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Chemical and biochemical composition of late periprosthetic fluids from women after explantation of ruptured Poly Implant Prothèse (PIP) breast prostheses

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ABSTRACT

In this study we have analysed the chemical composition of the silicone extracted from two explanted intact PIP breast prostheses and of breast late periprosthetic fluid (LPF) samples from $n = 4$ patients with ruptured PIP implants.

The results obtained by ATR-FT-IR spectroscopy, GC–MS, reverse phase HPLC-UV/DAD, SEC-UV/DAD and contrast phase microscopy demonstrated for the first time that the cloudy, viscous LPF found in the breast of women carriers of PIP implants is a multiphasic amphiphilic silicone/serum microemulsion that can migrate through the body (via lymphatic system) to accumulate first in the lymph nodes. The GC–MS and ATR-FT-IR data indicate that low and high molecular weight silicones (chemical markers: D4–D9, L8) can penetrate the elastomeric shell and periprosthetic capsule and enter the breast tissue via the periprosthetic fluid. Also serum influx from breast tissue into via periprosthetic fluid through the capsule and shell into implant filler silicone (chemical markers: cholesterol by GC–MS, and uric acid, globulins and albumin by HPLC-DAD).

To the best of our knowledge, the profile of the major constituents in PIP-induced LPF have been unequivocally characterised for the first time in this work. Further studies will be needed to evaluate the biological consequences of the current results. The potential toxicological implications of the results are discussed in the light of the current literature on the health effects of PIP implants.

1. Introduction

Poly Implant Prothèse (PIP) was a French company that manufactured breast implants starting from 1991 and during the following decade produced for the domestic and foreign market approximately 100,000 implants per year.

In March 2010, the French medical device regulatory agency (AFSSAPS) suspended the marketing, distribution and use of all silicone implants produced by PIP, after the discovery of their abnormally elevated rupture rate. The subsequent investigations indicated that manufacturers used a low-grade kind of industrial silicone gel to fill their implants. In the same year, the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK followed suit and issued a warning in March 2010, leading to the withdrawal of all PIP implants.

In a previous recent work we have demonstrated on analytical grounds the faulty chemical and physicochemical characteristics of these implants by comparison to an approved, high cohesive silicon gel [1]. In particular, we have shown that the reduced viscoelasticity of their filler silicone material is basically due to its highly reduced cohesive properties in such extent that the term 'gel' should be considered incorrect to identify its real nature (partially cured silicone oil). We have also shown the presence of significant amounts of cholesterol in the filler gel of intact explanted prostheses, a clear demonstration of the lack of impermeability of the PIP elastomeric silicone shells which leads to the absorption of biological material from the surrounding breast tissues.

After our first publication, different clinical European studies from public and private hospitals and clinics confirmed consistently the higher rate of rupture of this kind of implants (up to 34%), although evidencing controversies on the different procedures that may adopted for the management of women carriers of these implants [2–8].

Different clinical and case studies on the effects of implantation with PIP implants revealed several complications such as axillary lymphadenopathy, locoregional silicone spread, intra-mammary

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siliconomas, breast lumps, chronic breast pain and in one case also a bilateral supraclavicular swelling [2,5–13].

The massive involvement of axillary lymphonodes (up to those close to the neck), with reported enlarged lymph nodes far greater in size than in any other case of non-PIP implants [12], and the very low water solubility of low- and high-molecular weight polydimethylsiloxanes, suggested a active transportation mechanism of PIP silicone involving the lymphatic system, rather than its passive diffusion within the lipid rich breast tissue.

The accumulation of periprosthetic fluid, a clear serous fluid that may develop in wounds and in dissected spaces after surgery, that can occur months to years following breast augmentation or reconstruction surgery (LPF, or late seroma) is known in reconstructive surgery as an uncommon problem that may complicate management of patients with breast implants, with an overall frequency of 0.13% [14].

Before the PIP ban in 2010, Lahiri [15], Berry [16] and Khan have been the first surgeons to publish the appearance of a unusual 'thick yellow fluid' ('sterile puss') during the surgical explantation procedure of ruptured PIP implants in seven cases (of which five consecutive) of English women presenting silicone lymphadenitis, breast pain or breast swelling and cutaneous silicone dissemination [17–19]. These first observations were soon after confirmed by other surgeons from England, Holland and Germany [3,5–7,10,20].

Hence, to shed further light on the potential molecular mechanism(s) by which the PIP silicone can trigger the detrimental effects on the health of women carrier of this faulty breast implants, in this work we have:

(i) confirmed our first findings investigating the chemical properties of the silicone extracted from two additional explanted PIP prostheses and compared the results with those from a virgin, intact PIP implant;
(ii) studied for the first time the chemical/biochemical properties of the late periprosthetic fluids collected from the breast of $n = 4$ patients with ruptured PIP implants, using different analytical techniques including contrast phase microscopy;
(iii) combined our results with those previously reported in the literature to propose a possible molecular mechanism for the migration of PIP silicone to distal body sites such as lymphonodes.

2. Materials and methods

2.1. Patients, LPF and breast prostheses silicones

The study was conducted on $n = 4$ samples of LPF collected from three female patients in the beginning of 2013 (January–March). All LPF samples were cloudy, viscous and of white to yellow colour. To emphasise the difference between this fluids and the clear serous LPF associated to other implant brands, in the following parts of this study they will be identified as PIP-LPF1, PIP-LPF2, PIP-LPF-3, and PIP-LPF4, respectively.

The first sample (PIP-LPF1) was collected during the surgical procedure for the bilateral explantation of 330 cc PIP implants from a woman with capsule Backer grade I contracture for the right breast and Backer grade II for the left (serial numbers: 14307 116 (right), 14307-203 (left, ruptured); implanted in 2006). Substitution was done with Allergan TSX 445cc implants (Allergan Limited, Marlow, United Kingdom).

The second sample (PIP-LPF2) was from the right breast of a woman with Baker grade I contracture at the right breast, and grade II–III at the left. PIP prostheses serial numbers: 11300 126 (right, ruptured), 11300 107 (left); implantation date 2002. The prostheses (290 cc, old style) were replaced with Nagor IMP-HR 360 (Nagor, Glasgow, United Kingdom). The third sample (PIP-LPF3) was from the left breast of a women with PIP implants inserted 2004, presenting with Backer grade I (right) and II (left) capsule contracture (left implant with severe rupture, right implant intact, 290 cc PIP, serial numbers: right 09604-004, left 09104-0-49; replaced with Allergan TSX 560cc). PIP-LPF4 was from left breast of a patient implanted in 2004, who presented pain in both sides and capsules contracture Baker grade I (right) and grade II (left). PIP serial numbers: R 09604-004 (right, intact), 09104-049 (left ruptured). The prostheses were replaced with 560cc Allergan TSX implants.

The explanted PIP implants analysed were from two anonymous donors. The prostheses (size 305 cc and 145 cc; serial numbers: 98276 120 and 1350 016, respectively) were intact at visual and palpable inspections, the elastomeric shells were all of a yellowish colour, with evident signs of silicone bleeding (a slow and continuous leak of silicone fluid through its elastomeric shell).

The virgin, intact PIP implant used as control prosthesis was taken directly at the abandoned PIP factory during April 2013 in La-Seyne sur-Mer, France (350 cc; serial number 56007 020).

2.2. ATR-FT-IR

ATR-FT-IR spectra (spectral width 400–4000 cm^{-1}, scans: $n = 25$, resolution = 4 cm^{-1}) were recorded using an Alpha spectrometer equipped with an ALPHA's Platinum single reflection diamond ATR unit (Bruker Optics, Milan, Italy).

2.3. GC–MS

Qualitative and quantitative analyses were done using a 436 Gas Chromatograph coupled to a Bruker SCION SQ^{TM} mass spectrometer system (Bruker Daltonics, Macerata, Italy), equipped with a Factor Four capillary column (VF-5 ms = 30 m; i.d. = 0.25 mm, film thickness = 0.25 mm). The oven temperature was initially set at 60 °C (hold time 3 min), with a gradient from 60 °C to 150 °C (3.0 °C/min, hold 1 min), and from 150 °C to 240 °C (10 °C/min, hold 1 min); injector temperature $T = 250$ °C. Column flow 1.00 mL/min. Carrier gas helium 5.5; ionisation energy = 70 eV; split/splitless ratio = 1:30.

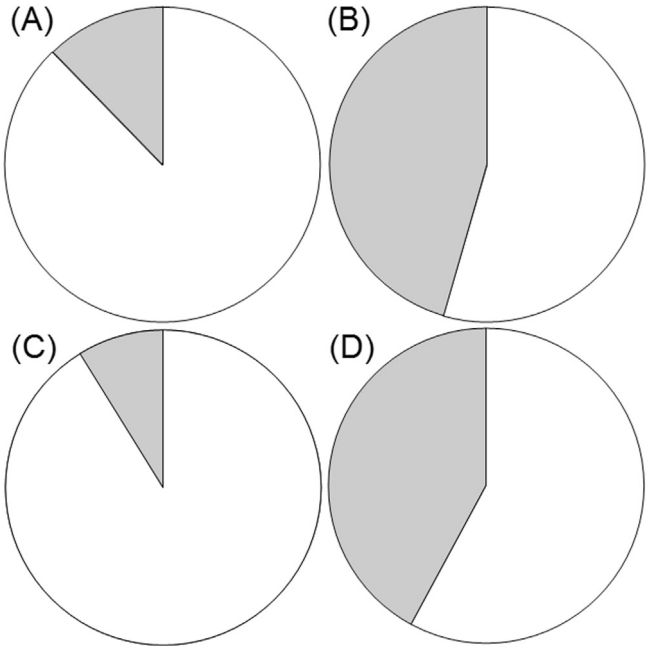

Fig. 1. Dry residue (grey) and water (white) percentage content of (A) PIP-LPF1, (B) PIP-LPF2, (C) PIP-LPF3 and (D) PIP-LPF4.

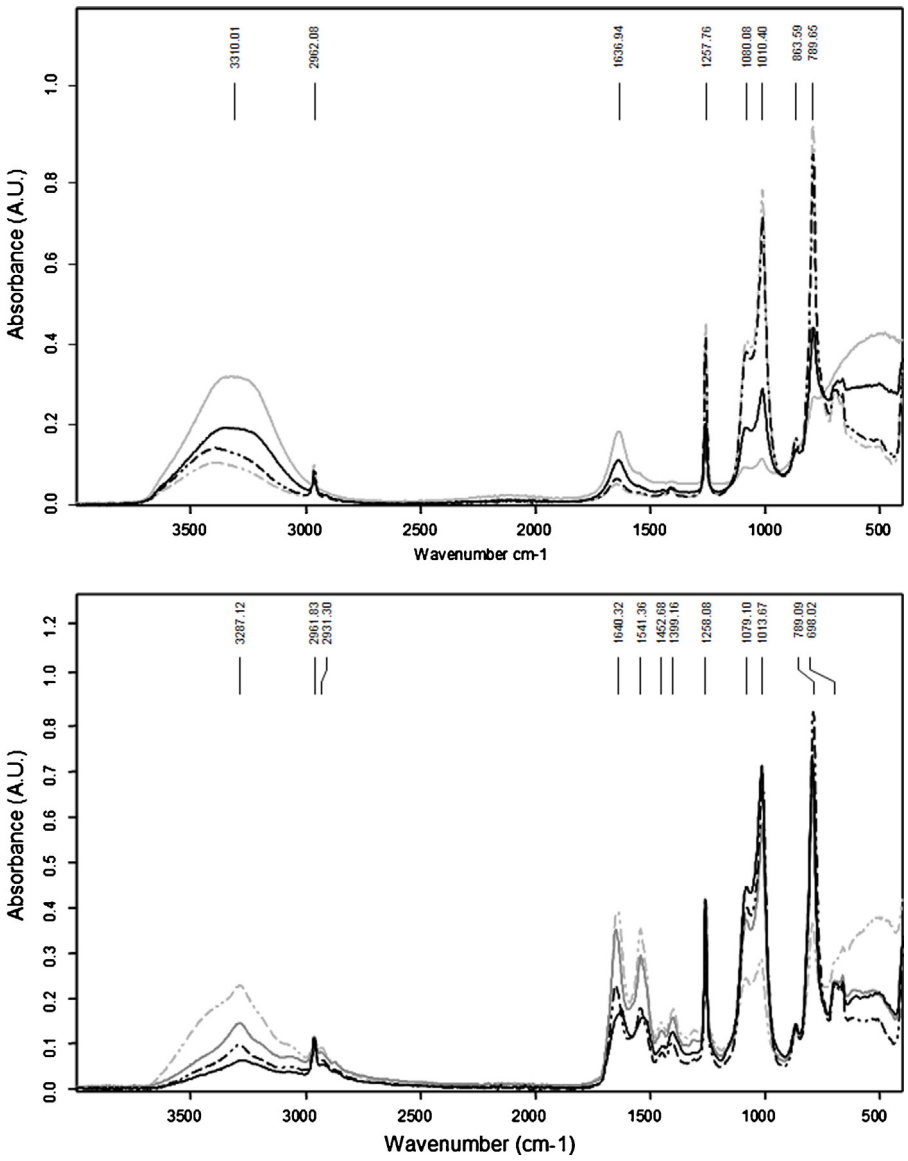

Fig. 2. ATR-FT-IR spectra of (A) PIP-LPF1 (black line), PIP-LPF2 (discontinuous black line), PIP-LPF3 (grey line) and PIP-LPF4 (discontinuous grey line) before (A) and after dehydration (B).

Peaks were confirmed by comparing the retention times with those of authentic standards when available, and final confirmation by matching with the spectra of the commercial NIST mass spectral database (NIST 11, software version 2.0g). For the analysis, 50 μL of PIP-LPF were extracted with 950 μL of acetone under sonication (at maximal power for 1 h) and, after centrifugation at 10,000 × *g* (20 min), 1 μL of this extract was submitted to GC–MS analysis. Cholesterol concentration in PIP-LPF and in silicones from explanted prostheses was estimated by comparison of peak areas with a calibration curve constructed in the concentration range 1–200 ng of injected cholesterol (1 μL, acetone). All analyses were done in triplicate.

2.4. Reverse phase HPLC-UV/DAD

PIP-LPF (50 μL), was extracted with (a) acidic mobile phase (1 mL final volume) or with (b) 100 mM aqueous NaOH/acetonitrile 1:1 (v/v), and 50 μL of the clear solutions obtained after centrifugation (10,000 × *g*) were analysed by reverse phase HPLC-UV/DAD. Chromatographic runs were done using a Varian LC-940 analytical/semipreparative HPLC system (Varian, Turin, Italy) equipped with a binary pump system, an autosampler, a fraction collector, a UV-DAD detector operating (200–400 nm) at λ_1 = 220 nm and λ_2 = 284 nm and a scale-up module. Analytical separations were done using a Supelcosil™ column (15 cm × 4.6 mm, 3 μm). The solvent system was aqueous formic acid 0.05%/acetonitrile 1:1 (v/v), flow rate 0.8 mL/min, run time 20 min.

2.5. SEC-UV/DAD

PIP-LPF aliquots (50 mg) were dissolved in mobile phase (1 mL final volume) and 50 μL of the clear solution obtained after centrifugation (22,000 × *g*) to remove insoluble material (suspended silicone, precipitated proteins, etc.) were analysed by SEC-UV-DAD. Chromatographic runs were done using the same apparatus used for the RP-HPLC-UV/DAD analysis. Analytical separations were done on a TSKgel G2000 SWXL column (300 mm × 7.8 mm and 250 mm × 21.2 mm, Tosoh Bioscience, Japan). The solvent system was NaCl (8.5 g/L)/NaH_2PO_4 (2 g/L)/Na_2HPO_4 (1 g/L) (pH 6.7), flow rate 1.2 mL/min. Analyses were done from 0 min to 30 min of the chromatographic run to ensure that analytes with the lowest MW were eluted.

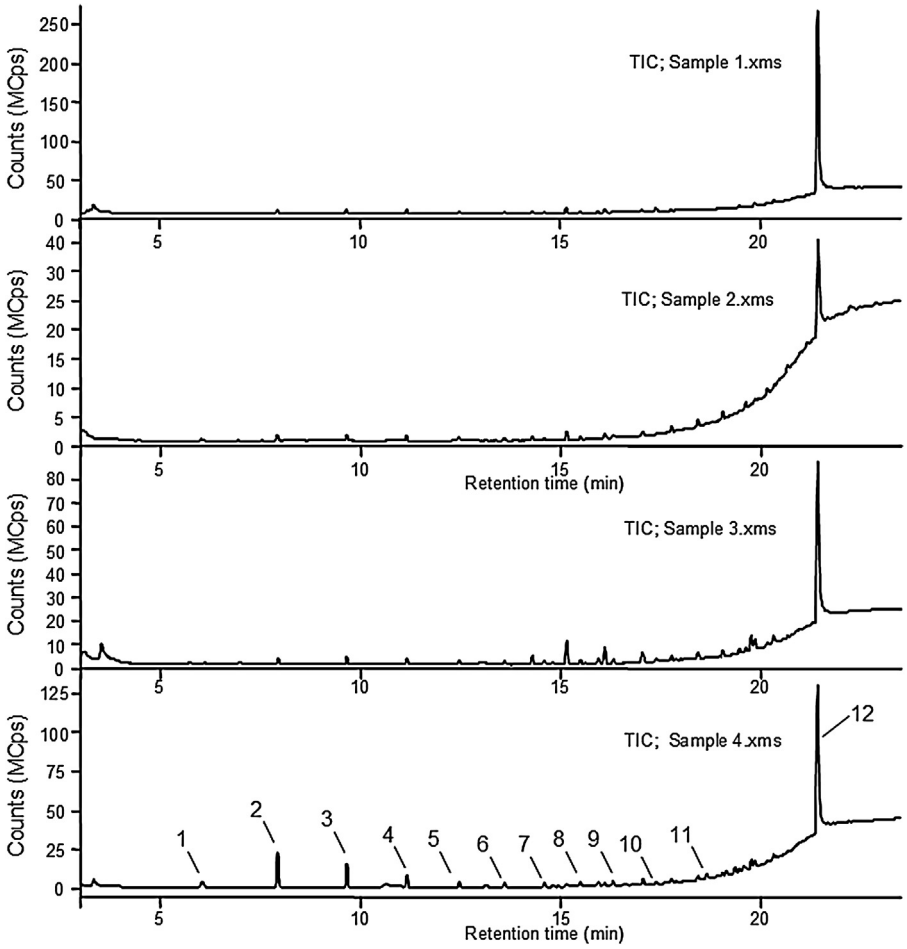

Fig. 3. GC–MS chromatographic profiles of the acetone extracts of PIP-LPF1, PIP-LPF2, PIP-PIP-LPF3 and PIP-LPF4. Compared to peaks from silicones, in all cases the peak of cholesterol is dominant. Cholesterol concentrations: C_{LPF1} = 161.9 ± 4.3 ppm, C_{LPF2} = 14.4 ± 3.7 ppm, C_{LPF3} = 48.0 ± 2.5 ppm, CL_{PF4} = 69.7 ± 5.4 ppm. Peaks are numbered according to Table 1.

2.6. Microscopy

Morphological analyses of LPF were carried out by phase-contrast microscopy (Eclipse TS 100, Nikon).

3. Results

3.1. Water loss test and ATR-FT-IR spectroscopy analysis

The non-volatile material contents of the PIP-LPF from patients 1 (PIP-LPF1), 2 (PIP-LPF2), 3 (PIP-LPF3) and 4 (PIP-LPF4) were evaluated indirectly by measuring the amount of water lost by heating.

After t = 2 h at T = 105 °C, PIP-LPF2 and PIP-LPF4 gave constant weights of dry residue which significantly higher compared to PIP-PF1 and PIP-PF3 (45 ± 2% (w/w) and 42 ± 3% (w/w), vs. 12.3 mean ± SD and 8.8 ± 2% (w/w); mean ± SD, P < 0.001; Fig. 1).

The chemical/biochemical nature of the bulk constituents of the PIP-LPF dry residues was first investigated by ATR-IR spectroscopy. Before water evaporation (Fig. 2A), the IR spectra of all PIP-LPF were dominated by IR absorptions typical of water (ν = 3328 cm^{-1}, O—H st) and of polydimethylsiloxanes (ν = 2962.2 cm^{-1} C—H stretch, ν = 1650.5 cm^{-1}, ν = 1258.1 cm^{-1}, CH_3 bend, ν = 1079.1 cm^{-1}, Si—O—Si st, ν = 1013.7 cm^{-1} Si—O—C st, ν = 863.4 cm^{-1}, $Si(CH_3)_2$ δ, ν = 789.1 cm^{-1}, Si—C st/CH_3 rock). According to the water/non-volatile percentage composition determined by the water loss test, the absorptions generated by water were stronger in PIP-LPF1/PIP-LPF3 (Fig. 2A, black trace), while those of silicone were stronger in PIP-LPF2/PIP-LPF4 (Fig. 2A, grey trace).

After water evaporation, the major absorptions from silicones were still present in the dry residue of all PIP-LPF (Fig. 2B), and in parallel to the disappearance of water absorption bands, new bands at 3293.4 cm^{-1} and in the range 1750–1500 cm^{-1} characteristic of protein amide I, II and III absorptions, were clearly observable in particular in PIP-LPF2.

This results were in good agreement with those recently published by Yildirimer et al. working on (intact) explanted PIP implants [21]. In the case of the implants filler silicones these authors found the presence of IR absorptions of different intensities between 3200 cm^{-1} and 3600 cm^{-1}, and between 1525 cm^{-1} and 1760 cm^{-1}, attributed to Si—OH bonds from silicone degradation and to protein-like bands, respectively. In our opinion, the absorption bands observed by these authors were due to water and proteins from LPF infiltrated inside the analysed prostheses.

3.2. GC–MS analysis of PIP implants and PIP-LPF

Beside bupivacaine (Table 1), the local anaesthetic drug injected to reduce pain after the surgical procedure, the GC–MS analysis of the PIP-LPF from all four patients showed the parallel presence of different concentrations of cholesterol and of trace amounts of LMW silicones: the highest concentration of cholesterol was found in PIP-LPF1 and the lowest in PIP-LPF2 (Fig. 3).

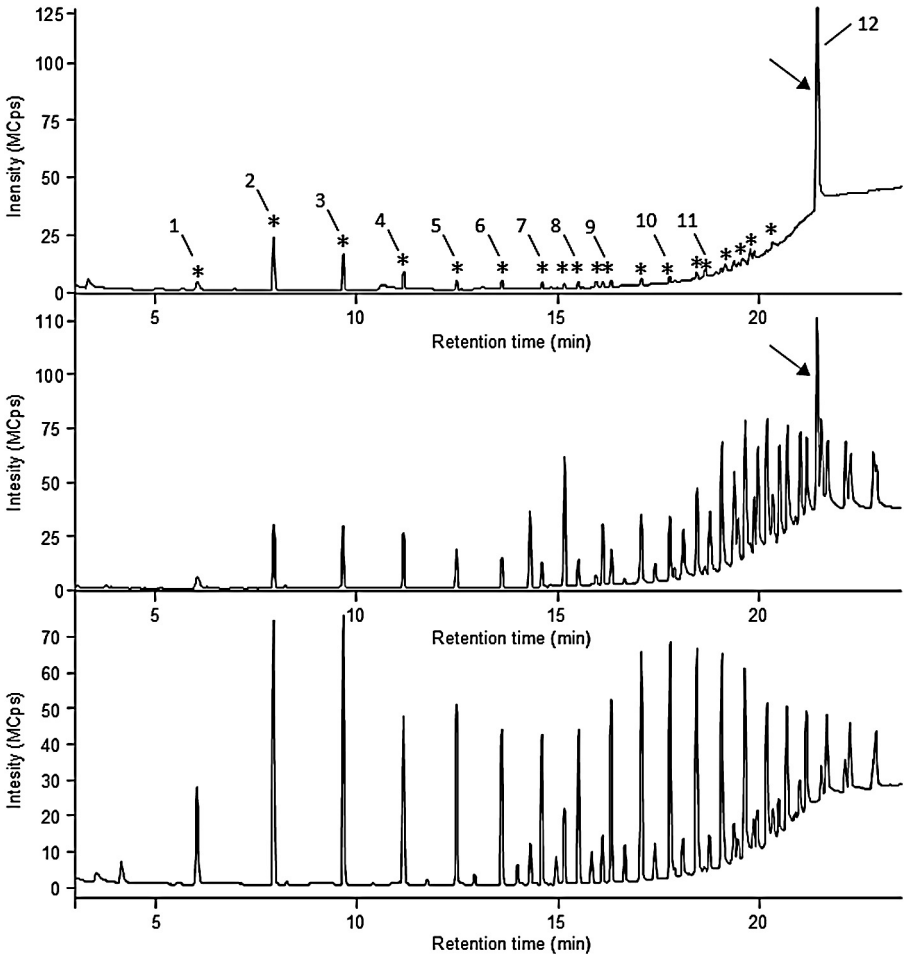

Fig. 4. Representative GC–MS profiles of the acetone extracts of: PIP-LPF4 (upper panel) and of silicone from an explanted PIP prosthesis (batch n. 98276 120, middle panel) and from a virgin non implanted PIP prosthesis (batch 56006 020, lower panel). See Table 1 for peaks attributions. *Peaks identified in both implant silicone and PIP-LPF. Arrows: cholesterol peak.

All the identified silicones (from D4 to D9, U1 and U2, see Table 1 for peaks attributions, see Ref. [1] for the proposed chemical structures of U1 and U2) matched the retention times and mass spectrometric data of LMW silicones extracted from different batches of explanted PIP implants and from one virgin, non implanted PIP prosthesis (Fig. 4).

3.3. HPLC-UV/DAD analysis of PIP-LPF

The presence of major semi-polar species and of serum derived biochemical metabolites that may have accumulated in the PIP-LPF, was investigated by reverse phase HPLC-UV/DAD analysis of their water/acetonitrile (1:1 (v/v)) extracts.

The chromatographic profile of PIP-LPF1 shown in Fig. 5 evidenced the presence of a dominant peak at RT = 3.6 min, generated by a species with both UV spectrum and RT matching those of uric acid (λ_{max} = 268 nm). To overcome the difficulties in the accurate and reproducible determination of this analyte, which is barely soluble in most polar and apolar solvent systems, and that in our case was present in a inhomogeneous sample (hindering its classical determination by colorimetric enzymatic assay), we developed an HPLC-UV method for the quantification of uric acid after its conversion into its water-soluble form (urate) at basic pH (100 mM aqueous NaOH/acetonitrile, 1:1 (v/v); see experimental section for details). In this conditions, the higher solubility of urate compared to that of native uric acid, and the bathochromic shift to λ = 284 nm of its UV spectrum, allowed a substantial improvement in terms of specificity, accuracy and reproducibility of its quantification. Using this methodology we determined in PIP-LPF1 a mean uric acid concentration of 4.0 ± 3.0 mg/dL (238 μM), a value falling in the normal range of human blood serum. Interestingly, in the case of PIP-LPF2, PIP-LPF3 and PIP-LPF4 the uric acid concentration was below the method LOD (not shown).

Table 1
Main compounds identified in PIP-LPF by GC–MS.

Peak	RT	Compound	R match
1	6,00	D4	886
2	7,90	D5	872
3	9,70	D6	761
4	11,20	D7	893
5	12,50	D8	747
6	13,60	D9	747
7	14,60	L8	823
8	15,10	U1	
9	16,10	U2	
10	17,40	Bupivacaine	934
11	18,40	D9 analogue	802 (for D9)
12	21,40	Cholesterol	891

3.4. SEC-UV/DAD analysis of PF proteins

According to the hypothesis that the PIP-LPF collected were originated by the emulsification of high and low molecular weight

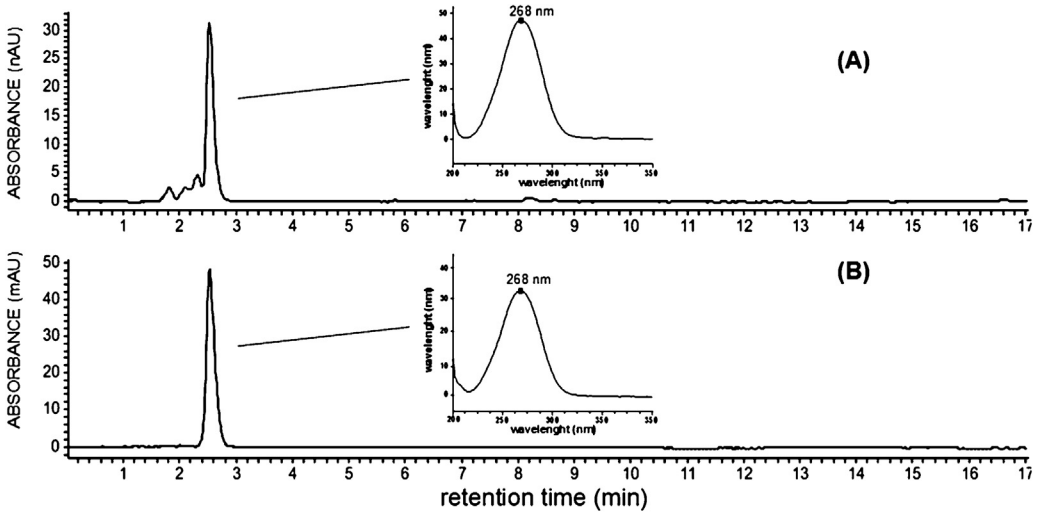

Fig. 5. HPLC-UV DAD profile (λ = 270 nm) of (A) the water/acetonitrile extract of PIP-LPF1 and (B) of standard uric acid. UV spectra of peaks at RT = 2.6 min in the insets.

silicone with blood components, the presence of water-soluble proteins associated with blood serum was investigated by SEC-UV/DAD analysis.

According to previous studies [22], our results (Fig. 6) showed a chromatographic profile clearly compatible with that of high molecular weight serum proteins (globulins, RT = 4–5 min; albumin, RT 5.5 min).

3.5. Microscopical examination

As expected on the bases of the results from ATR-IR experiments, the microscopical analysis of PIP-LPF, revealed a fluid morphology consistent with a complex multiphase system (Fig. 7).

All PIP-LPF samples displayed the presence of a background of round-shaped particles of small and intermediate size and of bigger, more structured particles consistent with oil-in-water emulsified silicone (diameter = 10–100 μm). The bigger structures also presented smaller embedded particles, consistent with a water-in-oil emulsion (see arrows in Fig. 7).

These results suggest that during the implantation time there is a slow conversion of big polydimethylsiloxane silicones particles into smaller multiphase silicone/blood components particles.

4. Discussion

In this work we have investigated the (bio)chemical properties of PIP breast implants silicone and of PIP-LPF induced in patients with ruptured PIP prostheses.

To the best of our knowledge, the only available analytical informations on PIP-LPF are those reported on the website of the Therapeutic Goods Administration of the Australian Government: "To date, the ruptured explants received by the TGA are associated with a 'milky fluid'. An aliquot of the milky fluid was chemically fingerprinting using FTIR and this indicated that the milky fluid was predominantly water with polydimethylsiloxane (silicone) [23]."

In this context, we believe our data add important informations: (i) beside water and polydimethylsiloxane silicone(s), PIP-LPF contains serum derived proteins such as globulins and albumin, and variable amounts of uric acid and cholesterol, (ii) at microscopical level, the PIP-LPF appears as a multiphase system, very likely induced by the emulsification of silicone with amphiphilic serum components, rather than a simple suspension of silicone in water (which should readily separate due to the extremely low solubility of polydimethylsiloxanes in water).

In particular, the comparison of the ATR-FT-IR and GC–MS data for intact explanted prostheses and PIP-LPF leads to the conclusion

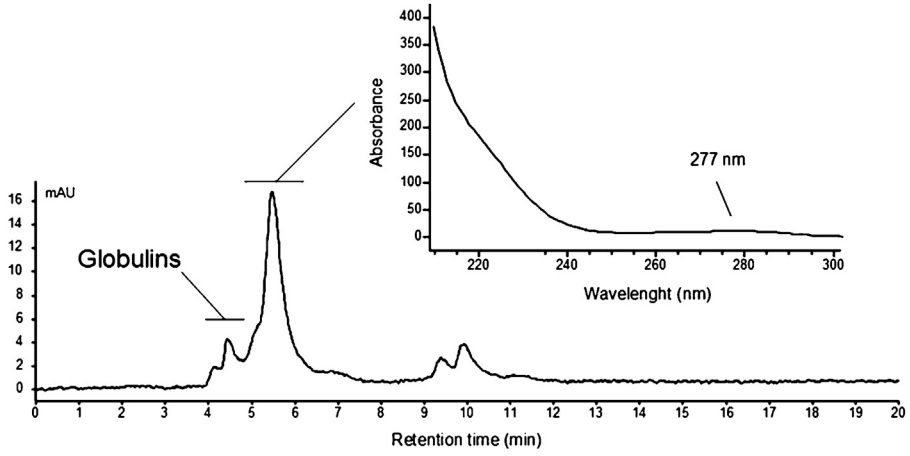

Fig. 6. Representative SEC-UV/DAD chromatographic profile of PIP-LPF proteins (λ = 280 nm, PF3). Albumin UV spectrum in the inset.

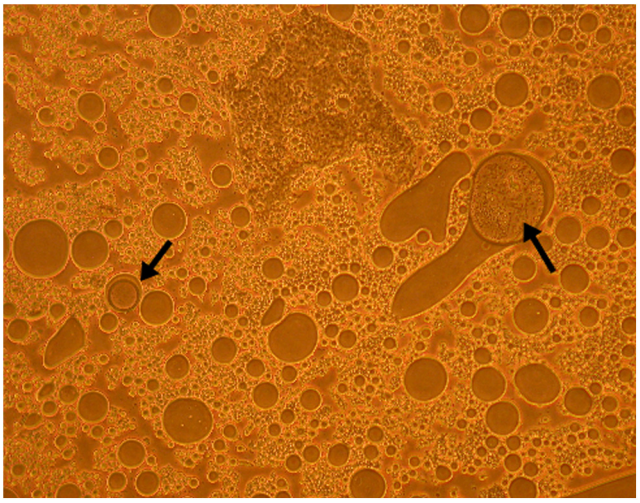

Fig. 7. Representative microscopical examination (original magnification: 20×, PIP-LPF2).

that the bulk of silicone found in PIP-LPF is made of high molecular weight polydimethylsiloxanes (beside low molecular weight polydimethylsiloxanes detected at trace levels). Both techniques did not evidence the formation of low or high molecular weight silicone-derived degradation products that may be induced by acidic environments, such as those produced by bacterial bio-films that may be present on the implant surface.

In Fig. 8 is summarised a proposed cascade of events based on the combination of our data with those reported by other groups, that can lead to silicone contamination of the breast tissue and other distal organs. The first step of silicone migration involves the PIP elastomeric shell.

Recently, Swarts et al. reported quality issues that may contribute to PIP elastomeric shell permeability, weakness and failure among which its variable thickness and the presence of sharp corners and porosities in the cavities of the textured surface, all potential areas of weakness and preferred rupture initiation sites [24]. The migration of silicones outside the implant, lead first to their accumulation in the layer between the implant outer surface and the inner surface of the periprosthetic capsule (Fig. 8, A1).

In the second step, the migrating silicone is slowly mixed and emulsified with the periprosthetic inflammatory exudate fluid (Fig. 8, B1) to form the small particles observed by contrast phase microscopy (Fig. 7).

This 'milky fluid', observed for the first time at explantation of PIP implants [14], basically differs from the classical seroma (a clear serous fluid, whose formation is known as a complication that may arise as a consequence of prosthesis implantation and/or rupture). Recently it has been further documented by different authors and surgeons [3,5–7,10,20], and it appears as a specific sign of breast tissue exposure to the silicone of PIP implants and not to that of other brands.

This is in line with the findings of our recent study in which we have reported the lack of cohesiveness of PIP filler silicone compared to a medical grade cohesive silicone gel [1].

The PIP filler silicone differs from that of other brands in that it has been produced mixing and curing different $n = 4$ components/reagents: silicone oil trimethylated Silopi W1000 or Rhodorsil H47V1000 (low viscosity polydimethylsiloxane oil; 90.2–94.3%), vinyl terminated silicone oil Silopi U165 (heavy silicone oil, mean MW = 165,000 Da; 4.4–8.3%), Rhodorsil RTV 141 Part A (1.1%) and Part B (0.2–0.4%; ratio Part A/Part B: 2.75–5.5%) [23]. According to the manufacturer instructions, the Rodhorsil products are used to produce silicone elastomeric systems for industrial

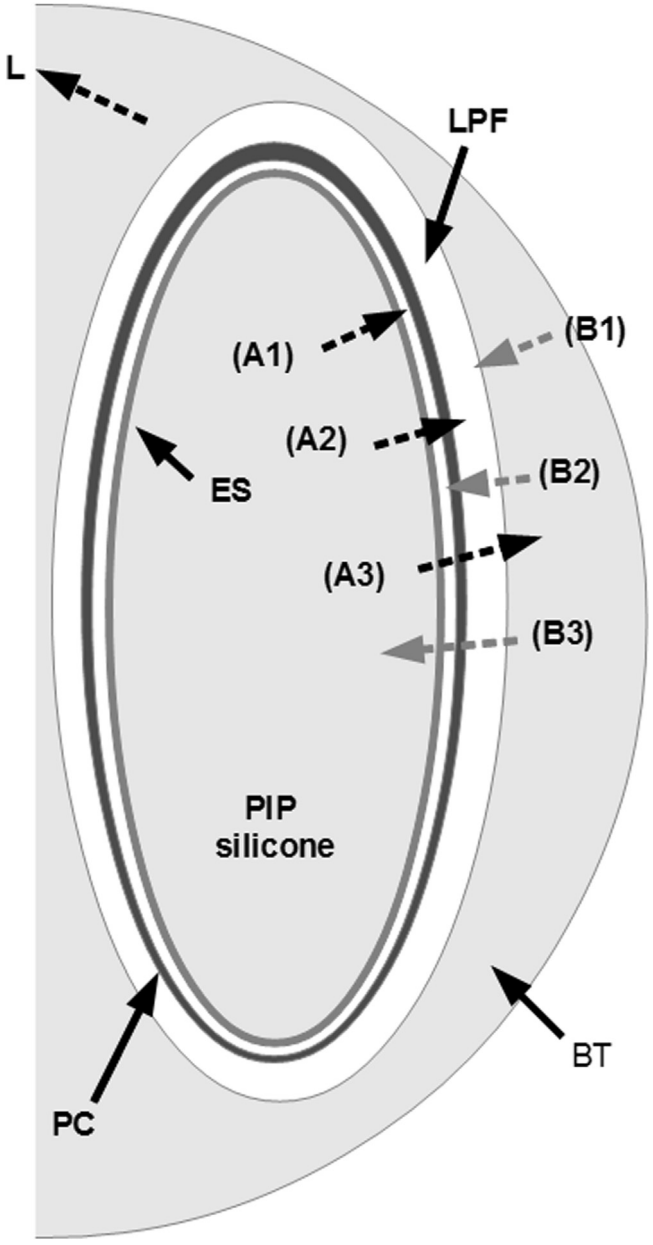

Fig. 8. Schematic representation of the proposed silicone diffusion mechanism into intra-capsular space (A1) and extra-capsular periprosthetic space (A2), breast tissue (A3) and breast draining lymphatic route (L); infiltration of inflammatory exudate/lymphatic effusion into extra-capsular (B1), intra-capsular (B2) and intra-implant compartments (B3). BT: breast tissue; PC: periprosthetic capsule; PIP-LPF: PIP late periprosthetic fluid; ES: elastomeric shell.

purposes by curing at $T = 150\,^{\circ}C$ for $t = 1$ h the mix of 100 parts of Rhodorsil Part A with 10 parts of Rhodorsil Part B. Hence, in the final mixture of PIP silicone, the sum of these components, which are of pivotal importance to confer the cohesive character to the producing silicone gel, have been found diluted with polydimethylsiloxane oils down to the 1.3–1.5% (w/w) of the total mixture weight. The high percentage of free silicone oils present in the only minimally crosslinked cured matrix explain the anomalous mobility of the PIP silicone compared to medical grade, cohesive silicone gels.

In this context we believe of great relevance what recently reported by Swarts et al. regarding the quality issues of the elastomeric PIP silicone shells that, in spite of the minimal evidence of shell degradation over a average time of implantation of 5.3 years, found several concerning quality issues (variable thickness,

sharp corners and porosities in the cavities of the textured surface, machining and identification marks) contributing to shell weakness or failure and, in our opinion, to the enhanced silicone bleeding of PIP implants [24].

Moreover, it has been reported that the anti-bleeding barrier layer usually present in medical silicone implants were removed from the PIP implants manufacturing procedure in 2007 [24].

The occurrence of the reciprocal exchanges between implant silicone, PIP-LPF and breast tissues (directly or through the inflammatory/lymphatic exudate) is further supported by the identification of cholesterol inside both intact explanted prostheses and PIP-LPF (Figs. 4 and 8, B2–B3). The osmotic effect induced by the significantly higher concentrations of cholesterol in the PIP-LPF compared to those found in the implants gel is probably the driving force that lead to its migration and incorporation into the prostheses silicone.

In this context, it is comforting to observe that the results obtained by Yildirimer et al., IR absorptions attributable to serum water and proteins in silicone from explanted PIP implants (attributed to Si—OH bonds from degraded silicone and protein-like groups in their interpretation, to water and serum proteins in the present study) are an objective evidence that not only cholesterol, but also the entire PIP-LPF may cross the elastomeric PIP implant shell, depending from the degree of its lack of permeability [21]. According to these authors, these water- and proteins-associated resonances were absent in explanted, non-PIP silicone gel prosthesis (Natrelle™, Allergan; Mentor Memory-Gel®, Johnson & Johnson Medical Limited; Impleo, Nagor) [21].

Moreover, a study by Hölmich et al. investigating in 2004 the effects of implant rupture of a variety of implant generations (produced before PIP introduction on the market) in a population of Danish women, concluded that implant rupture is a relatively harmless event that does not seem to produce significant clinical symptoms, at least at two years follow up after rupture [25].

In the third migration step, the small silicone-based emulsified particles permeate the periprosthetic capsule (made basically of connective tissue) reaching the breast tissue area. Due to the particular breast anatomical characteristics, we believe this step to be the one of highest concern.

It should be underlined that this emulsification mechanism can take place (i) before the implant rupture due to the abnormal shell permeability, and/or (ii) after the macroscopic rupture of the implant elastomeric shell.

The exposure of the mammary gland and of the deep, superficial and capillary breast lymphatic vessels to these small and diffusible particles, can lead to their drainage and transport to other distant body parts, something that would be less likely with native, highly insoluble high and low molecular weight silicone oil components.

This mechanism is supported by and explains the massive lymphadenopathy secondary to silicone deposition (siliconoma) in axillary lymphonodes that is often found in women carriers of PIP implants [2,6–8,12], in particular after implant rupture with consequent silicone invasion of breast tissue. Depending on implant positioning (sub-muscular or supra-muscular) the silicone migration can occur also towards intra-thoracic regions [26].

Previous studies reported that the direct tissue exposure to silicones in cases of illegal silicone oil injection for cosmetic breast and buttocks augmentations, leads to the formation of nodular and pseudo nodular groups of silicone, siliconomas, axillary lymph nodes, and silicone pneumonitis respectively, with severe signs of inflammation [27,28].

In this context, it is interesting to observe that in 1997, Naim et al. found that silicone gel/ovalbumin emulsified systems were able to elicit a systemic immunological response in the rat after intradermal injection. Their data showed that, among the tested samples, the silicone gel producing the highest immunological response also formed the greatest dispersion of HSA/saline within the silicone gel [29], supporting our hypothesis that the emulsification of silicone released by the prostheses plays an important role in the proinflammatory effect of PIP implants.

5. Conclusions

In this study we have analysed the chemical composition of (i) silicone extracted from two explanted PIP breast prostheses and from one non implanted, intact PIP implant, and of (ii) LPF samples collected from three patients with ruptured PIP implants.

The results obtained by ATR-FT-IR spectroscopy, GC–MS, reverse phase HPLC-UV/DAD, SEC-UV/DAD and contrast phase microscopy demonstrated that the cloudy, viscous PIP-LPF found in the breast of women carriers of PIP implants is a multiphasic silicone/water emulsion. To the best of our knowledge, the profile of serum and PIP silicone major constituents in PIP-induced PIP-LPF have been unequivocally characterised for the first time in this work.

The combination of our results with those reported by other research groups suggest that the particles produced by the emulsification process are small enough in diameter to be drained by the breast lymphatic system though deep, superficial and capillary lymph collecting vessels [30] and transported to the axillary lymphonodes and/or the skin. In the light of these results, we believe that urgent further investigations are needed to understand the toxicological consequences (in particular for the lymphatic/lymphoid systems) of silicone conversion into such small, emulsified and mobile particles into the periprosthetic capsule, breast tissue and into other excitable organs or body regions (i.e. lungs and thoracic cavity).

However, above all, we believe that the results of this study confirm the conclusion that was underlying our previous investigation [1], still in line with the position declared by the International Confederation for Plastic and Reconstructive Surgery (IPRAS) on its website at the beginning of 2012: “There is no further room for discussion. It is mandatory to recommend the explantation of PIP (. . .) implants”[31].

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