ORIGINAL ARTICLE

Polypropylene as a reinforcement in pelvic surgery is not inert: comparative analysis of 100 explants

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Abstract

Introduction and hypothesis Currently, most implants used for reinforcement in surgical treatment of pelvic floor disorders are knitted monofilament polypropylene (PP). While previously recognized as inert, PP is associated with high complication rates. Some recent literature suggests polyester prosthetics based on poly(ethylene terephthalate) (PET), which may be more inert in vivo.

Methods A sample of 100 implants explanted from patients due to complications was examined to evaluate the relative degradation characteristics of PP and PET prosthetics.

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Histological, microscopic (scanning electron microscopy, SEM) and chemical analysis (Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC)) were conducted on these explants.

Results Poly(ethylene terephtahlate) explants appeared to sustain less degradation in vivo than the PP explants observed in this cohort.

Conclusions This is the first study to evaluate synthetic implants used in a vaginal approach for pelvic floor reinforcement. The study provides evidence contrary to published literature characterizing PP as inert in such applications. Additionally, the study suggests the need for clinical trials comparatively investigating the performance of new types of monofilament prosthetics, such as those comprising PET.

Keywords Biomaterials · Histological examination · Pelvic floor disorders · Polyester · Polypropylene · Vaginal surgery

Abbreviations

PFD	Pelvic floor disorder
PP	Polypropylene
PET	Poly(ethylene terephthalate)
PPMF	Polypropylene monofilament
LDPPMF	Low density polypropylene monofilament
HDPPMF	High density polypropylene monofilament
NKNW	Nonknitted nonwoven polypropylene
PGA	Poly(glycolic acid)
FTIR	Fourier transform infrared spectroscopy
DSC	Differential scanning calorimetry
SEM	Scanning electron microscopy

Introduction

Pelvic floor disorder (PFD) treated surgically using autologous tissues have exhibited both high rates of failure and recurrence [1-3]. As such, the use of synthetic meshes for this application has gained increasing popularity since the 1980s [2, 4].

The majority of implants for reinforcement of PFD are knitted from polypropylene (PP) [5]. Polypropylene implants have been collectively recognized for good tolerance. Despite some promising results reported in the literature [3, 6–8], complications remain high for an elective surgery [7, 9–11].

Mesh implants are described and classified by material (PP and poly(ethylene terephthalate) (PET) being the most popular), yarn type (monofilament or multifilament), and textile process (knitting or nonwoven techniques). Surface density expressed in weight per square centimeter and/or pore size are critical parameters for predicting the quality of tissue integration. Usually, mesh material with surface density below 50–60 g/cm², and/or pores larger than 1.2 mm are considered low weight and/or high porosity [12, 13].

Both PP and PET are thermoplastic resins. The different yarns obtained from these materials can be prepared using different techniques leading to the following classification:

- When the yarn used to knit the mesh comprises a single thread, the mesh is considered monofilament. Single thread PP defines the prosthetic group polypropylene monofilament (PPMF). The amount and diameter of fiber used during the knitting process allows differentiation of implants between high density monofilament (HDPPMF) and implants of low density monofilament (LDPPMF).
- When the yarn used to knit the mesh consists of a multitude of fibers, the implant is called multifilament. This can be the case for both PET and PP-based materials.
- When the mesh is prepared by thermowelding of a multitude of yarns, it is called nonknitted nonwoven (NKNW) material.
- When an absorbable material is incorporated into the mesh, it results in a composite mesh. The aim of adding these materials is to improve the in vivo tolerance by decreasing the quantity of nonabsorbable material. This class of implants consists of yarns created from composites of PP and poly(glycolic acid) (PP/PGA).

The purpose of this study was to compare the state of alteration of different meshes commonly used in stress urinary incontinence (SUI) or pelvic organ prolapse (POP) surgery, explanted after clinical complication and to investigate potential causes of alteration.

Materials and methods

Sample collection

This prospective comparative study included 100 prosthetic explants surgically removed for one (or several) common

complications including exposure, infection, and/or shrinkage. The explantation procedures occurred between 2006 and 2007 by vaginal route in 13 collaborating French surgical centers that regularly use synthetic implant reinforcement in PFD surgery. The dimensions of the harvested samples were at least of 1.0 cm \times 0.5 cm. Each explant was rinsed and placed in a 4% neutral buffer formalin solution and then, sealed.

The samples were sent to the research team within 24 h of fixation. The brand and relevant clinical information for each explant was documented in an accompanying information file. This information file included the age of the patient, clinical indication for prosthetic placement, dates of implantation and explantation, trademark of the explanted prosthesis, reason for removal (exposure, infection, and shrinkage), and results of the bacteriological analysis (if available).

The Ethics Committee of St. George group clinic approved the study design. To protect patient identity, each sample was given a sequential specimen number unrelated to identifiable patient data.

Histological analysis

Samples were embedded in paraffin wax, sectioned, and then, stained with hematoxylin-eosine-safran for histological analysis.

Scanning electron microscope analysis

Morphological analysis of explants and pristine control mesh samples of the same trademark was conducted using scanning electron microscopy (SEM, JEOL 6700F). SEM images were collected at low voltage (1–5 kV).

Prior to imaging, explants and pristine samples were fixed and preserved in a 1% glutaradehyde solution in cacodylate buffer (0.1 M, pH 7.5). Samples were rinsed in a cacodylate buffer, then, postfixed by a 1% osmium tetroxide solution, which is added to the cacodylate buffer. Fixation times were adapted to the size of the sample. Samples were further rinsed with distilled water, then, dehydrated with a series of ethanol solutions of increasing concentration. Samples were then dried using hexamethyldisilazane (Carl Roth, Karlsruhe, Germany). Each sample was sputter-coated with a 3-nm gold–palladium coating prior to analysis with the SEM.

Chemical analysis

Chemical analysis of 32 mesh explants was carried out to characterize the degradation of mesh materials. Samples were divided into four groups (described below). Because of the small sample size and physical condition of the explanted materials, extensive and complete chemical analysis was difficult.

- Group1: degraded PP explants (as confirmed by SEM); seven LDPPMF, nine HDPPMF, one composite, and one NKNW;
- Group2: nondegraded PP explants consisting of six LDPPMF and four HDPPMF;
- Group3: four PET explants;
- Group4: a control group of pristine implants, which consisted of one pristine LDPPMF implant (Prolene Soft®/Ethicon), one pristine HDPPMF implant (Prolene®/Ethicon), and one pristine PET implant (Parietex®/Covidien).

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopy is a spectroscopic technique widely used to facilitate determination of chemical functional groups by their absorption frequency. Different functional groups have characteristic infrared (IR) radiation absorption frequencies, generally, presented as absorbance as a function of wave number.

A Spectrum 100 (Perkin Elmer) FTIR spectrometer in attenuated total reflectance mode was used to analyze the samples. The spectra were recorded using 12 scans and 4 cm^{-1} resolution as acquisition conditions.

Baseline IR spectra were recorded for all samples. To eliminate organic residue on explants from groups 1–3, samples were treated for 26 h with NaOCl solution (Chemie plus 12% active chlorine) at room temperature and washed with deionized water. Samples were then extracted with pure cyclohexane (Merck, Darmstadt, Germany) for 24 h at room temperature.

The control group samples (pristine samples of Prolene[®] and Prolene Soft[®]) were treated with the same protocol to determine if the cleaning process had chemically modified the material. Spectra from test groups were compared to their specific control spectra.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) aims to identify changes in polymer morphology through observation of changes in glass transition temperature, melting temperature, and heat of fusion.

Thermal characterization of the samples was performed using a jade differential scanning calorimeter (Perkin Elmer). Samples were hermetically sealed in aluminum pans, with an empty pan used as a reference. The samples were heated from 5 to 200°C at 10°C/min. The DSC thermograms of degraded and nondegraded LDPPMF and HDPPMF were compared to their respective pristine control thermograms. Statistical analyses

All statistical analyses were carried out using Minitab[®] 15 software. Chi-square or Fisher exact tests were used when relevant in order to compare:

- the histological results according the various families of implants; and
- the degradation rate according to the histological reaction type (infection as type 1/chronic inflammation as type 2/sclerosis as type 3).

The statistical significance level was fixed at p < 0.05.

Results

Sample collection and clinical data

Of 100 explanted samples, the information files were not complete for ten of them while the other six were either too small ($<2 \text{ mm}^2$) or dried during transportation. The average period before prosthetic removal was 790.6 days (ranging from 16 to 3,295 days).

The causes for removal were distributed as follows: isolated exposures (n=39, 46%), isolated infections (n=14, 17%), shrinkage or pain (n=12, 14%), associated exposure (n=19, 22%; exposure+infection (n=10 or 11.6%), exposure+shrinkage (n=9 or 9.3%)). The sampling of the series is shown in Table 1.

Histological analysis

The histological study revealed three types of periprosthetic tissue reaction.

Type 1 reaction has a characteristic of an infection (n= 37/84, 44%). The tissue reaction appeared identical to that observed in a periprosthetic abscess. A majority of altered polymorphonuclear neutrophils were found. This suggested an infectious process. There were no signs of periprosthetic colonization.

Type 2 reaction is presented as chronic inflammation (n=35/84, 42%) rich in giant cells and mononuclear cells. There could be a minor contamination process confirmed by the presence of some nonaltered polynuclear cells. A partial colonization of the implant was observed.

Type 3 reaction was sclerosis (n=12/84, 14%), whereby the implant was set in a pronounced fibrosis. This fibrosis was transformed to hardening with almost complete disappearance of the fibroblasts and maturation of the collagen. Implants were fully colonized, but a low infiltration rate of mononuclear cells without polynuclear cells was observed.

Designation		N	Туре	SEM Pictures	Weight (g/m ²)	Ø pores (mm)
PPMF N=51	LDPPMF	28	Low Density PolyPropylene Monofilament	X	≤50 <mark>-6</mark> 0	≥ 1,2
	HDPPMF	23	High Density PolyPropylene Monofilament		≥60	<mark>≤ 1</mark>
Other PP N=12	NKNW	8	Non Knitted Non Woven PolyPropylene		≥60	≤ 0,5
	PPmultifilament	4	PolyPropylene Multifilament			
Composite: PP/PGA		8	Polypropylene associated to polyglactine	AC.		
PET		13	Polyethylene terephtalate	II		

Table 1 Details of the 84 explants' sampling

All groups of implants showed evidence of type 1 and 2 reactions. A type 3 reaction was observed only in LDPPMF, HDPPMF, and in PET.

The results suggested a significant difference in infection and sclerosis between the type of PP explants. Multifilament PP, NKNW, and composite implants were more frequently associated with infection than PP monofilament implants (LDPPMF and HDPPMF), 70% versus 39%, respectively (p=0.02). On the other hand, PP monofilament implants were more frequently associated with sclerosis than other PP and composite implants, 20% versus 0%, respectively (p=0.05).

SEM analysis

As expected, SEM analysis of pristine meshes showed no prosthetic damage or alterations of their filaments. An explant was considered degraded if it showed morphological differences in comparison to the corresponding pristine implants. Analysis of different mesh explants showed evidence of damage to the prostheses (Fig.1). Mesh damage included superficial degradation, which appeared as a peeling of the fiber surface, transverse cracks in the implant threads, significant cracks with disintegrated surfaces and partially detached material, and superficial or deep flaking. Fractures were variable in number and depth. Specific deteriorations correlating to implant material were not observed.

SEM revealed that 42% of the implants were degraded (n=35/84), and 58% were intact (n=49/84; Fig. 2). Degradation was observed only in samples implanted for at least 3 months. Other than the 3-month implantation time, no

correlation between the duration of the implant and prosthetic damage was observed (Fig. 3).

Analysis of the damage observed on the prosthetic explants showed that all types of PP implants exhibited degradation but in an uneven way according to their nature and their manufacturing process. None of the PET implants were found to be altered and degraded (Fig. 2).

A significant difference in percentage of degraded samples between histological reaction in type 1 (infection) and histological reaction in type 3 (sclerosis) was found. Evidences of PP degradation were more frequently observed when the surrounding tissue reaction was classified as infection (59% of degraded PP samples with type 1 reaction versus 20% for type 3 reaction, p=0.031).

Chemical analysis

FTIR analysis

The FTIR spectra of explanted samples are shown in Fig. 4. The analysis results show that:

- The FTIR spectra of pristine Prolene[®] and Prolene Soft[®], before and after the treatment with NaOCl and cyclohexane, were similar to typical FTIR spectra of PP reported in the literature (Fig. 4a). Therefore, the chemical treatment had little effect on the material.
- FTIR absorption bands between 1,615 and 1,650 cm⁻¹ could be attributed either to carboxylate carbonyl or to residual products of biological origin. Therefore, these results cannot confirm the formation of carboxyl groups in vivo.

Fig. 1 SEM comparison between intact and degraded explants



- The absorption band at 1,730 cm⁻¹ could correspond to the absorption of ester carbonyl groups, which is likely from esterified fatty acids. However, some samples of group 2 also showed that the absorption band at 1,730 cm⁻¹, and they were not deemed damaged.
- The FTIR spectra of the PET sample after treatment revealed no change when compared to a typical PET spectrum. Therefore, the treatment had little effect on PET as well.

The DSC thermograms of treated degraded and nondegraded LDPPMF explants were similar to those of treated pristine Prolene Soft[®]. Additionally, the DSC thermograms of degraded and nondegraded HDPPMF explants were also similar to those of treated pristine Prolene[®] samples. No modification was observed in the melting temperature or heat of fusion of these samples. Thus, if an oxidation occurs in these prosthetics, it takes place in the amorphous zones, and crystallinity is preserved.



Fig. 2 Morphological state of explants according to their nature: PP implants did not perform uniformly; PET implants are not deteriorated. a Summary table of the morphological state of the various explants. b

Deteriorated versus nondeteriorated explants (X axis categories of explants, Y axis number of explants). c Percentage of degradation according to the category

Discussion

The primary objectives of this study were to objectively observe a series of prosthetic explants and to characterize potential degradation, which may occur in vivo.

Those histological, SEM, FTIR, and DSC analysis suggested the following:

a. There are classifiable histological reactions observed in standard complications of pelvic surgery with prosthetic reinforcement.

Three types of tissue reactions were observed in this study. The correlation analysis between the prosthetics groups and tissue reaction types agreed with and supported those found in the literature: multifilament PP and NKNW implants seemed to present histological reactions of type 1 [14, 15]. The unexpected observation of types 1 and 2 reactions in LDPPMF and HDPPMF prostheses suggested that, contrary to expectations, the monofilament polypropylene prosthetics were not exempt from these complications.

The type 1 reaction may correspond to an active debridement due to the presence of persistent pathogenic

agents in great quantities. Type 2 reaction may correspond to an incomplete debridement: the initial pathogenic agent may persist in tissues leading a succession of healing and debridement processes. This may explain the coexistence of partial prosthetic sheathing and the presence of polynuclear cells. Type 3 reaction may correspond to healing and aggravated foreign body reaction with an excessive collagen synthesis.

b. PP implants are altered in vivo.

PP implants did not perform uniformly. The LDPPMF was least damaged, while 100% of the NKNW was damaged. Generally, it appeared that monofilament explants were more intact than multifilament explants. This was probably due to the more frequent infection in the NKNW group. The duration of implantation did not appear to correlate to the degree of damage for samples implanted more than 3 months. A significant correlation between type 1 and 2 reactions and all degraded polypropylenes were found.

Several hypotheses concerning the degradation of the PP are described below. None of these, particularly direct oxidation, could be confirmed in this study.



Fig. 3 Deterioration of explants is not correlated to in vivo duration (ND nondeteriorated, D deteriorated, X axis categories of explants, Y axis number of explants)

i. Direct oxidation of the PP.

The in vivo oxidation of polypropylene implants has been reported in the literature. This oxidation should create carboxyl groups on the material [16, 17], which can be detected by FTIR analysis. The FTIR analysis neither confirmed nor excluded oxidation of PP in the in vivo environment.

ii. Fatty acid diffusion.

In previous works [18, 19], the authors suggested that the absorption band at $1,730 \text{ cm}^{-1}$ was related to cholesterol and esterified fatty acids that can diffuse in the amorphous zones of the polymer matrix. The diffusion of these organic molecules into the PP mesh filaments could affect the fiber physical and mechanical properties and generate the damage observed in some samples of group 1. Nevertheless, our study shows that some samples not showing evidence of degradation also absorb at 1,730 cm⁻¹.

iii. Oxidation due to free radical attack; radical oxidation without formation of carboxyl groups.

The chronic inflammatory reaction may infer free radical synthesis as peroxide and superoxide ions and hypochlorite acid. Once in contact with the PP implant, these radical species could infer an oxidation of C-H bonds. This oxidation could occur in the absence of oxygen, and the resulting free radicals could recombine and cross-link, altering the physical and mechanical properties of the polymer. These cross-linking reactions could be the origin of the observed damage [20] without formation of carboxyl groups.

This hypothesis may explain the observed degradation occurring only for specimens implanted beyond 3 months: this time would correspond to the necessary period for oxidation to affect the PP structure. This explanation is enhanced by the significant correlation between the reactions of type 1 and 2 and the number of degraded PP samples found in this study.

c. There was no alteration of the PET implants.

No alterations of PET were found with SEM analysis. The FTIR spectra of the PET samples after treatment



Fig. 4 FTIR Spectra of a typical PP spectrum and chemical structure, b representative FTIR spectrum of an excised PP sample before cleaning, c FTIR spectra of excised PP samples (group 1 and 2) after the cleaning procedure, and d typical PET spectrum and chemical structure

revealed no change when compared to a typical PET spectrum. The hydrophilic character of this polymer may limit the diffusion of the previously mentioned organic molecules. PET also appeared more stable regarding radical oxidation [19, 21].

Polypropylene, in particular, LDPPMF, is the most used material in the PFD surgery. It is generally considered an inert material [22]. This study contradicts this established fact and confirms the results of other studies on PP materials used in other areas of medical specialization.

A degradation related to the action of UV on PP threads used in ophthalmology has been described [23, 24]. In this study, the degradation of the polypropylene cannot be due to UV. More recently, studies were performed on the analysis of damage caused in implants, which were used in parietal surgery. These studies showed that PP meshes undergo degradation while in vivo, most likely due to fatty acids diffusion [17] or oxidation with formation of carboxyl groups [19, 20]. The main argument for the later one was based on a difference in the thermal transitions, as measured by DSC, between pristine and degraded PP implants [18]. The author advised subsequent researchers to analyze specimens by FTIR for confirmation of the degradation hypothesis. In this study, no difference between DSC thermograms of pristine and degraded samples was found. Additionally, FTIR analysis did not conclusively confirm that the degradation was due to oxidation.

The septic environment and large detachments of the vaginal approach resulting in collection and bruising hematoma could support both the accumulation of fatty acids and an increased risk of infection and makes the environment for the synthetic implants significantly more challenging. In these conditions, it is possible that degradation of PP can occur from mechanisms different from those found, for example, in parietal surgery. In the same

way, it is possible that the PP degradation is due to the association of the various expressed mechanisms and not only to oxidation phenomena.

For obvious ethical reasons, this study did not provide the opportunity to analyze vaginal implants from nonpathological situation. Therefore, prediction of normal in vivo material aging or the range of consequences in the clinical state beyond the observed samples is not possible. Due to small effective sample size, it is not possible to categorically conclude on the basis of statistical analysis even if a clear tendency is present.

A study of mechanical properties and an estimation and comparison of the strength and resistance of the various explants was not possible due to individual specimen size, as well as the degraded state of the samples. Moreover, a full chemical analysis of every sample in this series of explants was not possible for these same reasons. Additional chemical analysis such as thermogravimetric analysis and molecular weight determination, specifically, would further clarify the mode of prosthetic damage.

Conclusion

For transvaginal surgery, clinical experience indicates the use of low density, large pore implants knitted from a monofilament to facilitate tissue integration, and decrease the inflammatory reactions. This study, however, brings in to question the prevailing understanding of PP as inert when used in vaginal surgery for pelvic floor repair procedures.

In this work, not all types of PP implants degraded equally. The PP implants degraded more in the presence of an acute infection or chronic inflammation.

Several hypotheses persist concerning the nature of PP in vivo degradation. Large detachments and hematomas are one of the characteristics of the vaginal route and ultimately result in the massive accumulation of blood-derived fatty acids. The diffusion of organic molecules into the polymer (especially esterified fatty acids or cholesterol) may be a cause of the polymer structure degradation. Another explanation concerns radical oxidation due to the septic environment that accompanies acute infections and chronic inflammation. This results in an increase in free radicals generation. When radical oxidation occurs in the absence of oxygen, the formed radicals may promote cross-linking, which alters the physical and mechanical properties of the polymer.

PET exhibited greater resistance to radical oxidation. Additionally, the diffusion of nonpolar molecules such as esterified fatty acids or cholesterol appeared unfavorable. These properties may explain the stability of this polymer in the body. This preliminary study points to the need for clinical trials in order to comparatively investigate the performance of new types of monofilament meshes, such as PET, to existing monofilament devices in various surgical applications.

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