JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 477–485

Degenerative mineralization in the fibrous capsule of silicone breast implants

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The formation of a fibrous capsule made of long collagen fibers surrounding breast implants represents an unavoidable phenomenon as the patient's reaction to the presence of a foreign body. Depending upon the size and shape of the implants and the chemicals percolating through the shell, this fibrous capsule is continuously remodeled. The compaction of the foreign debris in the vicinity of the silicone shell is followed by the loss of cellular activity, shrinkage and necrosis. Calcification is the ultimate step. These phenomena were illustrated in the analysis of 18 explanted breast prostheses after 20 or more years of implantation. The degenerative mineralization was shown in scanning electron microscopy and light microscopy. The minerals proved to be bone-like hydroxyapatite by X-ray diffraction and Solid State NMR analysis. Whatever the characteristics of any sophisticated new model of breast implant, phenomenon of mineralization might be minimized but it is very unlikely that it would be totally eliminated.

1. Introduction

Silicone breast implants are being used again after they were banned by FDA in 1992 [1] because of multiple local complications such as ruptures, deflation and contractures with and without mineralization and severe systemic illnesses such as connective tissue diseases, neurology diseases, systemic complaints or conditions believed by women to be related to those implants.

Further to the report of the Institute of Medicine in 1999, the silicone breast implants were progressively reintroduced with preference to saline filled breast implants [2]. The new generation of gel filled breast implants are said to be far superior, without any silicone migration. They are made from a cohesive gel which substantially lowers risks of rupture and silicone migration. Unfortunately the manufacturers are keeping all the improvements achieved during the moratorium as trade secrets. There are no scientific data available. Complete technical description of almost all the countless models of implants manufactured since 1964 was never made available [3]. However, changes have been made to manufacture improved implants as plastic surgeons and manufacturers have learned from adverse reports: barrier shells, texturing, better valves in saline implants, stronger shells, to name a few improvements [4–5]. As the countless number of models led to similar problems of mineralization together with frequent local complications [6–8], revisiting these phenomena with innovative techniques of analysis such as NMR [9] could bring a better understanding of its origin, its evolution and the consequences for the durability of the implant.

Different types of breast explants were analysed. Deficiencies and failures of such implants, after long term of implantation, would be documented. Particular attention to unwanted mineralization would be done, using complementary methods of characterization.

2. Material and method

2.1. Selection of the prostheses

Eighteen breast prostheses from nine patients were selected for investigations based upon the high level of mineralization, i.e. clearly visible crystal formation. Those prostheses were harvested in different centers in USA, Canada and France. They were part of retrieval program conducted at the Quebec Biomaterials Institute, Quebec, Canada [6, 10]. The prostheses were

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shipped at patients' request after examination by the local pathologist, i.e. the fibrous capsules were usually dissected. The prostheses together with capsules were preserved in a 10% buffered solution of formaline.

2.2. Analysis of the prostheses

A thorough examination of the implant together with the capsule was conducted after receiving the explanted breast implant to classify the prostheses as: gel filled breast implants with Dacron® patches on the posterior surface, gel filled breast implants, and saline filled breast implants. Photographs were taken.

2.3. Scanning electron microscopy

2.3.1. Scanning electron microscopy of the surface in contact with the breast prosthesis

Representative samples of 5 × 5 mm were selected, with an average of 5 per capsule, and divided in 2 sub-samples. Each first sub-sample was post fixed in a buffered solution of glutaraldehyde, stained with osmium tetroxide, dehydrated in solution of ethanol of graded concentrations, and then transferred in absolute acetone. Final drying was achieved by critical point drying using liquid CO_2 as the transfer medium. The first sub specimens were then fixed on aluminum stubs. Further to gold-palladium coating in a sputter device, they were observed in scanning electron microscope (Jeol JSM 35-CF) at accelerating voltages ranging from 10 to 20 kV and photos were recorded. The second subsample was dehydrated according to the same protocol but without any post-fixation with osmium tetroxide. The specimens were fixed on carbon stubs prior to carbon coating in a sputter device. The scanning electron microscope observations were coupled to X-ray analysis with electron microprobe multichanel energy dispersion spectrometer PGT system 4 fitted to the scanning electron microscope.

2.3.2. Scanning electron microscopy of the section of the capsule

The selected samples were embedded in paraffin and cut in thin slices 5 microns thick. After deparaffinization, the slides were split in two groups for gold-palladium coating and carbon coating as above.

2.4. Light microscopy

Representative samples of the capsules were selected for histological investigations. Each sample was embedded in histological paraffin wax and sectioned with a microtome to produce thin slides 4 to 5 microns thick. The following stains were used for viewing in light microscopy: HE (hematoxylin-eosin) and Masson's trichrome stainings to identify the histological structure and differentiate collagen from fibrin, Weighert's staining to visualize the elastic fibers. The preparations were also stained after the method of Van Kossa as well as with Alizarin Red S, after the method of McGee-Russell (light green counter staining was applied) to identify the different steps of the mineralization.

2.5. Mineralogy

2.5.1. X-ray investigations

Powder X-ray diffraction experiments between 10° and 80° in 2θ were conducted using a Philips automatic diffractometer.

2.5.2. NMR investigations

Measurements were done on an ASX-300 Bruker spectrometer using Magic Angle Spinning (MAS) at a speed rate as high as 14 kHz, depending on the nucleus studied, at room temperature. Powered samples averaging 100 mg were necessary to fill up the rotor of the NMR probe; particular attention was paid regarding the duty cycle (1000 s) needed to acquire each spectrum due to a long spin-lattice relaxation time.

3. Results

3.1. Gross observations

The 18 prostheses were harvested from 9 patients at re-operation. All the patients were re-operated further to detected ruptures of the implants and/or grade IV contracture of the capsules (Baker scale). The following models of prostheses were collected:

- 12 Cronin implants silicone gel filled with various conformations of the Dacron® patches on the posterior face; the design was a contoured implant; none was intact and a thick gel was escaping in different locations. Those prostheses were implanted for more than 20 years. A very thick and stiff capsule was adhering to the anterior side of the prostheses. The surfaces of the capsule in contact with the silicone were heavily mineralized and well structured crystals were visible. Those crystals usually were poorly anchored to the capsule (Figs. 1–3).
- 2 gel filled round breast implants: the first one was still preserved however the shell was very sticky while the second one was received as totally destroyed during implantation and a non-cohesive gel was dispersed (Fig. 4).
- 4 saline filled silicone elastomer shell breast implants: all of them were deflated with ruptures and damages to the shell. They were implanted for more than 20 years. The capsules were heavily mineralized were strongly anchored to the silicone rubber (Fig. 5).

3.2. Scanning electron microscopy

The surface of the capsule in contact with the silicone rubber was frequently covered with large crystals that appeared to migrate from deeper collagen layers together with silicone particles. In the case of the saline filled implants the minerals were anchored to the shell (Figs. 6 and 7). Micro-analysis confirmed the presence of calcium and silicon.

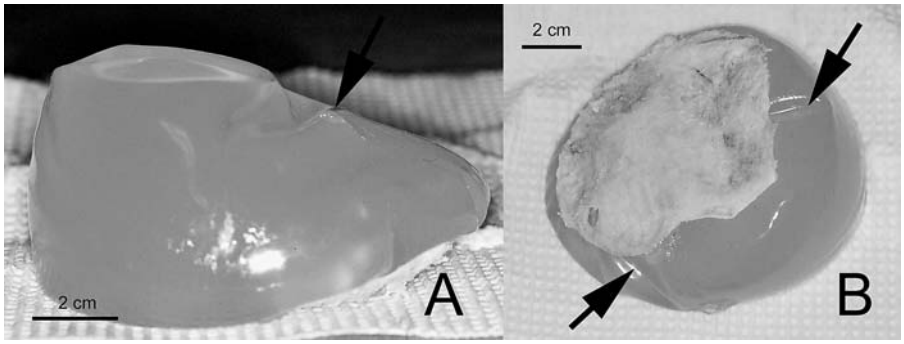

Figure 1 Contoured silicone gel filled breast implant of the Cronin type implanted for more than 20 years. A: Front side. The shell of the prosthesis was ruptured, but the gel did not escape because of its cohesivity (arrow). B: Back side. The Dacron® patches led to the development of a thick and contracted fibrous capsule, the shrinking of which caused major foldings in the shell of the prosthesis (arrows).

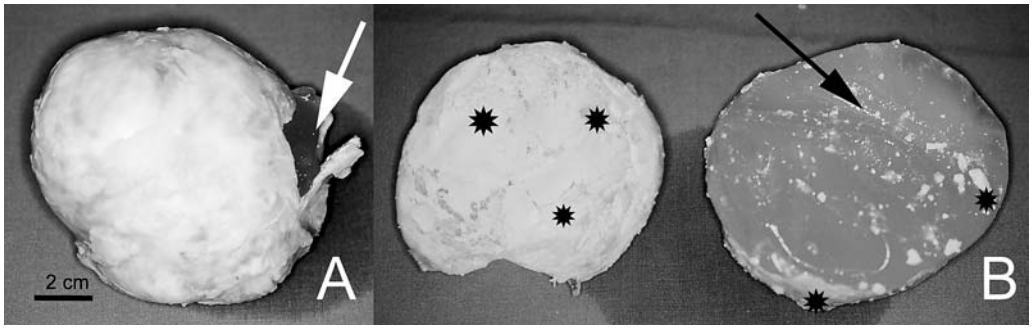

Figure 2 Cronin type prosthesis implanted more than 20 years. A: Encapsulated device. The contoured shape was lost as the result of the important contraction of the fibrous capsule. The resulting elevated pressure caused the device to rupture (arrow) without major gel dispersion due to its cohesivity. B: Dissected capsule and prosthesis. The surface of the capsule in contact with the prosthesis itself was heavily mineralized with well identifiable crystals (∗) while the prosthesis was heavily folded (arrow) with crystals anchored to the shell (∗).

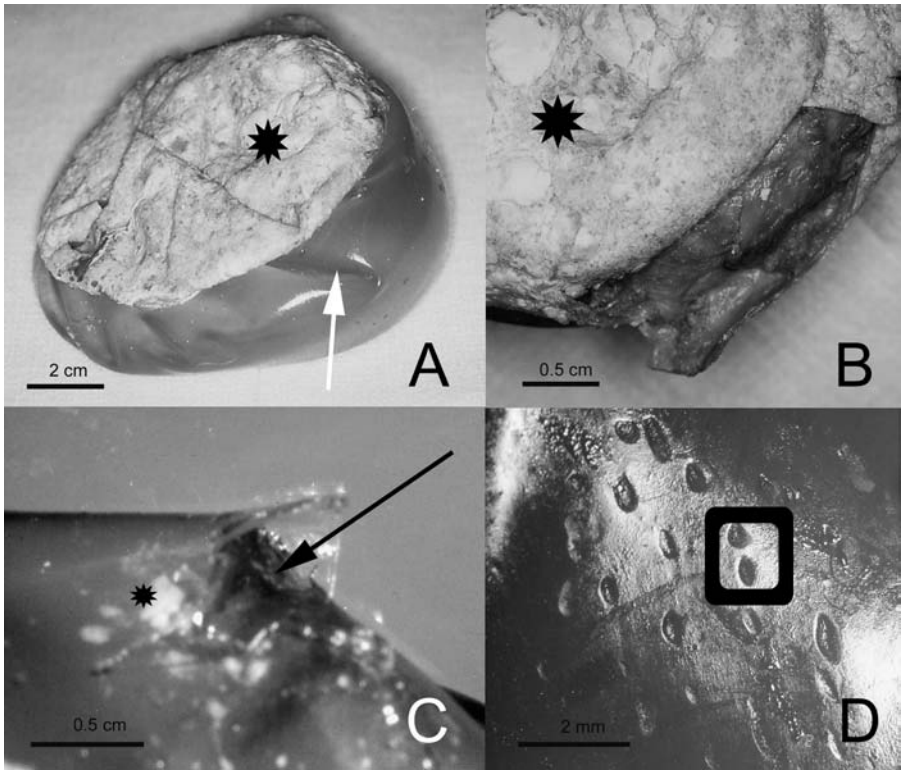

Figure 3 Contoured gel filled breast implant of the Cronin type. A: Side view illustrating the heavy mineralization of the capsule over the Dacron® patches (∗) the exacerbated shrinkage of which caused major folds (arrow). B: Rupture in the shell in the vicinity of the mineralized capsule (∗). C: Site of rupture in the shell without major gel dispersion (arrow) while some crystals penetrated within this gel. D: Surface of the shell. Shallow depressions were observed in many places likely to cause thinning of the silicone envelope.

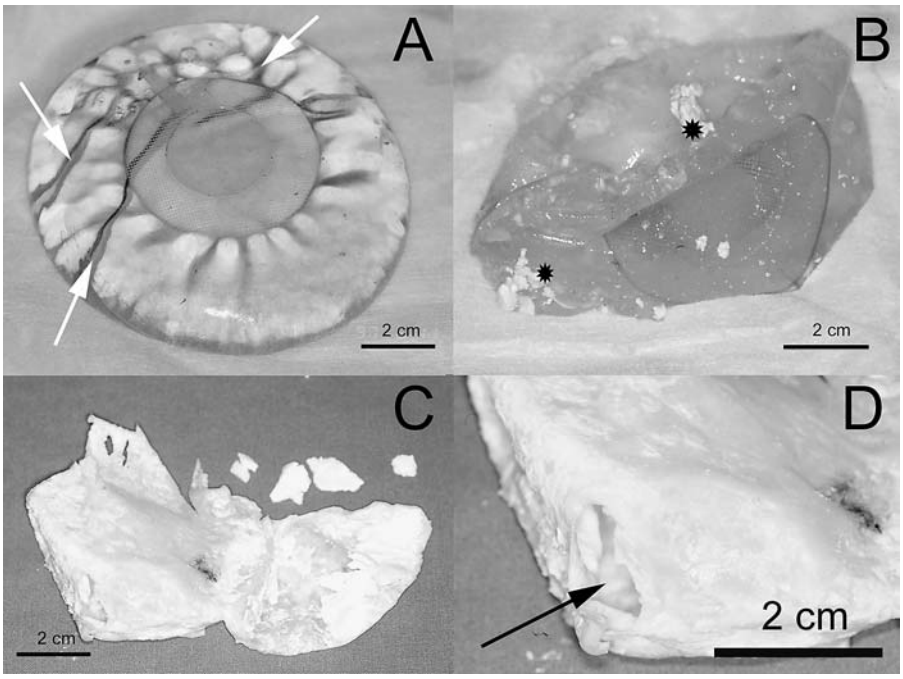

Figure 4 Round type silicone gel filled breast implants. A: Device capable to hold the silicone gel. It was not ruptured but the shell was very sticky, holding major folds caused by the shrinkage of the prosthesis (arrows). B: Device completely destroyed. The envelope was difficult to distinguish among the dispersive silicone gel including some crystals (∗). The thickness of the capsule (C) was very irregular incorporating empty pockets previously filled with silicone gel as illustrated in (D) (arrow).

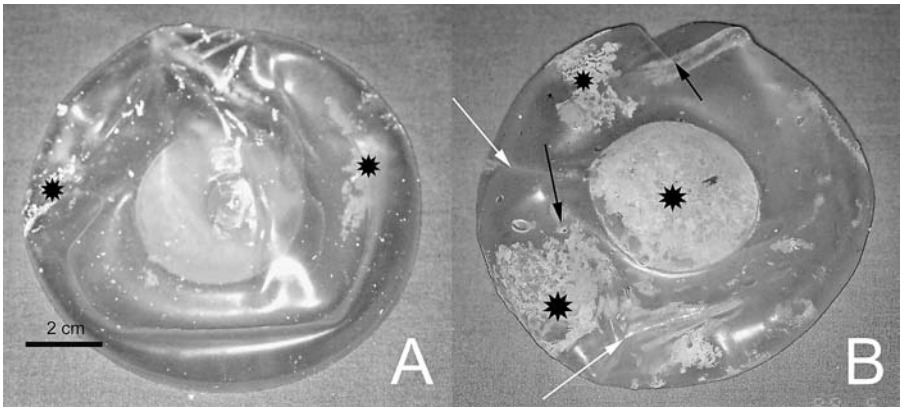

Figure 5 Deflated saline breast prosthesis implanted for more than 20 years. The front side (A) held adhesive minerals and showed multiple folds with holes and ruptures, while the back side was more damaged with holes and ruptures (black arrows) together with major folds caused by the pressure from the shrinking capsule (white arrow) while the mineral was strongly anchored to the silicone shell (∗).

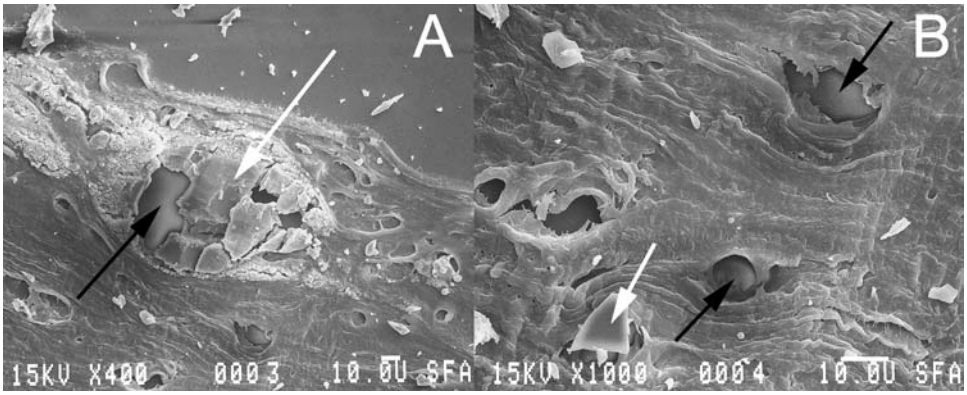

Figure 6 Scanning electron photomicrographs of the internal capsule contacting the silicone shell. A: Mixed vacuoles of silicone gel (black arrow). B: Vacuoles of silicone gel (black arrows) and crystals escaping the capsule (white arrow).

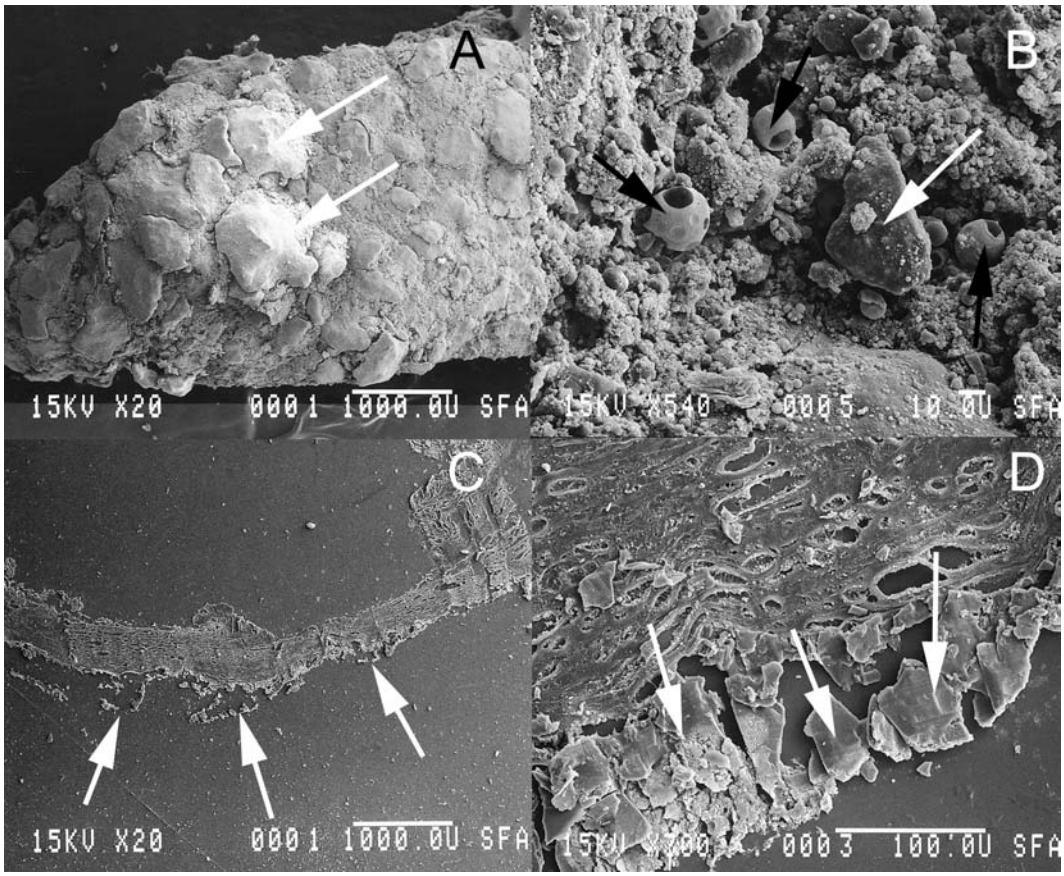

Figure 7 Scanning electron photomicrographs of the capsule of a Cronin type prosthesis. A: Heavy mineralized surface of the capsule in contact with the silicone shell with crystals taller than 1 × 1 mm (arrows). B: Detail of this surface illustrating the interpenetration of silicone gel (black arrows). C: Cross-section of the capsule with mineralized debris poorly fixed to the tissue at the contact of the silicone rubber (white arrows). D: cross-section evidencing the detachment of the crystals (white arrows).

3.3. Histology

The connective tissues forming the capsule surrounding the breast implants varied in thickness from 0.2 mm to 3 or 4 mm. Whatever, its thickness, the capsule was usually made of 3 well identifiable layers:

- interface layer: very thin part of the capsule adjacent and in contact with the surface of the prosthesis (Figs. 8–10);
- intermediate or transition layer: thick fibrous layer forming the most important part of the capsule;
- external layer: structure mimicked the structure of the *adventicia*.

3.3.1. Interface layer

It varied from one implant to another one and also within the capsule surrounding a specific implant. One could distinguish the sub layer of a hypercellular interface zone formed by a dense network of collagen fibers incorporating numerous dispersed cells (macrophages, lymphocytes, fibroblasts) occasionally covered by a layer of epithelium-like cells with dark elongated nuclei. Over this first sub-layer, there was a second very thin with thicker collagen fibers. The third sub-layer was highly cellularized with dark nuclei and irregular shapes. Mitoses were frequently observed. In the areas of intact layers, certain cells

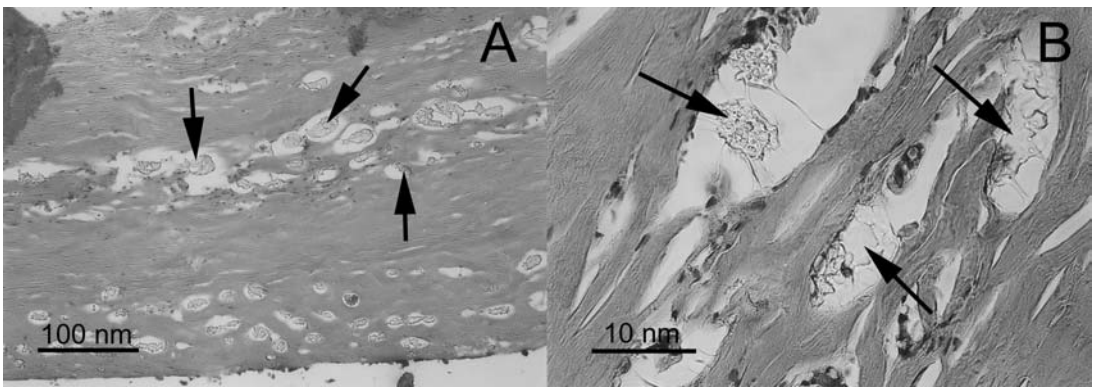

Figure 8 Capsule of a breast implant poorly cellularized with multiple vacuoles whose most of the silicone gel (white arrows) was eliminated during the processing.

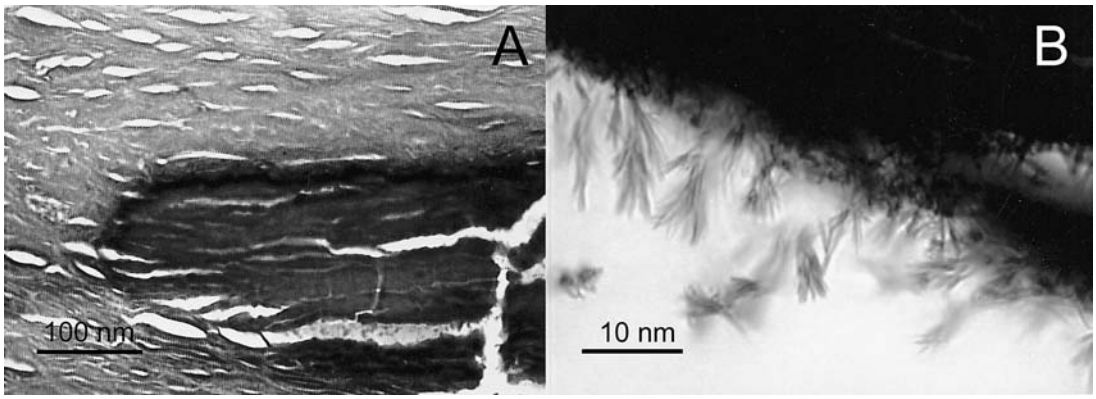

Figure 9 Mineralization process in the capsule of a Cronin type prosthesis. A: Longitudinal calcinosis of collagen fibers evidenced in Alizarine Red S light Green. B: Calcinosis of collagen fibers with fine needle shaped crystals attached to the collagen fibers in Van Kossa stain.

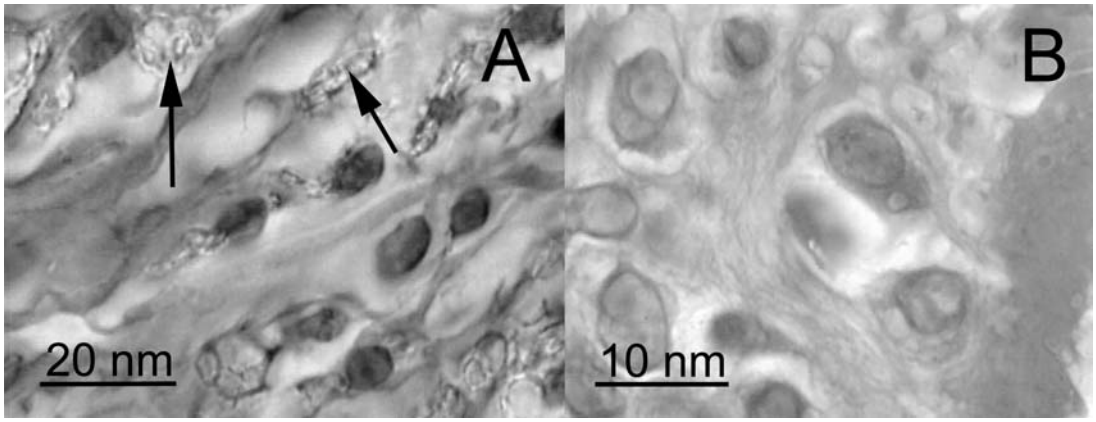

Figure 10 Areas with large numbers of cells in the capsule of a Cronin type prosthesis. A: Incorporating droplets of silicone (black arrows). B: With foamy cytoplasm and larger light nuclei incorporating micronized silicone gel.

with foamy cytoplasm and large light nuclei incorporated micronized silicone and resembled to xanthomic cells. Hydroxyapatite crystals were spread frequently and irregularly at the interface with the silicone shell of the breast implants. The accumulation of mineralization culminated in the vicinity or at the contact with polyester patches or any kind of irregularities. In all the three sub-layers, amongst the collagen fibers, droplets up to one hundred microns of the alien transparent material were evidenced: this micronized silicone droplets were generally not encapsulated *per se*.

3.3.2. Intermediate layer

It was quite difficult to distinguish sub-layers because of the presence of dense and thick bundles of collagen forming nodules. Fibroblasts as well as macrophages and lymphocytes were observed within the bundles. The intermediate layers were infiltrated by a great number of mononuclear and giant cells occasionally forming granuloma when the interface layer was hypercellular. Alternatively, when the interface layer was almost acellular, the cells were more scarce and limited to fibroblast-like cells. A few micro-vessels were also evidenced. Granuloma were evidenced beside vacuoles still partially or totally filled up with silicone droplets. Granuloma surrounding the silicone droplets were present. Some microfibers of polyester originating from the Dacron® patches were occasionally dispersed in the tissues.

3.3.3. External layer

It always was made of a loose connective tissue, incorporating numerous blood microvessels. Microemboli of silicone were detected in some of them.

3.4. Mineralogy: X-ray and NMR results

3.4.1. X-ray

The analysis of all the diagrams demonstrated that the only present phase was always hydroxyapatite (JCPDS9-0432) (Fig. 11). Hydroxyapatatite was previously found in cardiac bioprostheses (Fig. 12). The possibility of the existence of fluorohydroxyapatite (JCPDS 34-0010) was detected as well. The results of micro-analysis confirmed the presence of Ca and P for all samples. Si, Na, and Mg were detected.

3.4.2. ^{31}P MAS NMR

Spectra similar to that of a well-crystallized hydroxyapatite used as control were obtained (Fig. 13). The line-width of harvested samples was broader (3 ppm) than that of the control (0.5 ppm). Such broadening is generally observed in imperfect crystals. It is due

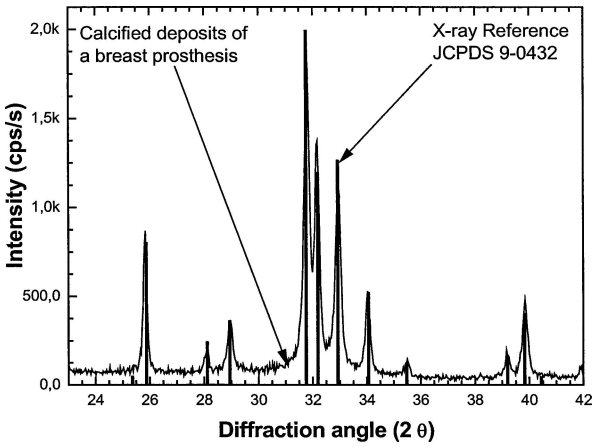

Figure 11 X-ray diffraction pattern of a typical mineral deposit on the surface of the capsule in contact with the silicone shell compared to the reference (hydroxyapatite JCPDS 9-0432).

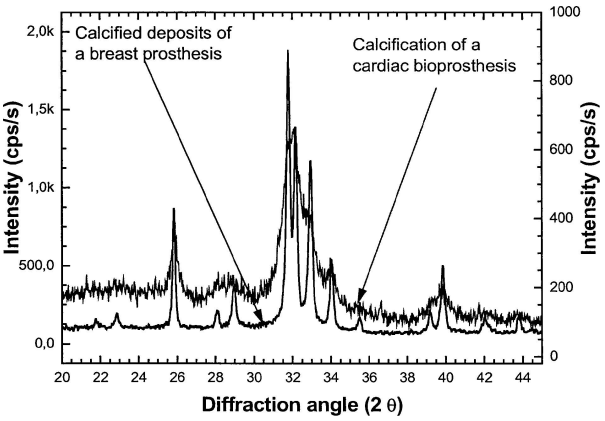

Figure 12 X-ray diffraction pattern of mineralizations in a breast prosthesis confirmed the presence of bone-like hydroxyapatatite previously found in a cardiac bioprosthesis.

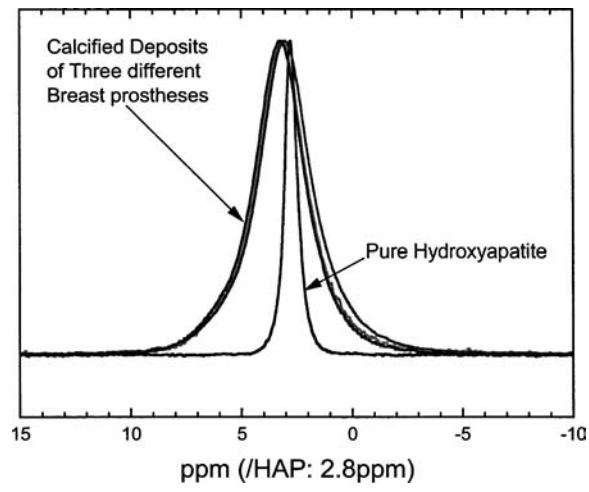

Figure 13 ^{31}P MAS-NMR spectra of hydroxyapatite compared to calcified deposits on breast prostheses. Recycle time 1000 s, rotation speed 5.3 kHz, frequency 121.49 MHz, number of scans 4.

to a loss of long range crystalline organisation of the material, in particular in presence of microcrystal of different sizes. Comparison with the observations carried out with bone demonstrated a similar organization of such calcified tissue.

3.4.3. ^{19}F MAS NMR

Fluorohydroxyapatite was detected as a trace and needed a very long accumulation time. The duty cycle was 100 s, MAS speed 13000 Hz, frequency 282.4 MHz, number of scans 200 to 500.

4. Discussion

In the early 1960's, when doctors began to augmenting the size of the breast of women, there was no study proving that silicone was safe. As implantations did spread before the FDA became operational, there was no obligation to submit any protocol of validation and the breast implants were "grandfathered" as safe medical devices.

The controversy that rose from the eighties to the end of the nineties sounds to be over. However a second thought is necessary. More than 120 000 American women, many of them in their teens and early 20 s, are giving breast implants a try [11]. Implants are more popular than ever. Any investigator raising questions is considered a bigot and/or his results frequently considered as junk science [12]. Corporate funded investigations show that implants are safe. In Canada, the funding agencies only support "University-Industry" grant applications, with contributions of the Dow Corning Corporation. Thus, after 40 years of breast implants availability, the number of patients with serious problems that harm their health or their quality of life is still unknown [13].

As the mineral deposit is mainly hydroxyapatite [14], the mineralization process is not related to a specific device but it is a phenomenon related to the procedure. Surrounding tissues appeared to be an arena of dynamic interaction between the prosthesis and the recipient's organism.

The presence of bacteria can also have some influence, and this idea must be further investigated, as results are still controversial. Ideally, the prostheses, together with the surrounding tissue capsules, must preserve their integrity and their softness. In addition, the potential durability of the prostheses must outlast the life expectation of the patient. The situation is still far from that goal [15].

The great variability in the structure of the capsule formed around the silicone mammary prosthesis hampers the comparison of data reported by single authors on this topic and often leads to misunderstanding [16, 17]. With respect to the three-layer structure hereby reported the capsule should be interpreted as a highly differentiated and complex structure:

- hypo/acellular interface layer of thick collagen fibers with very few flattened cells incorporating dark nuclei and cytoplasm, fibroblast and macrophage-like cells;
- hypercellular interface layer incorporating vascularization and loose network of fine collagen fibers with numerous dispersed cells similar to macrophages, fibroblasts and lymphocytes together with microvessels;

- capsule with synovial metaplasia of the interface layer which consists of several sub-layers of cells arranged as an epithelium, together with blood microvessels.

Extracellular silicone droplets are found amongst the collagen fibers of the interface and intermediate layers. Intracellular silicone is present in two micronized silicone in the cytoplasm of foamy cells (xanthoid reaction) and small droplets in the cytoplasm of macrophages and giant cells. Single calcification types differ mainly in their relation to the collagen fibers. Calcium precipitations related and not-related to the collagen fibers could be differentiated. In the first way, the granular deposits (focal calcinosis of the collagen fibers) and calcium-impregnated bundles of fibers (longitudinal calcinosis of the collagen fibers) are found [18, 19].

The role of silicone in the formation and evolution of the fibrous capsule is contradictory. It is assumed that either the implant as a whole or the silicone gel entering the surrounding tissues represents a stimulus to isolate the prosthesis, through a fibrous capsule. These two processes are mutually linked. The silicone liberated in the surrounding tissues as a 'bleed' is directly related to degenerative calcinosis of the periprosthetic capsule, according to observations of Rolland [20], who observed small crystals growing on the edge of intracellular silicone droplets, themselves included in macrophages.

Adverse effects of breast implants can be considered as the results of events initiated by an implant "being there" and whose relationship with the patient recipient is not as harmonious as anticipated. Depending upon the implant composition, shape and surface, also depending upon the chemicals percolating from the device, the fibrous membrane can acquire a number of pathological characteristics at different rates. With time this capsule matures and its properties change [21–28]. Calcification is the ultimate step where crystalline deposits form on the inner capsular surface, ultimately lining the interface between the implant and the capsule. As the capsule shrinks, the pressure exerted on the breast device increases dramatically. Therefore, the intracapsular space is poorly irrigated and collects debris of synthetic and natural origin that would be otherwise excreted by natural transport processes. The space between the implant and the tissue becomes gradually filled with stagnant body fluids and soluble implant impurities, leading to mineralized particles, in particularly poorly soluble calcium salts. The calcific material dispersed within the site causes the pH to rise dramatically, initiating hydrolysis of the silicone shell, evidenced by surface degradation and pitting of mineralized prosthesis implanted for 20 years or more. This alkali-rich mixture is very abrasive. It is the beginning of alkali-based tissue necrosis and silicone degradation. Perversely the capsule which was impermeable to fluids at its peak of maturity becomes permeable as the degradation process continues.

Calcification is almost universal in breast prostheses with polyester fabric fixation patches after 10 years of implantations. Devices without fixation patches also calcify in different ways after 15–20 years *in situ*. Saline filled implants manufactured prior to the mid eighties are often found with grossly calcified shells where the mineralization process has penetrated deeply in the silicone rubber rendering it brittle and permeable. Some pathologists routinely mischaracterize the mineralized entities as dystrophic breast calcification.

For an implant that is not life supporting and does not contribute to a key physiologic process, injuries from disturbances created in the implant environment can be severe and life-threatening. Even, if the breast prostheses are considered as benign, one must consider the systemic effects of the local injuries. The short range phenomena arising from mechanical action and efficient from breast implants are of degenerative nature culminating in necrosis of tissue with deeps mineralization. Therefore, "local injury" is at best an uninformative term and at worst a grossly misleading perception.

5. Conclusion

In summary the main observations were:

1. The structure of the capsule was constituted of three layers:
   (a) hypo/acellular with thick collagen fibers and few flattened cells, fibroblast and macrophage-like cells;
   (b) hypercellular with fine collagen fibers and dispersed cells similar to macrophages, fibroblasts, lymphocytes with microvessels;
   (c) synovial metaplasia capsule with sub-layers of cells as an epithelium and blood microvessels.
2. The silicone escaping from the prosthesis was found as:
   (a) extracellular silicone droplets amongst the collagen fibers of the interface and intermediate layers.
   (b) intracellular silicone: micronized silicone in the cytoplasm of foamy cells and small droplets in the cytoplasm of macrophages and giant cells.
3. The mineralization related and not-related to the collagen fibers were observed as:
   (a) granular deposits (focal calcinosis of the collagen fibers)
   (b) impregnated bundles of fibers (longitudinal calcinosis of the collagen fibers)
   (c) such calcium deposits were bone-like hydroxyapatite mineral.

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Received 1 July
and accepted 1 July 2004