thermal degradation of PET.[35] Hélène et al.[36] confirm that there is a degradation specific to DEG units, which occurs at 100 K below the degradation temperature of PET. The DEG groups provide some degree of flexibility to a relatively stiff PET backbone, which slows down its crystallization.[25]

Cyclic oligomer

In the process of producing linear polymers by condensation, cyclic oligomers are inevitably formed and their formation considerably affects the macromolecular weight distribution even though the total amount formed is less than 5%.[37]

The dominant oligomeric species in PET is cyclic trimer, which accounts for more than 77% of cyclic oligomers. The PET oligomers are located essentially on the fiber surfaces.[38]

Figures 8 and 9 show a remarkable reduction in the diethylene groups and PET cyclic oligomers for all explants compared to the virgin prosthesis. These results are in agreement with several authors,[36,39] who said that the increase in DEG content promotes hydrolysis and oxidative degradation. Holland and Hay[33,35] proved that DEG chain ends were more prone to degradation than the rest of the PET chain, leading to the formation of dioxane and carboxylic end-groups. Therefore, after aging, these long macromolecular chains (virgin prosthesis) have suffered severe degradation. This phenomenon is related to the formation of smaller molecules and it may proceed by random scission in DEG. Similarly, according to several authors,[36,37] the cyclic oligomers are released early during the degradation and correspond to the first mass-loss step observed for PET.

Therefore, degraded chains (explants) with low molecular weight and a remarkable reduction in DEG and PET oligomers were obtained.

This ^1H-NMR method is described as the most effective because this technique allows us to extract information and deduce the existence of structural anomalies (DEG and PET cyclic oligomer), which are very sensitive to degradation. These results confirm that the ruptures in macromolecular chain can occur during the *in vivo* stay (Fig. 10). Subsequently, it is interesting to study the impact of this degradation

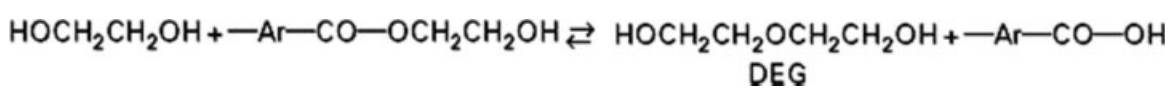

Figure 7 Formation of diethylene glycol (DEG).

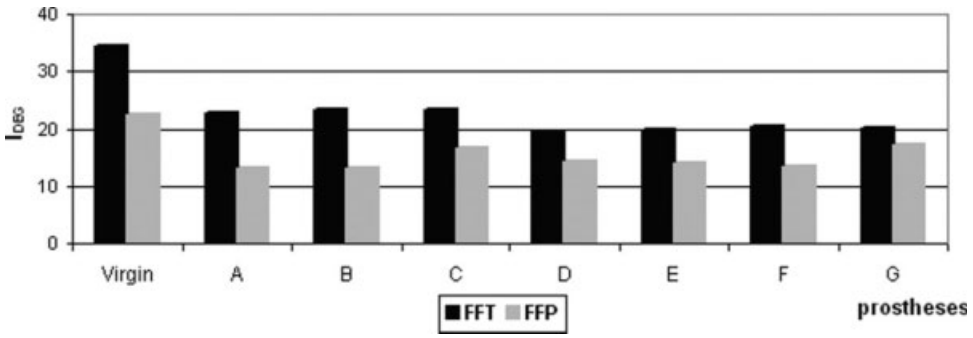

Figure 8 Variation of the diethylene glycol groups. IDEG is the integral peaks values of the diethylene glycol groups.

on the evolution of OH end-groups and the macromolecular weight of PET polymer.

Hydroxyl end-groups

Hydroxyl end-group analyses are an important method to investigate the chemical aging on PET fibers used for different explants and to determine the mechanisms of textile structure degradation.[40]

Figure 11 shows that the chemical aging induced an increase of hydroxyl end groups, mainly for texturized yarns. Another method "chemical titration" was adapted to validate the results obtained by using NMR method.

Concentrations of hydroxyl end-groups found by the chemical titration method

Chemical titration requires a large sample size ($\approx$ 20 mg), so only the textured yarns of standard knit (FFT) extracted from explanted prostheses were tested.

Figure 12 shows that there is a similar evolution between the hydroxyl end-group concentrations found by the chemical titration method and the area of hydroxyl end-group peaks by using ^{1}H-NMR spectra (Fig. 11). These analyses revealed that PET failures in vascular prostheses are susceptible to hydrolysis during an *in vivo* stay. Thus, the hydroxyl end-group contents of PET can be used for the characterization of this polymer[41] and therefore to describe the chemical aging of the vascular prostheses during *in vivo* stays.

To clarify the effect of chemical aging, the macromolecular weights of PET fibers extracted from various vascular prostheses, using NMR technique, were quantified.

Determination of molecular weight using ^{1}H-NMR method

Polymer properties are strongly dependent on the number of monomer units that comprise the macromolecular chain. Mathematically, the average number of macromolecular weight (M_n) is given by the following formula:

$$M_n = \sum_i \left(M_i \times \overline{\mathrm{DP}_i}\right) + M_G \qquad (3)$$

where M_i is the molecular weight of repeating unit; $\overline{\mathrm{DP}_i}$ is the average number of repeating unit; and M_G is the residual molecular weight and characterized end-groups and/or insertions.

In this article, an important method to evaluate PET macromolecular weight (M_n) using ^{1}H-NMR was presented. Direct ^{1}H-NMR technique is not sensitive to the carboxyl end-groups because of line broadening of the signal caused by fast chemical exchange within impurities. COOH end-groups are very low in comparison to OH end-groups, even

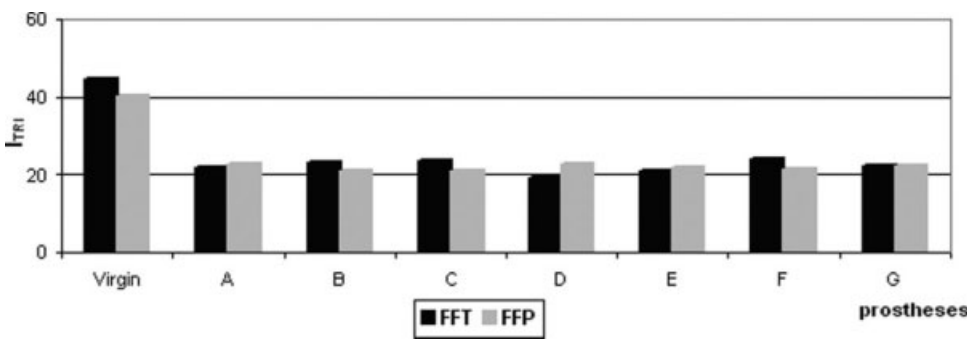

Figure 9 Variation of the cyclic oligomers. ITRI is the integral peaks values of the cyclic oligomers.

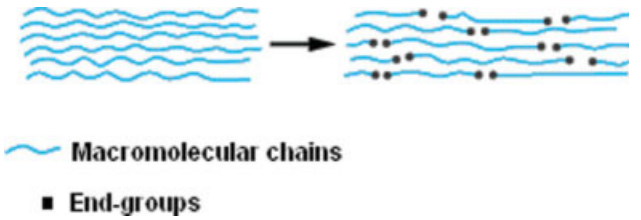

Figure 10 Chain scission in macromolecules. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

after aging (COOH < 20% and OH > 80%; these results are verified by chemical titration); therefore, the COOH groups have little influence on the evolution of (M_n) values. To calculate PET (M_n) values, the corresponding linear chains contain only hydroxyl end-group OH, but the PET cyclic oligomers were not excluded. Therefore, the M_n can be calculated by:

$$M_n = \frac{(\%M_{n1}) \times M_{n1} + (\%M_{nol}) \times M_{nol}}{100} \quad (4)$$

where $\%M_{n1}$ is the percentage of principal polymer chain (without oligomers); M_{n1} is the macromolecular weight of principal polymer chain; $\%M_{nol}$ is the percentage of oligomer; and M_{nol} is the molecular weight of oligomer.

The macromolecular weight of principal PET chain (M_{n1}) can be calculated by the following formula (Fig. 13):

$$M_{n1} = (M_{n01}) \times n + (M_{n02}) \times m + M_{n(\mathrm{HO}-(\mathrm{CH_2})_2-\mathrm{OH})} \quad (5)$$

where n is the average number of ethylene repeating unit; and m is the average number of diethylene repeating unit.

Molecular weight (M_{n01}) for the ethylene repeating unit is equal to

$$M_{n01} = (10 \times 12) + (4 \times 16) + (8 \times 1) = 192\,\mathrm{g/mol}$$

Likewise, the molecular weight of diethylene repeating unit (M_{n02}) is equal to

$$M_{n02} = 192 + 44 = 236\,\mathrm{g/mol}$$

Thus, the average number macromolecular weight can be represented by the following formula:

$$M_n = n \times 192 + m \times 236 \quad (6)$$

Generally, for all groups, the integral value of ^{1}H-NMR signal (integration) is proportional to their proton number and this group's repeating number. Thus, this integral value (I_i) can be determined by the following formula:

$$I_i = (Nber\,H) \times (Nber\,M) \times (Nber\,C) \quad (7)$$

where *Nber H* is the proton number in this group; *Nber M* is the number of repetitions of this group in one macromolecular chain; and *Nber C* is the number of macromolecular chain repetitions.

Using the above equation, the equalities are calculable (Fig. 14):

$$I_6 = (4H) \times (m) \times (Nber\,C\,H) \quad (8)$$

$$I_2 = (4H) \times (n) \times (Nber\,C\,H) \quad (9)$$

where I_6 and I_2 represent the integral values of the diethylene glycol (H_6) and ethylene glycol (H_2), respectively [The H_2 and H_6 signals are represented by four protons (Fig. 14)]; n and m are the average numbers of ethylene and diethylene repeating unit, respectively; and *Nber CH* is the number of repetitions of macromolecular chain.

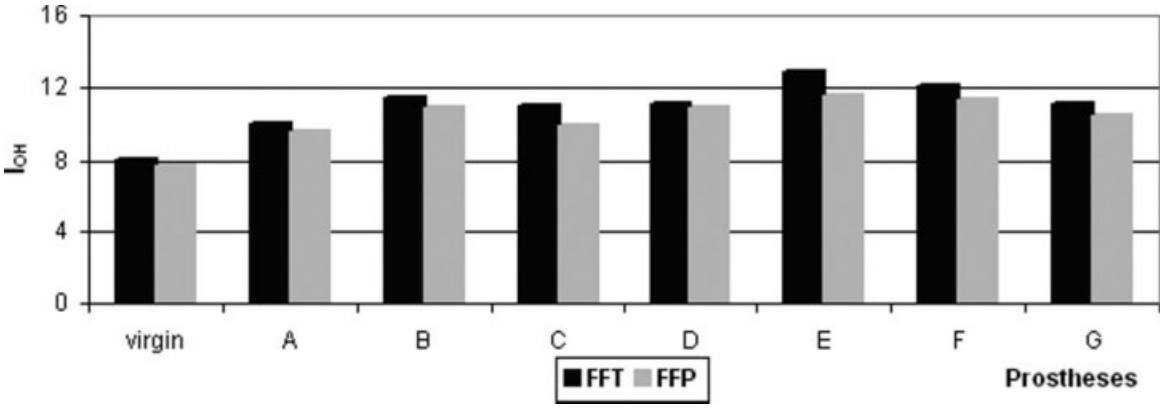

Figure 11 Variation of the hydroxyl end-groups by ^{1}H-NMR method. IOH is the integral value of the hydroxyl end-groups peak.

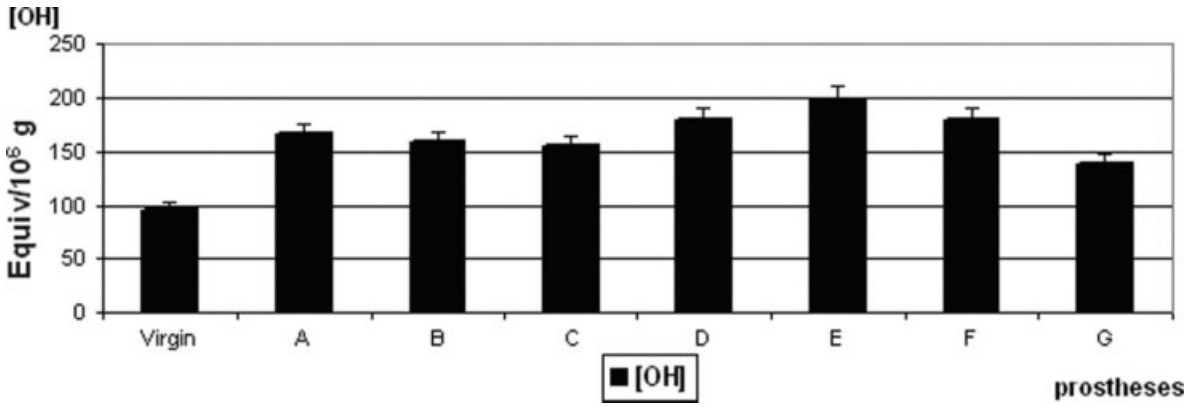

Figure 12 Variation of the hydroxyl end-groups by chemical titration method.

The quantities of hydroxyl end-groups in the macromolecular chain are determined by the following formula:

$$I_3 = (2H) \times 2 \times (Nber\,C\,H) \tag{10}$$

where I_3 is the integral value of the CH_2 in α-position of hydroxyl end-groups (H_3). The H_3 group is repeated two times by chain and it contained two protons.

The average number of diethylene repeating unit (m) was calculated by using the ratio (I_6/I_3).

Thus,

$$m = \frac{I_6}{I_3} \tag{11}$$

The average number of ethylene repeating unit (n) was calculated by using the ratio (I_2/I_3):

$$n = \frac{I_2}{I_3} \tag{12}$$

H_2 signal is much more intense than the two end-group (COOH and OH) signals. Therefore the ratio (I_2/I_3) variation will be interpreted differently. Consequently, the results of the calculations are not accurate. Thus, instead, to use the ratio (I_2/I_3), the area of (H_2) ^{13}C satellite (I'_2) was compared with the area of (I_3) to determine the average number of ethylene repeating unit.

Knowing that

$$I_{13_{CH_2}} = 0.55\% I_{H_2}$$

$$n = \frac{I'_2}{I_3 \times 0.55 \times 10^{-2}} \tag{13}$$

the molecular weight of oligomer (M_{nol}) is given by the following formula:

$$M_{nol} = 3 \times M_{n01} = 3 \times 192 = 576\,\text{g/mol}$$

The percentage of oligomer ($\%M_{nol}$) can be determined by using H_1 and H_7 bands:

$$\%M_{nol} = \frac{I_1}{I_1 + I_7} \times 100 \tag{14}$$

where I_1 is the integral value of the aromatic protons of principal polymer; and I_7 is the integral value of the aromatic protons of oligomer.

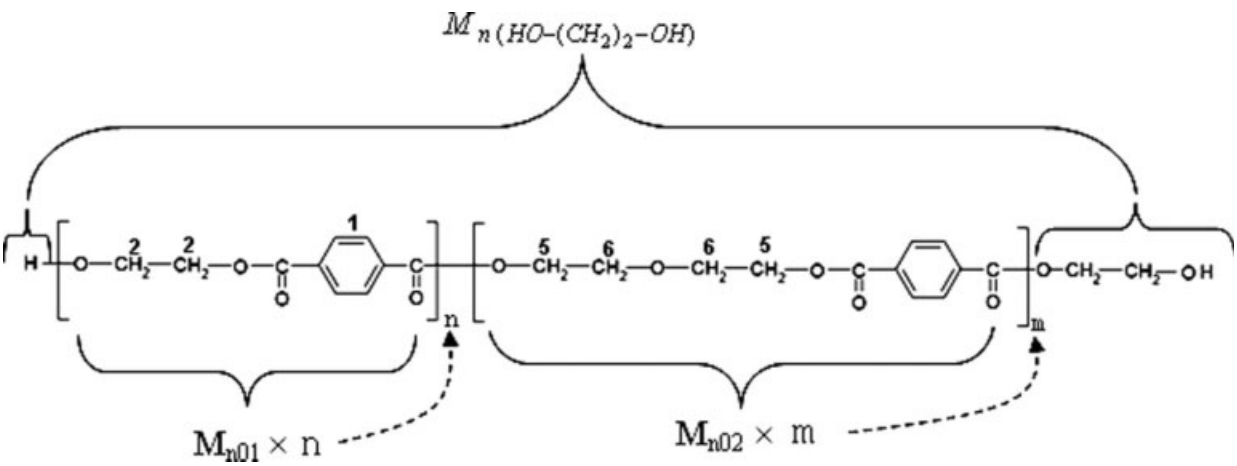

Figure 13 Macromolecular chain with hydroxyl termination.

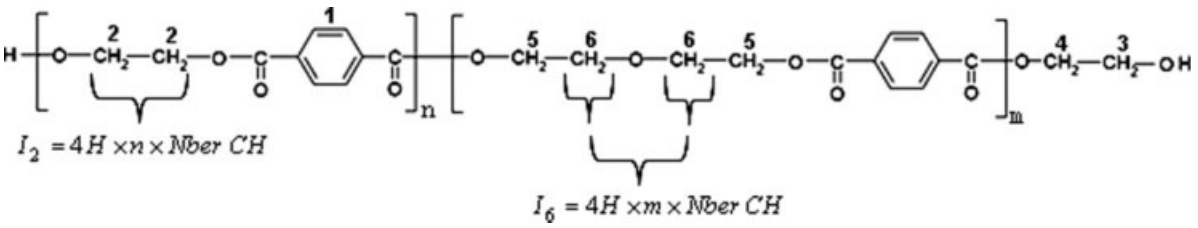

Figure 14 PET group intensities in macromolecular chain with hydroxyl termination.

^{1}H-NMR technique has been used to compare the macromolecular weight of a series of PET vascular prostheses, collected after aging at different durations of *in vivo* stay. Other methods are required to prove the validity and usefulness of this technique.

Determination of macromolecular weight by chemical titration method

Polyesters are essentially linear molecular chains terminated in hydroxyl and carboxyl groups. The average number macromolecular weight (M_n) is easily calculated from the concentration of these two end-groups[21,23,26]

$$M_n = \frac{2 \times 10^6}{[\mathrm{COOH}] + [\mathrm{OH}]} \tag{15}$$

The concentrations of hydroxyl and carboxyl end-groups ([OH] and [COOH], respectively) are expressed in mequiv/g polymer. This method requires a large quantity of sample ($\approx$ 40 mg) to determine the two end-groups concentrations ([OH] and [COOH]) and therefore the (M_n).

Macromolecular weight analysis

Prior to determining the macromolecular weight for various vascular prostheses using the ^{1}H-NMR methods, this method is used to test many PET standard samples with different macromolecular weights. Other techniques (viscosimetry and titration methods) have been used to confirm the found results.

Standard samples

Four samples were used as standards for determining the macromolecular weight values, as follows:

- The commercial PET of the company Olympic Fibers, S.A. (Costa Rica).
- The commercial PET of the company Meadox Medical.
- A thermal degraded PET of Meadox Medical. The sample has been degraded for 30 min at 300°C.
- A thermal degraded PET of Meadox Medical. The sample has been degraded for 60 min at 300°C.

The thermal degradation of the PET was achieved by using the illustrated method of Susumu and Haruhiko.[34] The samples were charged into the glass tube and sealed by fusing under a nitrogen atmosphere. The glass tube was immersed in a salt bath at a temperature of 300°C for a specific duration [for 30 min for sample No. 3 and 1 h for sample No. 4 (Table II)] and then was cooled in liquid nitrogen. Finally, the samples were dried at 50°C under vacuum. The macromolecular weight values (M_v and M_n) obtained from the four PET standards are listed in Table II.

Table II shows that there is the same (M_n) evolution using both the ^{1}H-NMR and the titration methods comparing to the obtained viscosity macromolecular weight (M_v) by the viscosimetric method [the measurement was repeated two times for each

TABLE II
Macromolecular Weight Analysis for Various Sample References

No.	Reference samples		Viscosimetric (M_v)			^{1}H-NMR (M_n)			Titration (M_n)		
			Test 1	Test 2	Average	Test 1	Test 2	Average	Test 1	Test 2	Average
1	Olympic Fibers, S.A., Costa Rica	Macromolecular weight (g/mol)	23,508	23,562	23,535	22,083	22,083	22,083	19,105	19,118	19,112
2	Meadox Medical de Oakland, USA		18,384	18,328	18,356	17,762	17,762	17,762	15,080	15,076	15,078
3	Thermally degraded PET (300°C, 0.5 h)		11,233	11,253	11,243	9572	9572	9572	8430	8425	8428
4	Thermally degraded PET (300°C, 1 h)		8365	8311	8338	7343	7343	7343	6227	6221	6224

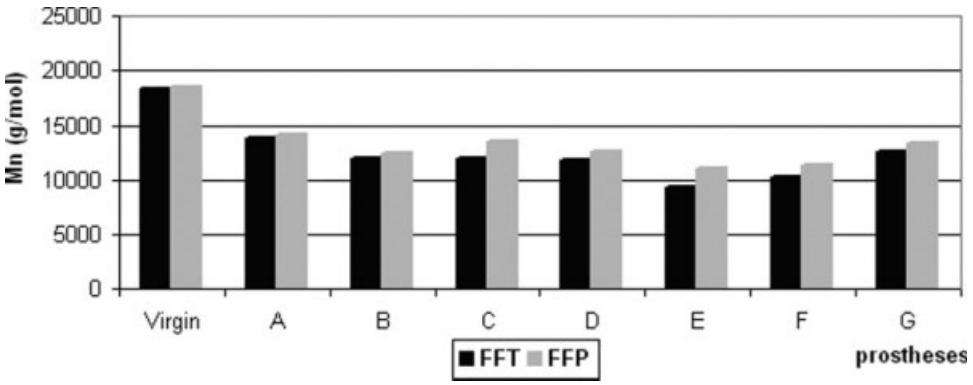

Figure 15 Variation of the macromolecular weight.

sample and their average was used for analysis (Table II)].

Although the same trend is observed of the (M_n) found using two methods (NMR and chemical titration), the results show a difference between the (M_n) values found by these methods, especially for the samples No. 3 and 4 (Table II), with a high macromolecular weight. These differences in values can be explained by the limitation on sensitivity of the chemical titration for the macromolecular weight (M_n) beyond 15,000 g/mol.[42] This limitation can be justified by a few quantities of the end groups presented in the polymers with high macromolecular weight.

Consequently, the (M_n) results in the ^{1}H-NMR method is useful to give a judgment on the explant's degradation state.

Prostheses

Figure 15 shows a significant reduction of macromolecular weight (M_n) for the explanted prostheses. In addition, it was observed that the textured standard yarns (FFT) were more degraded than the flat standard yarns (FFP) for different explanted prostheses (between 28 and 51% for FFT and between 26 and 42% for FFP). These results are in good agreement with the results found in the literature[27,40,41,43] by proving that the PET chemical degradation is a change in the properties, end-group concentrations, and macromolecular weight, under the influence of one or more environmental factors such as heat, temperature, or chemicals. This degradation can be explained by a random scission of the ester linkages, which in turn leads to a decrease in the macromolecular weight of the polymer.[21,43,44]

While the results show that the macromolecular weight values (M_n) are significantly influenced by aging following implantation, the impact of the duration of in vivo stay on the chemical degradation is difficult to evaluate [for example, the prosthesis (E: 156 months) is more degraded than the prosthesis (A: 204 months) despite the fact that the prosthesis (A) is more aged than the prosthesis (E)]. Therefore, even if the time clearly influences the level of degradation, the results highlight other mechanisms of the degradation that are probably related to the human metabolism, and these results are in agreement with the literature.[45]

We will continue to analyze the PET macromolecular weight of various vascular prostheses by NMR spectroscopy. Derivatization method was used to extend this NMR method to determine the carboxyl and hydroxyl end-group concentrations and therefore the exact macromolecular weight. Previous work on polyesters[24,46] found that trichloroacetyl isocyanate (TAI) reacts rapidly and quantitatively with both carboxyl (COOH) and hydroxyl (OH) chain ends to form derivatives that can be readily determined by ^{1}H-NMR spectroscopy.

Thereafter, in the further studies, it is interesting to prove a correlation between (M_n) and mechanical properties to couple the chemical aging with mechanical aging.[47–49]

CONCLUSIONS

The chemical aging in vascular prostheses during *in vivo* stay is detectable by using NMR spectroscopy. NMR spectroscopy has been applied to investigate the modifications in PET fibers extracted from explanted prostheses and to illustrate the mechanisms of structure degradation. Examination of these PET fibers proves that implantation involves a transformation of the original structure of fibers found in prostheses.

The (M_n) can be used to describe the chemical aging of the vascular prostheses. Thus, all explants have suffered severe degradation, especially for texturized yarns, which can be explained by the chain rupture by hydrolysis and oxidative degradation. The degradation level of vascular prostheses does

not directly depend on the duration of the *in vivo* stay.

References

1. Voorhees, H. B.; Jorretski, A.; Blakemore, A. H. Ann Surg 1952, 135, 332.
2. Chakfe, N.; Dieval, F.; Riepe, G.; Mathieu, D.; Zbali, I.; Thaveau, F.; Heintz, C.; Kretz, J. G.; Durand, B. Eur J Vasc Endovasc Surg 2004, 27, 33.
3. Chakfe, N.; Riepe, G.; Dieval, F.; Lemagnen, J. F.; Wang, L.; Urban, E.; Beaufigeau, M.; Durand, B.; Imig, H.; Kretz, J. G. J Vasc Surg 2001, 33, 1015.
4. Chakfe, N.; Dieval, F.; Thaveau, F.; Taghavi, I.; Lemagnen, J. F.; Laroche, G.; Hassani, O.; Riepe, G.; Durand, B.; Kretz, J.-G. Eur J Vasc Endovasc Surg 2003, 25, 360.
5. Chakfe, N.; Beaufigeau, M.; Durand, B.; Kretz, J. G. In Séminaire Intensif de Thrombose; Enghien-les-Bains; Société Chirurgie Vasculaire: France, 1997; p 1.
6. Chakfe, N.; Riepe, G. Urban, U. XII[e] Congrès Annuel 1997, Société Chirurgie Vasculaire de Langue Française; Société Chirurgie Vasculaire: Nice, France, 1997; p 11.
7. Chakfe, N.; Kretz, J. G.; Durand, B.; Riepe, G.; Urban, U.; Cottin-Bizonne, S.; Lemagnen, J. F.; Hassani, O.; Beaufigeau, M.; EdahTally, S. J Méd Strasb 1997, 28, 17.
8. Sweet, G. E.; Bell, J. P. J Polym Sci Polym Phys Ed 1978, 16, 2057.
9. Sladen, J. D.; Gerein, A. N.; Miyagishima, R. T. Am J Surg 1987, 153, 453.
10. Batt, M.; King, M.; Guidoin, R. La presse médicale 1984, 13, 1997.
11. Feldstein, M.; Pourdeyhimi, B. J Mater Sci Lett 1990, 9, 1061.
12. Edwards, W. S. Arch Surg 1978, 113, 1225.
13. Berger, J. H.; Sauvage, L. R. Ann Surg 1981, 193, 477.
14. Riepe, G.; Loos, J. H.; Schroder, A. Eur J Vasc Endovasc Surg 1997, 13, 540.
15. Conix, A. Makromol Chem 1958, 226.
16. Pohl, A. Anal Chem 1954, 26, 1614.
17. Maurice, J. M.; Huizinga, F. Anal Chim Acta 1960, 22, 363.
18. Ma, Y.; Agarwal, U. S.; Sikkema, D. J.; Lemstra, P. J. Polymer 2003, 44, 4085.
19. Jabarin, S. A.; Lofgren, E. A. J Appl Polym Sci 1986, 32, 5315.
20. Berkowitz, S. J Appl Polym Sci 1984, 29, 4353.
21. Tate, S.; Narusawa, H. Polymer 1996, 37, 1583.
22. Duh, B. J Appl Polym Sci 2002, 83, 1288.
23. Zhang, H.; Rankin, A.; Ward, I. M. Polymer 1996, 37, 1079.
24. Postma, A.; Davis Thomas, P.; Donovan, A.; Richard, L.; Moad, G.; Mulder, R.; O'shea Michael, S. Polymer 2006, 47, 1899.
25. Yin, Z.; Koulic, C.; Pagnoulle, C.; Jérôme, R. Macromol Chem Phys 2002, 203, 2021.
26. Zhang, H.; Ward, I. M. Macromolecules 1995, 28, 7622.
27. Abdulrazzak, S.; Lofgren, E.; A Jabrin, S. Polymer 2002, 51, 174.
28. Dannoux, M.; Cassagnau, P. H.; Michel, A. Can J Chem Eng 2002, 80, 1075.
29. Tang, W.; Murthy, N. S.; Mares, F.; Mcdonnell, M. E.; Curran, S. A. J Appl Polym Sci 1999, 74, 1858.
30. Kern, V. W.; Munk, R.; Schmidt, K. H. Makromol Chem 1956, 17, 219.
31. Kurata, M.; Tsunashima, Y.; Iwana, M.; Kamada, K. In Polymer Handbook, 2nd ed.; Bandrup, J.; Immergut, E. H., Eds.; Wiley-Interscience: New York, 1975; p 85.
32. Kenwright, A. M.; Peace, S. K.; Richards, R. W.; Bunn, A.; MacDonald, W. A. Polymer 1999, 40, 2035.
33. Holland, B. J.; Hay, J. N. Polymer 2002, 43, 1797.
34. Susumu, T.; Haruhiko, N. Polymer 1996, 37, 1583.
35. Holland, B. J.; Hay, J. N. Polymer 2002, 43, 1835.
36. Lecomte, H. A.; Liggat, J. J. Polym Degrad Stab 2006, 91, 681.
37. Jun, S. C.; Ji, H. Y.; Won, H. J.; Suk, W. K.; Wan, S. H.; Dong, I. Y. Macromol Chem Phys 2001, 202, 998.
38. Rwei, S. P.; Ni, S. K. Textil Res J 2004, 74, 581.
39. Hosseini, S. S.; Taheri, S.; Zadhoush, A.; Arjomand, M. Z. J Appl Polym Sci 2007, 103, 2304.
40. Ma, Y.; Agarwal, U. S.; Vekemans, J. M.; Sikkema, D. J. Polymer 2003, 44, 4429.
41. O'Neill, A.; Cavaco, P. A. Biocatal Biotransformation 2004, 22, 353.
42. Patrick, C.; Isabelle, E. Physique des Polymères, Tome I: Structure, Fabrication, Emploi; Hermann Editeurs des Sciences et des Arts: Paris, 2005; p 42.
43. Sammon, C.; Yarwood, J.; Everall, N. Polym Degrad Stab 2000, 67, 149.
44. Lee, K. H.; Won, C. Y.; Chu, C. C.; GITSOV, I. J Polym Sci Part A: Polym Chem 1999, 37, 3558.
45. Maarek, J. M.; Guidoin, R.; Aubin, M.; Prud'homme, R. E. J Biomed Mater Res 1984, 18, 881.
46. Donovan, A. R.; Moad, G. Polymer 2005, 46, 5005.
47. Jordens, K.; wilkes, G. L.; Janzen, J.; Rohlfing, D. C.; Welch, M. B. Polymer 2000, 41, 7175.
48. Marl, H. F.; Atlas, S. M.; Cernia, E. Man-Made Fiber Sci Technol 1968, 2, 273.
49. Grosvenor, M. P.; Staniforth, J. N. Int J Pharm 1995, 135, 103.