The papain pocket delivery impairs the capsule fibrous healing around textured silicone implants in rats.

MARCIO MOREIRA
DJALMA JOSÉ FAGUNDES
Surgery Department
Federal University of São Paulo – UNIFESP
R. Botucatu, 740 - Zip Code 04023-900 - São Paulo, SP
BRASIL
marciomoreiradr@brturbo.com.br http://www.marciomoreira.com.br
djfagundes.dcir@epm.br

Abstract: - To study the tissue repair around the textured mammary implants under the action of papain (PA). **Methods:** Thirty-six Wistar rats were evaluated and randomly distributed into two groups (n = 18): papain (PA) and control (CT). Each group was equally distributed into 3 subgroups (n = 6) and observed on seventh, thirtieth-fifth and ninth pos-operative days. Each animal received a textured implant in the left dorso-axillary region (sham - SH), on were instilled 0.5 mL saline solution 0.9%, and another textured implant on the right dorso-axillary region (papain - PA), on were instilled 0.5 mL of water-soluble solution of papain. The control group (CT) received only textured implant in the left dorso-axillary region with prior instillation of 0.5 mL of saline solution 0.9%. The histological analysis of the 3 subgroups was carried out using picrosirius-red stain and an image analyzing system using the Image Pro Plus™ program to evaluate the thickness and maturation and deposition of collagen fibers. Immunohistochemical evaluation was performed, using micrometric reticules of Weiss (Olympus Labstore™), for myofibroblasts counting only in the 90th day subgroup. **Results:** At 35th and 90th days, the papain group (PA) presented reduction on the fibrous capsule thickness around the implant, in the number of collagen fibers and myofibroblasts, comparing to the control group (CT). **Conclusion:** The papain drug decreased the fibrous capsule formation around the textured silicon implants in rats.

Key-Words: - Mammary implant, capsular contracture, inflammation, capsular thickness.

1 Introduction

The capsular contracture after breast implant is the most common adverse effect of this surgical procedure[1]. The severity of capsule contracture is directly related to the degree of the local inflammatory reaction[2] and does not depend on the implant surface employed[3]. Although it is not clear the pathogenesis of capsular contracture, this phenomenon seems multifatorial[4].

Currently, there is no effective preventive measure for capsule contracture[4]. Two conventional treatments may be applied: surgical, by capsulectomy (or capsulotomy) and implant replacement[5], or pharmacological, through steroids, anti-leukotrienes, anti-TGF-B, antibiotics or antiinflamatories[6].

Papain is a thiol endopeptidase plant whose activity is similar to the lysosomal cathepsin B enzyme with fibrinolytic and proteolytic action on the normal healing mechanism[7].

Some authors have speculated on the possible modulator action of certain proteolytic enzymes present around the implants in the early stages of healing[8, 9].

It has been suggested that the papain could be helpful when used locally around the implant at the surgical procedure, promoting tissue repair with less fibrotic tissue, thus avoiding the capsule contracture.

The aim of this study was to investigate the papain effects on the thickness, collagen fibers density and myofibroblasts of fibrous capsule around textured implants in rats.

2 Methods

The experimental protocol (1082-06) was approved by the Ethics Committee of the Federal University of São Paulo - Escola Paulista de Medicina (UNIFESP - EPM).

Thirty-six male Wistar rats weighing 250-300g, kept in individual cages at room temperature with

photoperiod of 12 hours (light / dark) were used, receiving water and food freely.

Anesthesia was given intramuscularly with hydrochloride 2-(2,6-xylidine)-5,6-dihydro-4H-1,3-thiazin (Ronpun ® - Bayer, Germany) and ketamine hydrochloride (Ketalar ® - Parke-Davis, Belgium) in 1:1 ratio, using 1mL.Kg-1. A single dose (60 mg/kg) of cefazolin (Kefazol ™ - Eli Lilly do Brasil Ltda - São Paulo - Brazil) was given intramuscularly as a prophylaxis of infection.

A textured surface silicone implant was used (pore diameters between 0.05 and 0.25 mm), shell-shaped with two centimeters in diameter and volume of 2 mL of silicone gel (Silimed ® Brazil - São Paulo).

Two parallel incisions (1.5 cm) to the left and right of the spine just below the neck were made in eighteen animals. Two bags were set up at 4 cm from the incision under the *panniculus carnosus* muscle where silicone implants were placed.

Before placing the implant, 0.5 mL of saline solution (sham group - SH) was instilled in the left bag. The right bag received a solution of 0.5 mL of 30mg/kg-1 papain (USP 27 ® - Papain Tablets for Topical Solution Papaverine - Vermizym, Germany - Formula & Ação Farmácia Ltda. Brazil) (papain group - PA). Other eighteen animals were subjected to only one incision to the left, bag dissection and instillation of saline solution (0.5 mL), followed by implant insertion (control group - CT) (Figure 1).

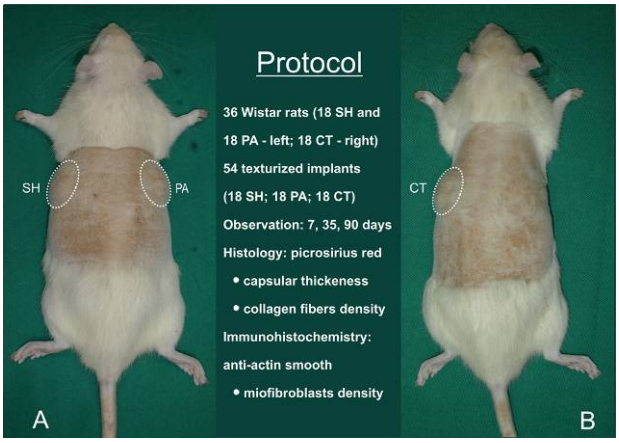

Figure 1 - Schematic drawing of the protocol: rat A - two textured implants - sham group (SH) without drugs; papain group (PA) with drugs, rat B - a textured implant - the control group (CT)

Euthanasia was performed on the 7th, 35th and 90th day, under anesthesia, through an intravenous injection of potassium chloride until the animal's cardiac arrest.

The piece removal was through an incision around the center of the implant (4cm²) (Figure 2).

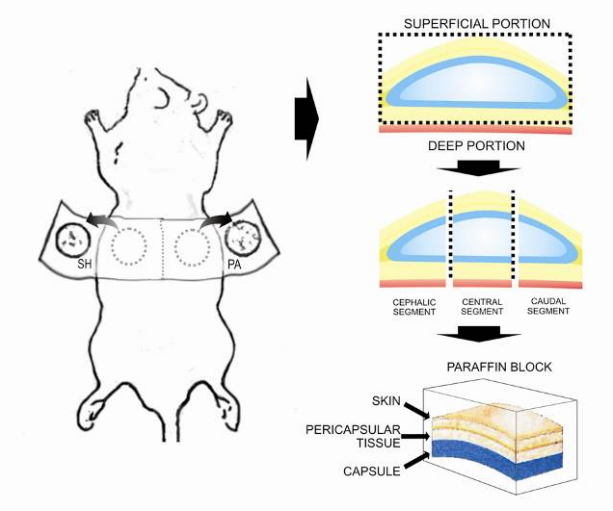

Figure 2. Single block with the implant and capsule around it, removed from the rat after 24 hours. The capsule was dissected from the silicone implants and three sections (cranial, medial and caudal) were sent for histological processing.

The specimens were immersed in 10% buffered formalin. After 24 hours, the capsule around the implant was dissected (Figure 2), cut into three fragments (cranial, medial and caudal), embedded in paraffin and prepared for histological sections of 5μm stained with picrosirius red to measure the capsule thickness (using an increase of 200x) and to calculate the collagen fibers density with polarized light (using an increase of 400x). The software Image Pro Plus ® was used in both cases.

Other sections were prepared for immunohistochemistry for myofibroblasts counting (Dako Cytomation™ - Biogen Inc™ - Cambrigde, MA - USA), using a micrometric reticule of 100 points (Weiss Olympus ® - Labstore ®). Muscles and brown vessels were stained and excluded from the percentage of myofibroblasts population found in the fibrous capsule.

Statistical analysis was performed using factorial variance tests (one-way and two-way). These were carried out to compare the groups means and test whether there was interaction between time versus treatment. Tukey test (SPSS version 11.0) was employed to identify the "two-way" variation within the same group and it was considered significant when less than 5% ($p \leq 0.05$).

3 Results

Papain was effective to decrease the capsule thickness at the 35 and 90 days when compared with the other two groups (control and sham). These

results suggest that the drug had a local action in the healing process (Table 1 and Figures 3 and 4).

Table 1. Values (Mean ± SD) of the capsule thickness (μm), at 7th, 35th and 90th days of observation in control groups (CT), sham (SH) and papain (PA).

	7 days	35 days	90 days
CT	589.97 (±150.89)	745.08 (±156.37)	705.79 (±81.75)
SH	587.69 (±119.28)	553.33 (±119.2)	611.61 (±135.69)¤
PA	464.53 (±20.65)	397.84 (±92.59)§	443.7 (±55,42)¥

7 days: CT = SH = PA
35 days: CT > SH > PA§ ($p \leq 0.0094$) ($p \leq 0.006$)
90 days: CT = SH¤ > PA¥ ($p \leq 0.1389$) ($p \leq 0.0123$)

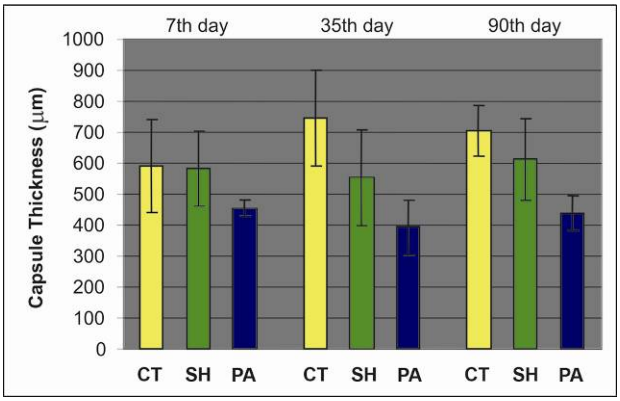

Figure 3. Values (Mean ± SD) of the capsule thickness (μm), at 7th, 35th and 90th days of observation in control groups (CT), sham (SH) and papain (PA).

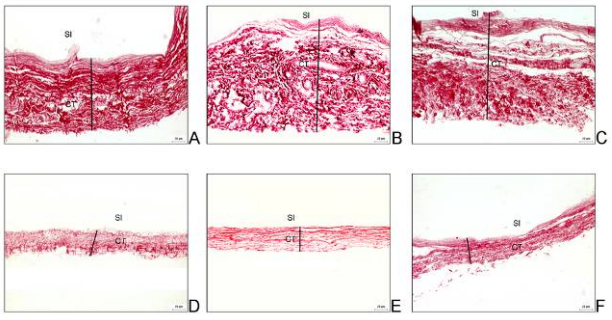

Figure 4. Photomicrography of the capsule thickness: Control group (CT) at the 7 days - 739.79 μm(A), 35 days 898.62 μm (B) and 90 days 809.34 μm (C); Papain (PA) at 7 days 394.40 μm (D), 35 days 337.45 μm (E) and 90 days 377.14 μm (F). (Picrosirius red - 200X). SI = silicone. FC = fibrous capsule.

Papain was effective to decrease the collagen density at 35 days, but also worked in the sham group (SH), which probably suggests a systemic action in the formation of fibrous collagen in healing. (Table 2 and Figures 5 and 6).

Table 2. Values (Mean ± SD) of collagen density (μm^2) at 7, 35 and 90 days of observation in control groups (CT), sham (SH) and papain (PA).

	7 days	35 days	90 days
CT	8884.38 (±4726.81)	7235.4 (±1061.04)	6964.31 (±1242.69)
SH	6969.93 (±3706.6)	5880.95 (±575.28)	8108.43 (±1127.45)¤
PA	6525.97 (±2175.66)	3470.4 (±90.66)§	3594.11(±531.95)¥

7 days: CT = SH = PA ($p \leq 0.3284$) ($p \leq 0.7997$) ($p \leq 0.2141$)
35 days: CT > SH > PA§ ($p \leq 0.0491$) ($p \leq 0.0001$) ($p \leq 0.0001$)
90 days: CT = SH¤ > PA¥ ($p \leq 0.1239$) ($p \leq 0.0001$) ($p \leq 0.0001$)

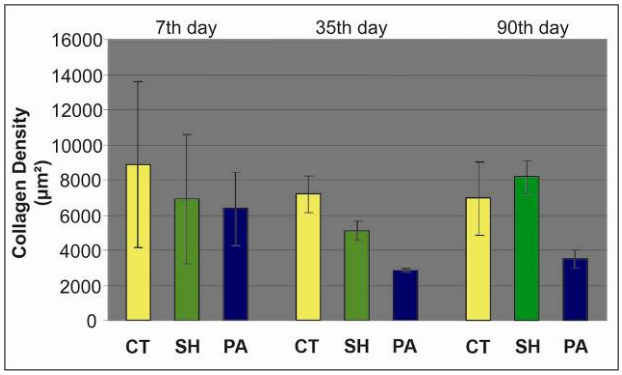

Figure 5. Values (Mean ± SD) of collagen density (μm²) at 7, 35 and 90 days of observation in control groups (CT), sham (SH) and papain (PA).

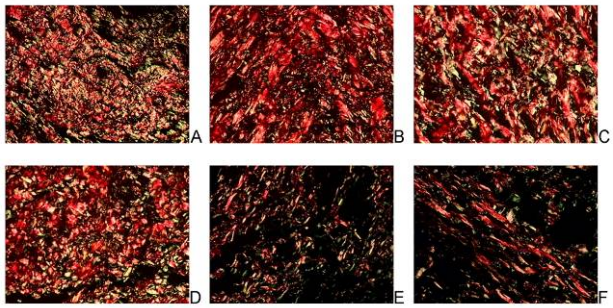

Figure 6. Photomicrography of collagen density: control group (CT) to 7th (A) 7681.17 μm², 35th (B) 6318.67 μm² and 90th day of observation (C) 7856.83 μm²; group papain (PA) at 7th (D) 7173.60 μm², 35th (E) 3301.70 μm²;, and 90th (F) 3510.30 μm². The number of collagen fibers is smaller at the 35th day (B). (Picrosirius red, polarized light - 400X). Images captured on screen (Image-Pro Plus ™ software).

Papain at the 90th day was associated to a lower count of myofibroblasts in the PA group when compared to the control (CT) which could be related to a local effect of the drug. On the other hand, the number of myofibroblasts was significantly lower in the sham group (SH) which indicates a systemic action of drugs (Table 3 and Figures 7 and 8).

Table 3. Values (Mean ± SD) of myofibroblasts (%) after 90 days of observation in control groups (CT), sham (SH) and papain (PA).

	CT	SH	PA
90 days	32,7 (±13.62)	10,13 (±3.19)§	4,28 (±1.66)$^{\square}$

90 days: SH§ < CT > PA$^{\square}$ ($p \leq 0.0001$) ($p \leq 0.0001$) ($p \leq 0.0001$)

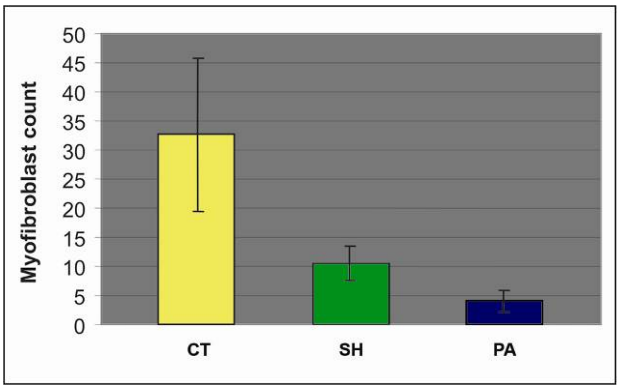

Figure 7. Number of myofibroblasts in a hundred fields (Mean ± SD) after 90 days of observation in control groups (CT), *sham* (SH) and papain (PA).

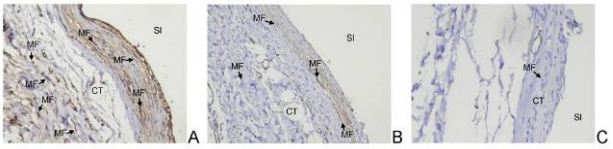

Figure 8. Photomicrography of the myofibroblasts counting at 90 days of observation (Immunohistochemistry - 200X). Control group (CT) (A), sham (SH) (B) and papain (PA) (C).

Animal models have often been used to study the capsule contracture around implants[4-6]. The prolonged and exacerbated tissue reaction has a positive linear correlation with the fibrosis and contraction levels around the implant[2].
The papain properties are known to reduce the swelling, or as an anti-inflammatory, antithrombosis and analgesic as well as its proteolytic action[7].
The drug regulates the function of mast cells by reducing the production capacity of cytokines and growth factors, reducing the inflammatory phase and limiting the proliferation of myofibroblasts and collagen deposition. The excess of mast cells produce growth factors, such as TGF-beta that will finally promote contracture, scar formation and myofibroblasts proliferation. It seems that the papain main mechanism of action is the regulation of IL-6 cytokines which has a key role in the tissue repair and regeneration[7, 8].
Some authors measured the contractile activity of myofibroblasts and demonstrated its relaxation with the use of papaverine (papain) [9.10].
Bastos et al.[5], in an experimental study in rats, obtained a reduction on the capsule thickness and on the collagen density, but not on the number of myofibroblasts after peritoneal injection of zafirlukast.
Batra et al.[11], in an experimental study, found less collagen fibers and myofibroblasts around textured implants after the third month due to the presence of certain factors in the transudate that limits the transformation of fibroblasts into myofibroblasts and deposition of collagen fibers. Wyatt et al.[12], in a histologic study of fibrous capsules in humans, described a natural decrease in the density of collagen fibers around textured implants over time and with the reduction of fibroblasts proliferation due to the presence of fibrinolytic enzymes, which may reduce the local inflammatory process.
Earlier studies of Ajmal et al.[13], with rabbits, with local instillation of 2-mercaptoetane sodium sulphate (Mesna) and Frangou et al.,[14] in mice, with mitomycin C, reduced the capsule thickness, the collagen density and the number of fibroblasts.
Karaçal et al.,[15] using an experimental model in rats, instilled human amniotic fluid containing hyaluronic acid, in the implant bag and obtained a reduction on the pressure and on the capsule thickness after six months.
It is believed that, similar to the study of Karaçal et al., papain may offer a tissue repair with more regeneration and less scar formation. As it was hypothesized, papain promoted a significant reduction on the fibrous capsule thickness at 35th and 90th days, when compared to the control group. It also reduced the amount of collagen fibers at 35th and 90th days and the number of myofibroblasts at 90th days of observation, when compared to the control group.

4 Discussion

Animal models have often been used to study the capsule contracture around implants[4-6]. The prolonged and exacerbated tissue reaction has a

positive linear correlation with the fibrosis and contraction levels around the implant[2].

The papain properties are known to reduce the swelling, or as an anti-inflammatory, antithrombosis and analgesic as well as its proteolytic action[7].

The drug regulates the function of mast cells by reducing the production capacity of cytokines and growth factors, reducing the inflammatory phase and limiting the proliferation of myofibroblasts and collagen deposition. The excess of mast cells produce growth factors, such as TGF-beta that will finally promote contracture, scar formation and myofibroblasts proliferation. It seems that the papain main mechanism of action is the regulation of IL-6 cytokines which has a key role in the tissue repair and regeneration[7, 8].

Some authors measured the contractile activity of myofibroblasts and demonstrated its relaxation with the use of papaverine (papain) [9.10].

Bastos et al.[5], in an experimental study in rats, obtained a reduction on the capsule thickness and on the collagen density, but not on the number of myofibroblasts after peritoneal injection of zafirlukast.

Batra et al.[11], in an experimental study, found less collagen fibers and myofibroblasts around textured implants after the third month due to the presence of certain factors in the transudate that limits the transformation of fibroblasts into myofibroblasts and deposition of collagen fibers. Wyatt et al.[12], in a histologic study of fibrous capsules in humans, described a natural decrease in the density of collagen fibers around textured implants over time and with the reduction of fibroblasts proliferation due to the presence of fibrinolytic enzymes, which may reduce the local inflammatory process.

Earlier studies of Ajmal et al.[13], with rabbits, with local instillation of 2-mercaptoetane sodium sulphate (Mesna) and Frangou et al.,[14] in mice, with mitomycin C, reduced the capsule thickness, the collagen density and the number of fibroblasts.

Karaçal et al.,[15] using an experimental model in rats, instilled human amniotic fluid containing hyaluronic acid, in the implant bag and obtained a reduction on the pressure and on the capsule thickness after six months.

It is believed that, similar to the study of Karaçal et al., papain may offer a tissue repair with more regeneration and less scar formation. As it was hypothesized, papain promoted a significant reduction on the fibrous capsule thickness at 35th and 90th days, when compared to the control group. It also reduced the amount of collagen fibers at 35th and 90th days and the number of myofibroblasts at 90th days of observation, when compared to the control group.

5 Conclusion

The local instillation of a single dose of papain was effective to prevent the formation of fibrous capsule around the textured silicone implants after an evaluation on the capsule thickness, on the collagen fibers density and on the number of myofibroblasts.

References:

[1] Prantl L, Schreml S, Fichtner-Feigl S, Pöppl N, Eisenmann-Klein M, Schwarze H, Füchtmeier B. Clinical and morphological conditions in capsular contracture formed around silicone breast implants. Plast Reconstr Surg. 2007;120(1):275-84.

[2] Siggelkow W, Faridi A, Spiritus K, Klinge D, Rath W, Klosterhalfen B. Histological analysis of silicone breast implant capsules and correlation with capsular contracture. Biomaterials. 2003;24(6):1101-9.

[3] Poeppl N, Schreml S, Lichtenegger F, Lenich A, Eisenmann-Klein M, Prantl L. Does the surface structure of implants have an impact on the formation of a capsular contracture? Aesthetic Plast Surg. 2007;31(2):133-9.

[4] Gancedo M, Ruiz-Corro L, Salazar-Montes A, Rincón AR, Armendáriz-Borunda J. Pirfenidone prevents capsular contracture after mammary implantation. Aesthetic Plast Surg. 2008;32(1):32-40.

[5] Bastos EM, Neto MS, Alves MT, Garcia EB, Santos RA, Heink T, Pereira JB, Ferreira LM. Histologic analysis of zafirlukast's effect on capsule formation around silicone implants. Aesthetic Plast Surg. 2007;31(5):559-65.

[6] Spano A, Palmieri B, Palmizi TT, Nava MB. Reduction of capsular thickness around silicone breasts implants by zafirlukast in rats. Eur Surg Res. 2008;41(1):8-14.

[7] Rose B, Herder C, Löffler H, Meierhoff G, Schloot NC, Walz M, Martin S. Dose-dependent induction of IL-6 by plant-derived proteases in vitro. Clin Exp Immunol. 2006;143(1):85-92.

[8] Desser L, Holomanova D, Zavadova E, Pavelka K, Mohr T, Herbacek I. Oral therapy with proteolytic enzymes decreases excessive TGF-beta levels in human blood. Cancer Chemother Pharmacol. 2001;47**:**S10–S15.

[9] Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971;27(5):549-50.
[10] Baker JL Jr, Chandler ML, LeVier RR. Occurrence and activity of myofibroblasts in human capsular tissue surrounding mammary implants. Plast Reconstr Surg. 1981;68(6):905-12.
[11] Batra M, Bernard S, Picha G. Histologic comparison of breast implant shells with smooth, foam, and pillar microstructuring in a rat model from 1 day to 6 months. Plast Reconstr Surg. 1995;95(2):354-63.
[12] Wyatt LE, Sinow JD, Wollman JS, Sami DA, Miller TA. The influence of time on human breast capsule histology: smooth and textured silicone-surfaced implants. Plast Reconstr Surg. 1998;102(6):1922-31.
[13] Ajmal N, Riordan CL, Cardwell N, Nanney LB, Shack RB. The effectiveness of sodium 2-mercaptoethane sulfonate (Mesna) in reducing capsular formation around implants in a rabbit model. Plastic Reconstr Surg. 2003;112(5):1455-61
[14] Frangou J, Kanellaki M. The effect of local application of mitomycin-C on the development of capsule around silicone implants in the breast: an experimental study in mice. Aesthetic Plast Surg. 2001;25(2):118-28.
[15] Karacal N, Cobanoglu U, Ambarcioglu O, Topal U, Kutlu N. Effect of amniotic fluid on peri-implant capsular formation. Aesthetic Plast Surg. 2005;29(3):174-80.