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ORIGINAL ARTICLE

The Influence of External Ultrasound on the Histologic Architecture of the Organic Capsule Around Smooth Silicone Implants: Experimental Study in Rats

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Abstract
Background Capsular contracture is the main complication related to breast silicone implants, and its prevention remains a medical challenge. The authors present experimental research examining the effect of external ultrasound on the formation and contracture of peri-implant capsules.
Methods In this study, 42 male Wistar rats had a 2-mm smooth surface implant placed in a dorsal submuscular pocket. They then were separated into "ultrasound" and "control" groups that received repeated external applications either with or without the ultrasound power on. Ultrasound applications were given three times a week for a period of 90 days. After that, both groups were housed under the same conditions with no application scheduled. Five animals of each group, killed at 30, 60, 90, and 180 days, had their implants removed along with the capsule, which received a special histologic preparation via annular sectioning that provided wide circumferential observation of the capsular tissue. Sections were stained with hematoxylin/eosin stain, Masson's trichrome stain, and Pricrosirius Red stain for regular microscopic evaluation under normal and polarized light.
Results Histologic data showed that capsules from the ultrasound and control groups had statistically significant differences. Ultrasound application developed a capsular architecture similar to that shown within textured silicone implants, and its effect had an early definition with subsequent stabilization.
Conclusion The authors conclude that early and repeated external ultrasound application enhances the thickness, cellular count, and vascularity of smooth silicone capsular tissue, whereas it diminishes the pattern of parallel orientation of collagen fibers.

Keywords Breast silicone implants · Capsular contracture · External ultrasound

In 1984, Silversmith [1] published a letter describing the case of a patient with unilateral capsular contracture refractory to closed capsulotomy procedures who had experienced a curious softening process after receiving therapeutic ultrasound sessions at the upper thoracic region because of an orthopedic problem without connection to the contracture. According to his records, after these findings, the patient was submitted to sessions of ultrasound directly over her contracted breast three times a week over a period of 30 days and presented with complete and stable recovery of the contracture.

In the same year, Herhahn [2] described his personal experience using ultrasound for the management of capsular contractures. Although he had never published his records, considering them subjective and scientifically inadequate, he claimed to have achieved favorable results in treating such contractures by associating therapeutic ultrasound sessions and the closed capsulotomy procedure.

In 1997, Planas et al. [3] published a treatment protocol applied to 24 breast-implanted patients with 34 capsular contractures, which were submitted to closed capsulotomy followed by a sequence of ultrasound sessions delivered through four fixed transducers attached to their bra. The

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results were remarkably encouraging, and the group kept using that protocol for their patients until 2001. Then they finally presented an extended experience of 5 years, showing an improvement rate of 82.6% over 12 months of follow-up evaluation [4].

These experiments made us consider the potential modulating capacity of external ultrasound to affect contractures, and its prophylactic application during the initial process of capsule formation around silicone implants. Facing the absence of published essays on this topic, we decided to research the morphologic and evolutional behavior of external ultrasound in an experimental model. This study aimed specifically to evaluate external ultrasound effects on the histologic architecture of the organic capsule around smooth silicone implants in rats.

Methods

Animals

The study protocol was approved by our Ethical Committee of Animal Experiments, and 42 male Wistar rats (*Rattus norvegicus*) were used, without any kind of genetic manipulation and a medium weight of 184 g. The specimens were maintained in the Biotery of the Experimental Surgery Laboratory of the Botucatu Medical School, UNESP, in a safe environment with controlled light and temperature conditions, good air circulation, and usual feeding with a balanced ration and filtered water ad libitum. The administration of analgesic drugs, antibiotics, or complementary medication was not prescribed in any phase of the experiment other than surgical anesthesia and final killing of the animals.

Silicone Implants

For this study, 42 sterile Silimed (Rio de Janeiro, RJ) smooth surface silicone implants were used, each with a diameter of 22 mm, a height of 7 mm, a circular base, and a volume of 2 cm^3. All were inserted the same day, which was considered the beginning of the experiment.

Anesthetic induction was obtained through intraperitoneal injection of sodium pentobarbital (30 mg/kg). After dorsal tricotomy and ventral decubitus, antisepsis took place with topical 10% iodine alcohol solution and a sterile operative environment. A 2-cm median scalpel incision on the dorsal interscapular line of each animal provided access to the unilateral submuscular undermining (latissimus dorsi) just large enough to receive the silicone implant. The wound edges were directly sutured through three or four simple stitches using 5.0 monofilament nylon, which were removed 7 days later.

External Ultrasound

The ultrasound device used was the Ultrasonator Plus produced by Industra (São Carlos, SP), which was made to order with a 3-MHz handpiece and a radiant circular plane surface diameter of 3.4 cm, most appropriate to the small dimensions of the animals and implants used in this experiment.

To determine the standard ultrasound potency, different increasing intensities were delivered to the dorsum of several animals. They then were comparatively tested to verify the maximum tolerated levels before they presented pain signals or evident discomfort.

The calibration and verification of the true delivered ultrasound energy was accomplished by an acoustic measurement instrument that uses a mass dislocation procedure produced in a liquid medium (natural temperature water) through ultrasonic waves emitted by the handpiece over a conical diffuser placed at the bottom of a press tube and attached to a high-precision digital weighing apparatus. The effective ultrasonic potency chosen for this experiment was 1.73 W (0.19 W/cm^2), obtained with the generator functioning at 3 MHz in continuous emission mode.

Studied Groups

After implantation, the animals were randomly allocated to two equal groups: the ultrasound and control groups.

Ultrasound Group

By postoperative day 8, the animals were exposed to a series of external ultrasound applications over the implanted area using soft and circular movements of the transducer, enhanced by the use of a common transmission gel with permanent contact to the skin (Fig. 1) for 90 s

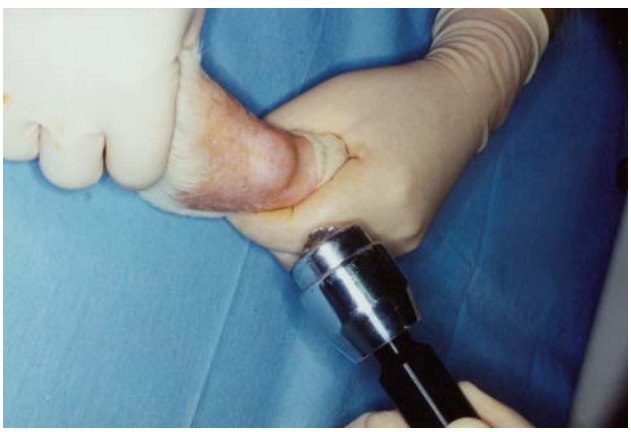

Fig. 1 External ultrasound being applied to the dorsal implanted area of the rat

three times a week. The initial transducer temperature was the same for all the animals, and the ultrasound emission was verified with water drops before every session. The tricotomy of the area to be treated by the ultrasound was systematically repeated in periods of 10 days. The applications were terminated 90 days after surgery, and the animals were kept in rest for 90 more days.

Control Group

Kept separately from the other animals but exposed to the same environmental conditions, the control group was submitted to the same protocol based on the use of the ultrasound device. However, the machine was always turned off. Otherwise, the same procedure was adopted with regard to time and frequency of applications, handpiece movements, transmission gel, and tricotomies. As with the former group, after the 90th day, the animals were kept in rest without any further application.

Removal and Histologic Preparation of the Pieces

Five animals of each group were killed 30, 60, 90, and 180 days after implant inclusion. Their implants were removed along with the surrounding capsules, which were submitted to histologic preparation and evaluation through optical microscopy. Capsulotomies were performed to allow implant extrusion through a small opening, ordinarily created in the cephalic segment of the piece to register spatial identification with regard to its original location in the dorsum of the animals.

After regular 10% formaldehyde fixation, the empty capsular bags were divided to produce complete rings of tissue with 3 to 4 mm of width and transversely oriented to the cranial caudal axis of the capsule (Fig. 2). This maneuver allowed an equated harvesting of the capsular tissue to be included within the paraffin blocks to provide complete capsular circumference sheets with no retractions and standardized angulation of the histologic incisions. Therefore, slices of 5 μm were cut for hematoxilin-eosin and Masson trichrome staining, whereas a thickness of 6 μm was used for the Pricrosirius Red technique.

Histologic Evaluation

All the incisions and histologic colorations were evaluated together at the end of the experiment. After production of the slides, they were distributed and identified by passwords so the observer did not know which group was being studied or its harvesting time. A protocol involving microscopic observation of the capsules was determined according to thickness, cellularity, vascularization, and parallel alignment of the collagen fibers.

Thickness

The evaluation of capsular thickness was performed by observing Masson trichrome slides through a Leica DM LS

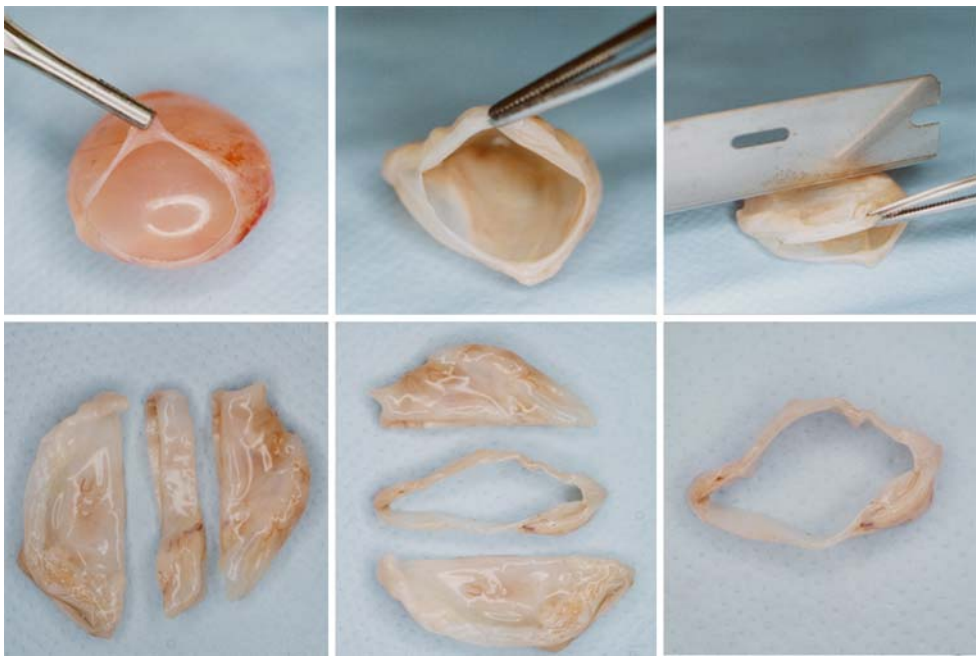

Fig. 2 Capsular tissue harvesting for histologic evaluation

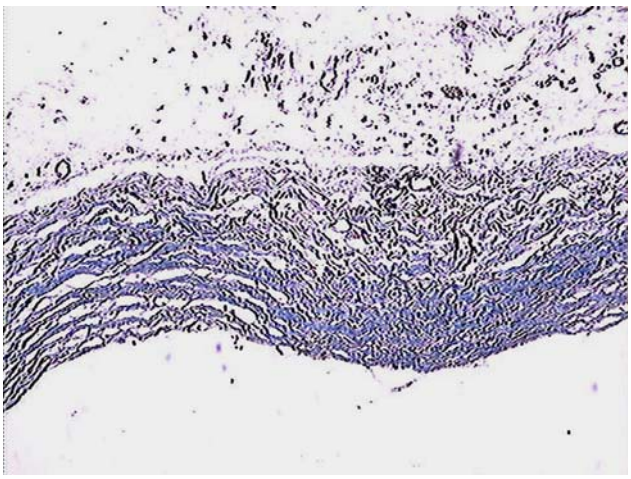

Fig. 3 Masson trichrome–stained slide for thickness evaluation (magnification, ×10)

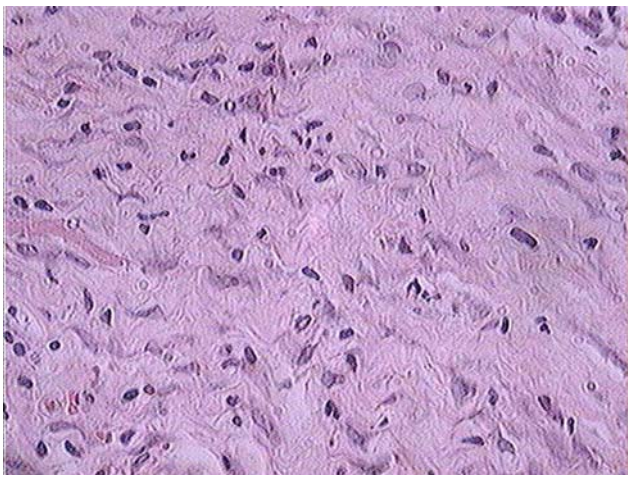

Fig. 4 Hematoxylin-eosin–stained slide for cellularity evaluation (magnification, ×40)

microscope attached to a video camera with digital capture commanded by an IBM-compatible computer connected to the system. Each slide had 10 studied fields uniformly captured over the capsular ring with a magnification of ×10 (Fig. 3). The software Image ProPlus (Media Cybernetics, Bethesda, MD) was used to perform the linear measurement of the capsule thickness. Each of the 10 analyzed fields was measured at six different sites, randomly distributed, to establish an average value for each field, and afterward, for each slide. Therefore, 60 measurements were registered for each studied slide. These data were registered in tables created with Excel software (Microsoft, Redmond, WA).

Cellularity

The count of the cellular cores stained by hematoxylin-eosin was performed through the same optical system with ×40 magnification and integrated into the computer program Image ProPlus. The images of 10 uniformly distributed fields throughout each capsular ring slide were captured, and the existing cores were individually marked by the software count tool, which at the end registered the total number of cells in each studied field (Fig. 4).

Vascularization

The evaluation of capsular vascularization degree was obtained through individual counts of vessels stained with hematoxylin-eosin, visualized with ×40 magnification. Again, the observation was taken by 10 different fields in each slide uniformly distributed throughout the capsular ring. The visual count of the vessels was performed by the same observer, with the tactical assistance of a fragmentation grid in the microscopic field. The numbers obtained were registered in an Excel table.

Parallel Alignment of the Collagen Fibers

Through the polarized light microscopic observation of Picrosirius Red-stained slides, the parallel orientation of the collagen fibers was registered to determine the alignment degree (Figs. 5 and 6). Ten fields on each slide were studied, with ×20 and ×40 magnifications, and each one of these fields received a mark regarding the percentage of the parallel alignment of the fibers based on the personal evaluation of the same observer. The sum of the numbers obtained provided sufficient data for elaboration of the average values for each slide, also expressed as percentage values.

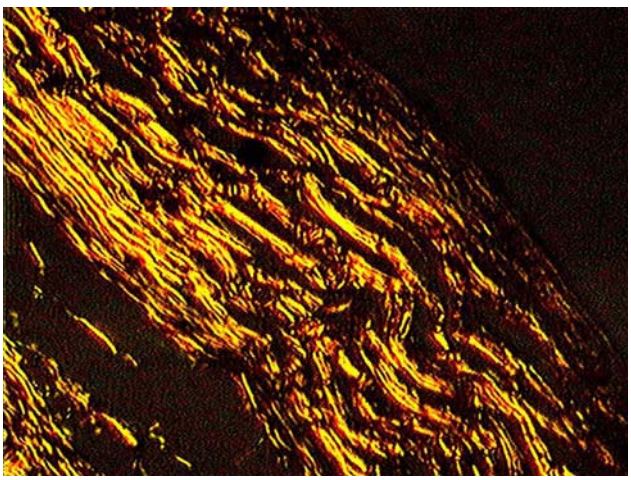

Fig. 5 Picrosirius Red–stained slide showing a high percentage of parallel orientation of the collagen fibers (magnification, ×40)

Fig. 6 Picrosirius Red–stained slide showing a low percentage of parallel orientation of the collagen fibers (magnification, ×40)

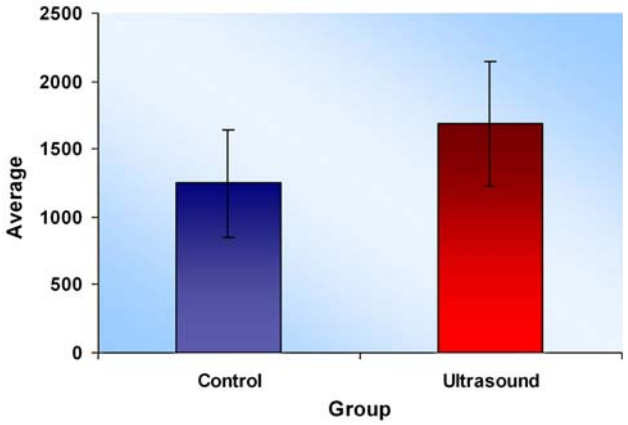

Fig. 7 Average and standard deviation of thicknesses from both groups ($p = 0.003$)

Results

Two rats initially died during the anesthetic procedure and were therefore removed from the experiment. Another animal, from the control group, was found dead on the morning of the 122th day of the experiment for no apparent reason. The surgical healing process evolution was positive in all cases, and no dehiscence, infections, or extrusions of implants were registered. Moreover, no signs of self-mutilation by the rats were observed.

Both groups demonstrated good and similar acceptance of the transducer application, with some levels of individual irritability. Two animals in the ultrasound group presented with second-degree burns in their dorsal region, near the implantation site, which became evident on days 33 and 35. The wounds, approximately 1.5 cm^2 in size, evolved with early formation of a rind in the dorsal region, which was naturally eliminated within 2 weeks, with no need for any specific treatment. The external ultrasound sessions were not discontinued, and total skin recovery was observed.

With regard to histologic tissue, our methods allowed the production of standardized representative ring sections. These covered all the circumferential aspect of the peri-implant capsule, with an insignificant occurrence of artifacts. According to the established protocol, microscopic observations of the capsule attributes were registered as thickness, cellularity, vascularization, and parallel alignment of collagen fibers.

Thickness

The variance ratio testing of the average thicknesses in both groups showed a statistically significant difference ($p = 0{,}003$), proving that thicker capsules developed in the ultrasound group compared to the control group (Fig. 7). There was no such significance when different moments and groups were taken into consideration ($p = 0.979$), indicating that the thickness of the capsules did not vary after the first 30 days of the experiment (Fig. 8).

Cellularity

The analysis of the variation in cell counts highlighted a statistically significant difference ($p = 0.002$), with the ultrasound group showing capsules that had a higher level of cellularity in comparison to the control group (Fig. 9). On the other hand, the evaluation involving averages of different moments and groups showed no statistically significant difference ($p = 0.98$), proving that the cellularity of the capsules did not vary during different periods (Fig. 10).

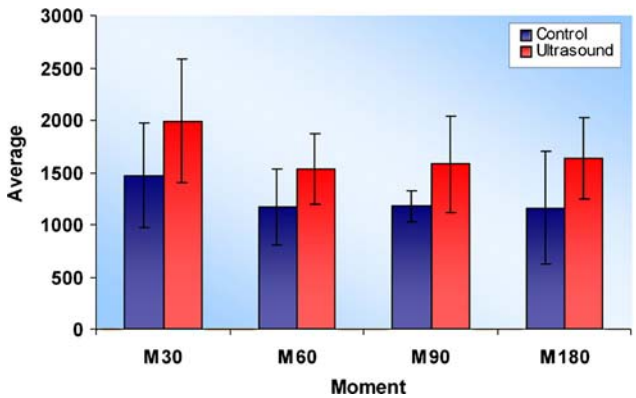

Fig. 8 Average and standard deviation of thicknesses considering groups and moments ($p = 0.979$)

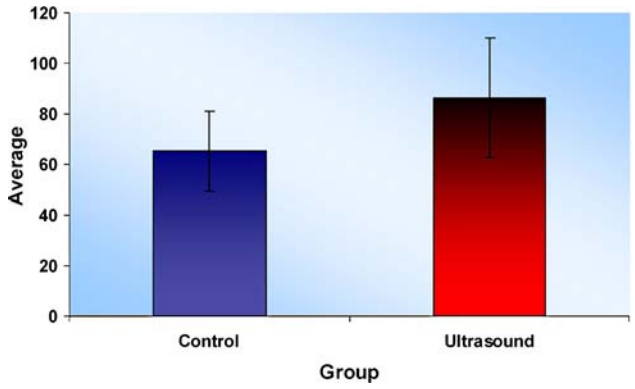

Fig. 9 Average and standard deviation in cell counts in both groups ($p = 0.002$)

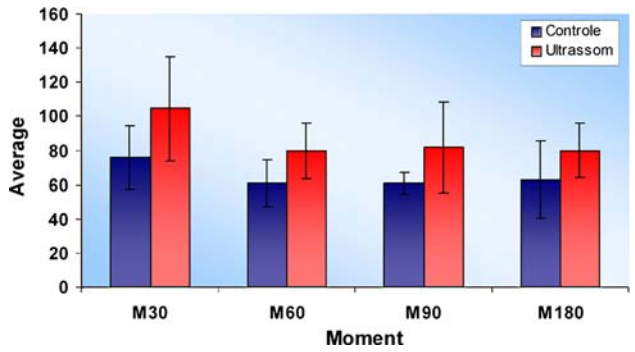

Fig. 10 Average and standard deviation in cell counts considering groups and moments ($p = 0.98$)

Vascularization

The analysis of the variation in vessel counts presented a statistically significant difference ($p < 0.01$), proving that capsules in the ultrasound group were more vascularized than those in the control group (Fig. 11). There was no such significance when comparison of different moments and groups was taken into consideration ($p = 0.98$), indicating that the vascularization of the capsules did not vary throughout the experiment (Fig. 12).

Parallel Alignment of Collagen Fibers

According to Fig. 13, capsules developed in the ultrasound group with a lower parallel alignment of the collagen fibers than in the control group. That is shown through variance ratio test for the standard alignment percentage, which was statistically different in the two groups ($p < 0.001$). On the other hand, the statistical evaluation involving different groups and moments was not significant ($p = 0.98$), showing that the degree of parallel alignment of the collagen fibers in the capsules did not vary throughout the experiment (Fig. 14).

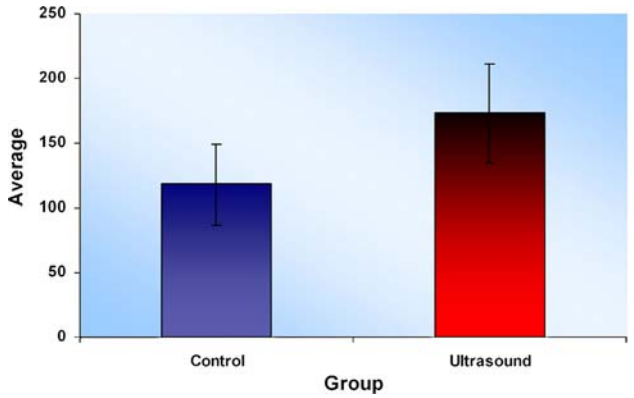

Fig. 11 Average and standard deviation in vessel counts from both groups ($p < 0.01$)

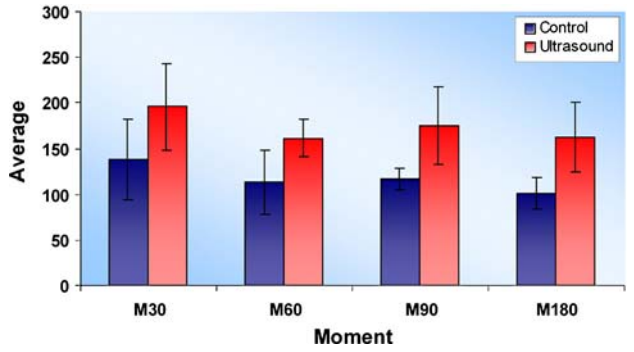

Fig. 12 Average and standard deviation in vessel counts considering groups and moments ($p = 0.98$)

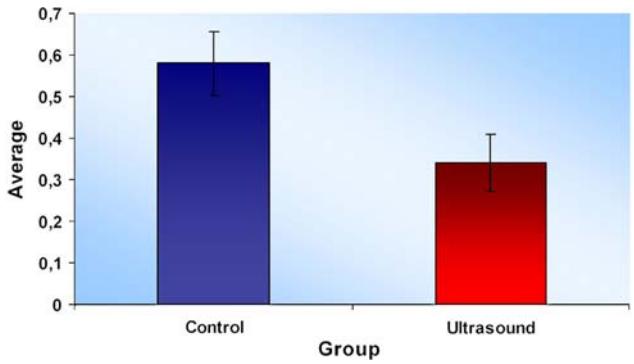

Fig. 13 Average and standard deviation in the percentage of parallel alignment, considering both groups ($p < 0.001$)

Discussion

It was not possible to find any clinical or experimental work in the medical literature that examined the influence of external ultrasound on the structural capsular formation around silicone implants. Herhahn's [2] (1984) and Silversmith's [1] (1984) letters report their personal experience in the treatment of established contractures by the association of the closed capsulotomy procedure and

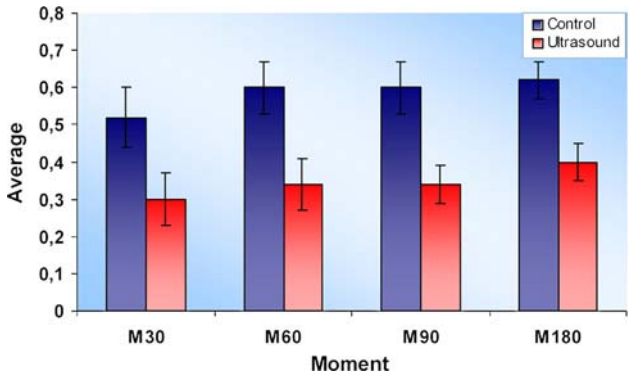

Fig. 14 Average and standard deviation in the percentage of parallel alignment, considering groups and moments ($p = 0.83$)

ultrasound sessions. However, they do not relate a scientific method that can support valid conclusions.

Many years later, Planas et al. [3] published their clinical experimentation with the aforementioned association, presenting some preliminary results in 1997, followed by their complementary observations with 5 years of experience in 2001 [4]. Although their results were encouraging, with about an 83.8% reduction in the contracture amplitude after 1 year of attendance, their methodologic description was not satisfactory with regard to the standardization and isolation of the possible variables in the research model. The lack of a control group submitted to the capsulotomy without the use of ultrasound waves seems to be a methodologic mistake that made it impossible to provide definitive conclusions regarding the obtained results. On the other hand, the publications of Planas et al. [3, 4] were responsible for drawing our attention to the need for experimental research that could prove some preventive effects of external ultrasound on the formation of capsular contracture.

The majority of published studies about the behavior of organic silicone capsules have used rats as the best experimental model [5]. Some authors, using rabbits in their studies, registered several doubts concerning the suitability of this model because its healing process seems to be very different from that of human beings, especially with regard to textured surface implants [6–8]. On the other hand, many studies using rats have found them to be an appropriate model, providing relevant scientific conclusions with accurate histologic extrapolation to the human tissue [5, 9–11].

Male animals were chosen for isolation of ultrasound delivery as the only variable of the model, thus excluding the widely known hormonal instability of female rats. We did not compare two implants in the same animal, one with and one without ultrasound, because their dorsal area was quite small, and it would have been impossible to ensure that ultrasound delivered to one side would not reach the implant on the control side. Our number of animals ($n = 42$) represented the approximate average of similar experiments in the literature, with a variation between 5 [12] and 96 [11].

With regard to the surveillance period, because the rat lifetime is approximately 3 years, 6 months was considered sufficient time to estimate the formation and stability of a peri-implant capsule, comparable with 15 years for a female human patient [13].

The frequency of 3 MHz was adopted to provide a 2.5-cm-deep effective ultrasound field through the skin [14, 15]. Previous reports showed ultrasound intensities varying from 0.1 W/cm^2 (cutaneous scar) [16] to 0.5 W/cm^2 (muscular lesions) [17]. We agree with the conclusions of Speed [18], who considered the detailed information concerning the characteristics of the ultrasonic fields as a fundamental methodologic requirement for validation of such experiments.

In the future, the protocol of ultrasonic emission needs to be tested on the basis of different methodologic approaches to determine the ideal intensities and frequencies for the optimization of the effects [19, 20]. Continuous emission of external ultrasound with a mobile transducer has proved to be superior to the pulsatile mode in fixed transducers. Moreover, some experiments have reported very low rates of skin burns [21–25]. However, it is necessary to highlight the importance of adequate soft and circular movements during the transcutaneous applications [26].

With regard to the histologic preparation, it was fundamental to adopt a method that provided a standardized observation of the entire capsule circumference. It is known that different areas of the same capsule present with different histologic characteristics [27]. Capsular tissue harvesting for histologic studies have failed to describe a representative and proper standardization of the procedure [28–32], and our method has provided it in detail. In relation to histologic observations of the capsular rings, it was noted that the ultrasound group presented with thicker and more vascularized capsules, a larger cellular contingency, and a lower parallel alignment of the collagen fibers than in the control group. All these factors have been described as indicators for a reduced tendency toward capsular contracture because they were observed in textured, as compared with smooth implants [5, 7–11, 33, 34].

Another important observation in the comparison of these histologic parameters is that the groups presented with differences between them. However, there was not a statistically significant difference among the different moments of evaluation, which considered periods of 30, 60, 90, and 180 days. This finding probably indicates early stabilization of histologic changes in the initial phase of capsular formation around the implants.

Further studies involving similar models should help to clarify a possible modulation of the capsular contracture itself, using applanation tonometry, saline infusion pressure, and other methods. A qualitative observation of the cellular population found within the two groups also could be implemented in future studies for a more complete evaluation of the healing process. Other kinds of implants, with textured and polyurethane surfaces, also may have their capsular formation changed by external ultrasound, and therefore should be part of new research projects. The possible consequences of the ultrasound application with regard to the integrity of these implants as well as the presence of bacteria and biofilm formation around the implant also should be considered.

In summary, we believe the findings provided by this study will create a path for future clinical and experimental research, which probably will evaluate and elucidate the real role of external ultrasound in the biologic mediation of capsules around silicone implants, reflecting on the treatment and prevention of capsular contractures.

Conclusion

On the basis of the results obtained with the current method, we conclude that the early and seriate external delivery of ultrasound waves over the smooth silicone implanted area in rats promotes a significant augmentation in the thickness, cellularity, and vascularization of the organic capsule, with evident disarrangement in the deposition pattern of collagen fibers.

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