UNIVERSITÀ DEGLI STUDI DI CATANIA

DIPARTIMENTO DI SCIENZE BIOMEDICHE E BIOTECNOLOGICHE

DOTTORATO DI RICERCA IN *BASIC AND APPLIED BIOMEDICAL SCIENCES*

XXX CICLO

TESI DI DOTTORATO

THE ROLE OF OMEGA 3 FATTY ACIDS IN CAPSULAR CONTRACTURE AROUND THE BREAST IMPLANTS

Dott. Giuseppe A.G. Lombardo

Coordinatore :

Chiar.mo Prof. Ferdinando Nicoletti

Tutor:

Chiar.mo Prof. Luigi Maiolino

Anno accademico 2016-2017

A Serena,

Alla mia Famiglia

INDEX

Introduction

In 2013 more than 360,000 women, in the United States alone, underwent breast implant placement for augmentation mammoplasty or heterologous reconstruction (Segreto 2016).

One of the most common complications of the use of foreign material, both in reconstructive and cosmetic breast surgery, which often leads to re-intervention, is capsular contracture. It consists in pathological hardening of the fibrous shell developing around a breast prosthesis [Escudero 2005, McCoy 1984 Handel 2006, Araco 2009]. The development of connective tissue around any foreign body is physiological, but progression into a thick and firm capsule, as around breast implants, is responsible for varying degrees of local inflammation, dislocation, and deformation of the prosthesis.

Infection, hematoma, and granulomatous response to free silicone, as well as many other conditions, have been considered possible causes of capsules formation and pathological progression around some breast implants [Adams 2009].

As mentioned above, capsular contracture is based on a physiologic foreign body reaction [Anderson 2008]. This process is supposed to protect the human

organism from potentially hazardous materials, but it can turn into an excessive extra-cellular matrix formation with contractile properties [Wick2010]. One of the mechanisms apparently affecting the most capsular formation is the direct immunostimulation through silicone particles and, indirectly, through biofilm formation [Wilflingseder 1983, Tamboto 2010]. Both pathways induce and maintain a chronic inflammatory reaction, which in turn provokes proliferation of fibroblasts.

Thus, this interplay of factors leads to either implant tolerance or progressive peri-implant inflammation and subsequent fibrosis, generating capsular contracture.

In particular, when the implant is not tolerated, synthesis of collagen becomes inadequate, thus causing the fibrotic capsule formation and turning in palpable hardness, visible deformities, and progressive pain by immuring nerves and vessels [Anderson 2008, Wick 2010].

Nowadays, the only sufficient treatment to overcome capsular contracture is surgical revision, with dissection of the capsular tissues and silicone implant replace, or, eventually, conversion in autologous breast reconstruction. [Collis 2000, Gurunluoglu 2013, Young 2008].

Historically, researches on capsular contracture focused mainly on prevention of the excessive fibrosis using antibiotic and reducing bacteria contamination, identifying the principle pathogenic cause on subclinical infection. [Bartsich 2013, Thorton 1988, Burkhardt 1981, Deva 1999, Mah 2001, Mah 2003, Borriello 2004Fux 2004, Fux 2005, Deva 2013, Leid 2002, Jesaitis 2003, Tamboto2010, Paikos, 2003, Netscher 2004].

Only secondary studies were focused on pharmacological control of the inflammation process.

Considering the impossibility to use systemic steroids to prevent capsular contracture in first attempts, the colleagues tried to irrigate the implant pocket using steroids [Peterson 1974]. Successively, in an experimental model, it was used a liposome-delivered prednisolone which resulted in a capsular contracture reduction. [Moreira 2010]

Considering the same pathway, the arachidonic acid cascade, a leukotriene antagonist (Zafirlukast), was used for the first time in 2002 in contracted breast, leading to the fibrosis decreasing [Schlesinger 2002]. These empirical findings were supported by numerous experimental studies. [Spano, Bastos 2007].

An important role in the arachidonic acid cascade is also played by the omega-3 fatty acids.

The omega-3 polyunsaturated fatty acids (n-3 PUFAs), eicosapentaenoic acid and docosahexaenoic acid, are found mainly in oily fish and commercially available supplements, which are available either over the counter (as fish oils) or as concentrated pharmaceutical preparations. Such supplements are now becoming increasingly popular, since several health benefits have been attributed to them.[Saravan 2010].

Increased consumption of marine n-3 PUFAs results in their dose-dependent incorporation into cell phospholipids, thus taking the place of arachidonic acid. A decrease in arachidonic-acid content turns in a decreased amount of substrate available for synthesis of the classic pro-inflammatory eicosanoids.

According to this finding, an increased intake of n-3 PUFA in animals and human beings has been reported to decrease production of a large range of pro-inflammatory eicosanoids [Rees 2006, Calder 2009]

To our knowledge, there is no study in literature which has examined the effect of dietary supplement of omega-3 fatty acids on the fibrosis forming around silicone breast implants.

Thus, the goal of the present thesis is to investigate the effects of omega-3 supplements on capsule contraction, evaluating the thickness, and TGF-B expression on sample of capsule collected in a rat living model.

Materials and Methods

Animal Care

32 Wild-type C57BL/6 knockout mice were bred and maintained in a university vivarium. Mice were operated on at 8 weeks of age and following implant surgery were housed one animal per cage. We divided the mice in two group. The group omega 3 received daily by gavage omega-3 oil (EnerZona Omega 3 RX Enervit Italia) 300mg/Kg (0,002 gr EPA+0,001 gr DHA). The control group received daily by gavage water.

The Institutional Animal Care and Use Committee approved all experiments in this study.

Rodent Surgery

On day 0, the mice were implanted bilaterally with custom-made, 300-mg, silicone gel implants (Mentor Corp., Santa Barbara, Calif.)(Fig.1).

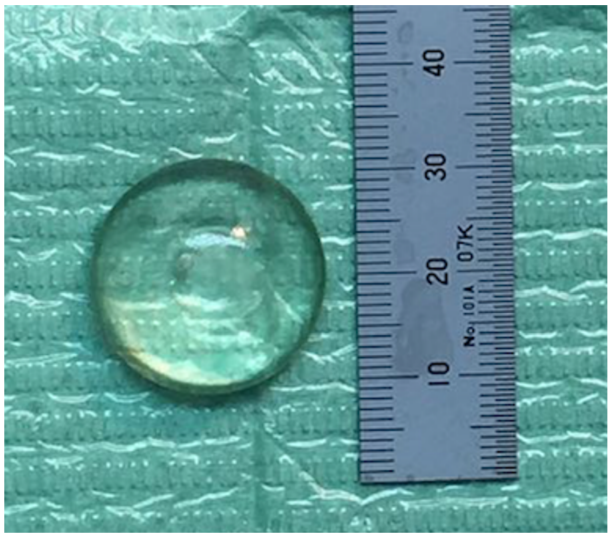

Fig.1 Custom made implant designed for rodent surgery

Mice were anesthetized using Altadol (5 mg/kg) and Avertin (400 mg/kg). The surgical site was then shaved and prepared with an iodine solution. A 2-cm transverse incision was performed on the dorsal aspect of the mouse at the level of the sacral spine. One tunnel was then dissected to the level of the rib cage, and the prosthesis were placed over the ribs in a pocket beneath both the skin and the thin *panniculus carnosus* muscle- fascial layer . The incision was then closed with interrupted sutures using 6-0 nylon (Ethicon, Inc., Somerville, N.J.).(Fig.2)

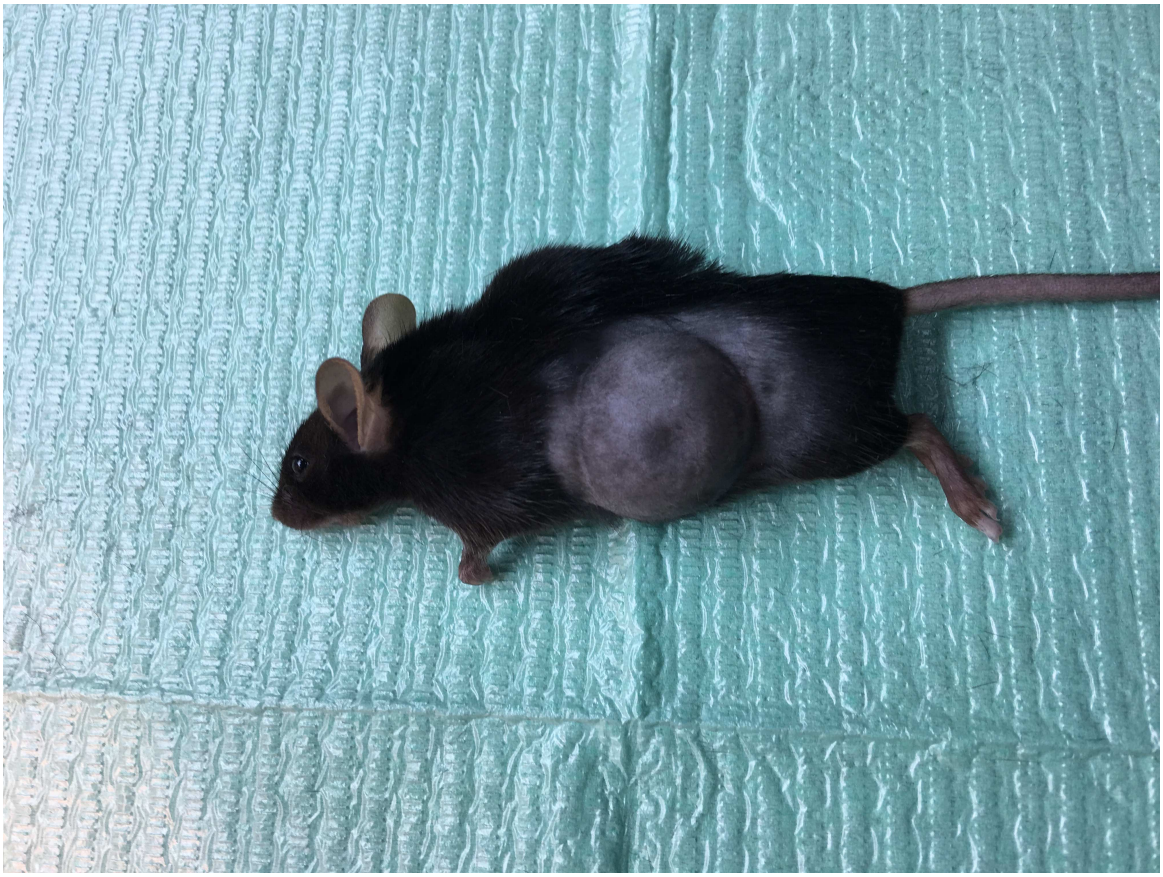

Fig.2 The implant was inserted under the panniculus carnosus layer after the shaving of the skin.

After 12 weeks, mice were euthanized using a carbon dioxide inhalation and implant and all tissues surrounding implants were harvested in one piece. (Fig. 3)

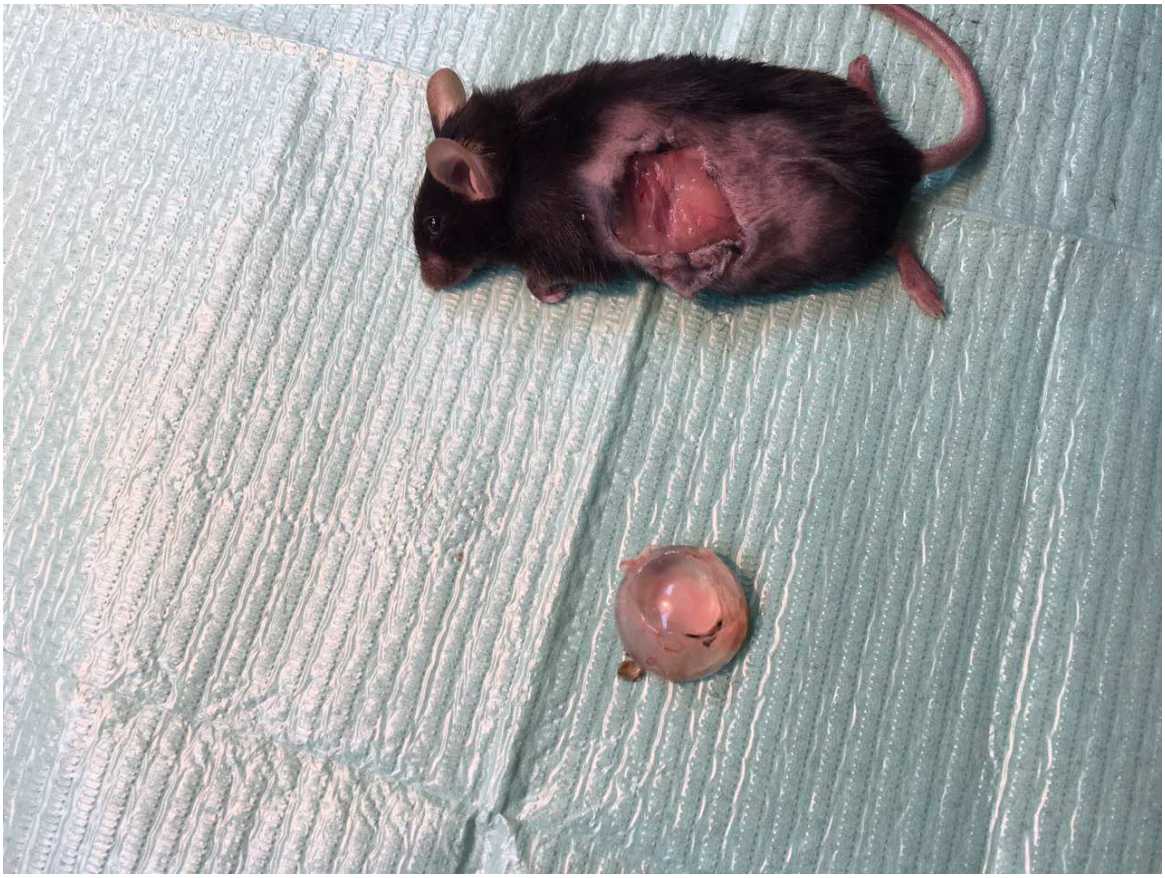

Fig.3 The mice was euthanized and the implant and the capsule was harvested in one piece

Histology

Capsular tissue was harvested on postoperative day 84. Harvested tissues were then fixed in 10% neutral buffered formalin for 36 hours. Fixed tissues were dehydrated in a gradient of alcohols and embedded in paraffin blocks. Serial, longitudinal, 3 µm, paraffin-embedded sections were prepared and stained with hematoxylin and eosin. Capsule thicknesses were measured by two authors independently (L.S and G.L) using the digital slide scanner Aperio ScanScope SC (Aperio Technologies, U.S.A.). Each author performed five

measurements per specimen, and the mean of the 10 measurements was recorded as the capsule thickness for that specimen. Both authors were blinded to treatment of the samples.

Real-time PCR analysis

At the end of the experimental period, the animals were sacrificed, the capsule tissue was excised (approximately 25 mg) and stored in RNAlater (Invitrogen) at 4°C until further processing. Total RNA was extracted using the RNeasy Mini Kit (Quiagen) according to the manufacturer's protocol. Briefly, specimens were first dissected in RLT buffer using surgical scalpels, then homogenized by passing them through a blunt 20-gauge needle fitted to an RNase-free syringe. One volume of 70% ethanol was added to the homogenates, and subsequently the samples were centrifuged for 15 s at≥8000 x g in a Rneasy spin column. Columns were then washed with the RWI and RPE buffers. Finally, 20 µl of Rnase-free water were added to the spin column and centrifuged for 1 min at≥8000 x g to elute the RNA. The concentration of RNA for each sample was determined by measuring the absorbance at 260nm (A_{260}) in a spectrophotometer. The A_{260}/A_{280} ratio was above 1.8 for each sample. Reverse transcription reactions were performed using retro-transcription reagents from

the High Capacity RNA-to-CDNA kit (Thermo Fisher). The quantification of gene products was performed by real-time PCR using LightCycler 480 SYBR green I master mix (Roche, Indianapolis, IN). Real-time PCR for TGF-beta2, COL2A1 and Beta-actin (used as an endogenous control) analyses were carried out using the following primers:

TGF-beta2 Forward: TCGACATGGATCAGTTTATGCG

TGF-beta2 Reverse: CCCTGGTACTGTTGTAGATGGA

COL2A1 Forward: GGTGAGCCTGGTCAAACGG

COL2A1 Reverse: ACTGTGTCCTTTCACGCCTTT

Beta-actin Forward: CATCATGAAGTGTGACGTTGAC

Beta-actin Reverse: GCATCCTGTCAGCAATGCC.

Gene expression levels were evaluated according to the formula:

$2^{-\Delta Ct}$

where ΔCt = Ct (target gene) - Ct(Beta-actin) (Livak KJ et al. 2001)

Statistical analysis

Differences between vehicle and Omega3-treated samples were analyzed by unpaired two-tailed Student's t test, with <0.05 being considered statistically significant.

Results

Gross Findings

There were no gross signs of infection or inflammation of the surrounding capsular tissue. The capsules in the omega 3 group were thinner and more transparent than those in the control group.

Hystology evaluation

The average capsular thickness was 205.09 µm in the omega 3 group, compared with 361.63 µm in the control group. This difference was statistically significant (P = 0.0004) (FIG.4-5)

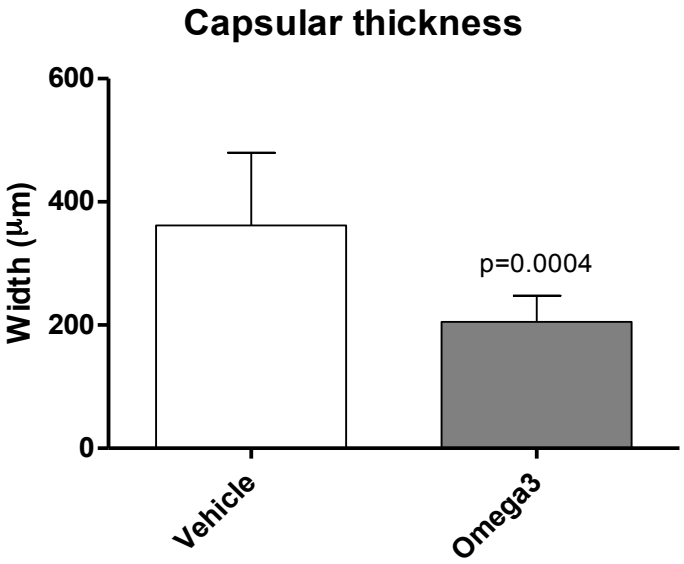

Fig.4 The capsular thickness was significantly lower in the omega 3 group. (p=0.0004)

Real-time PCR analysis evaluation

The effect of omega 3 on the gene expression of TGF-β2 and COL1A2 in capsules was examined by real-time quantitative PCR. The TGF-β2 gene expression was higher in the control group than in the omega 3 group (p=0,048); COL1A2 gene expression was higher too in the control group than in the omega 3 group (p=0,039). Based on these observations, TGF-β2 and COL1A2 gene expression decreased significantly in the omega 3 group compared to the control group (Fig.6).

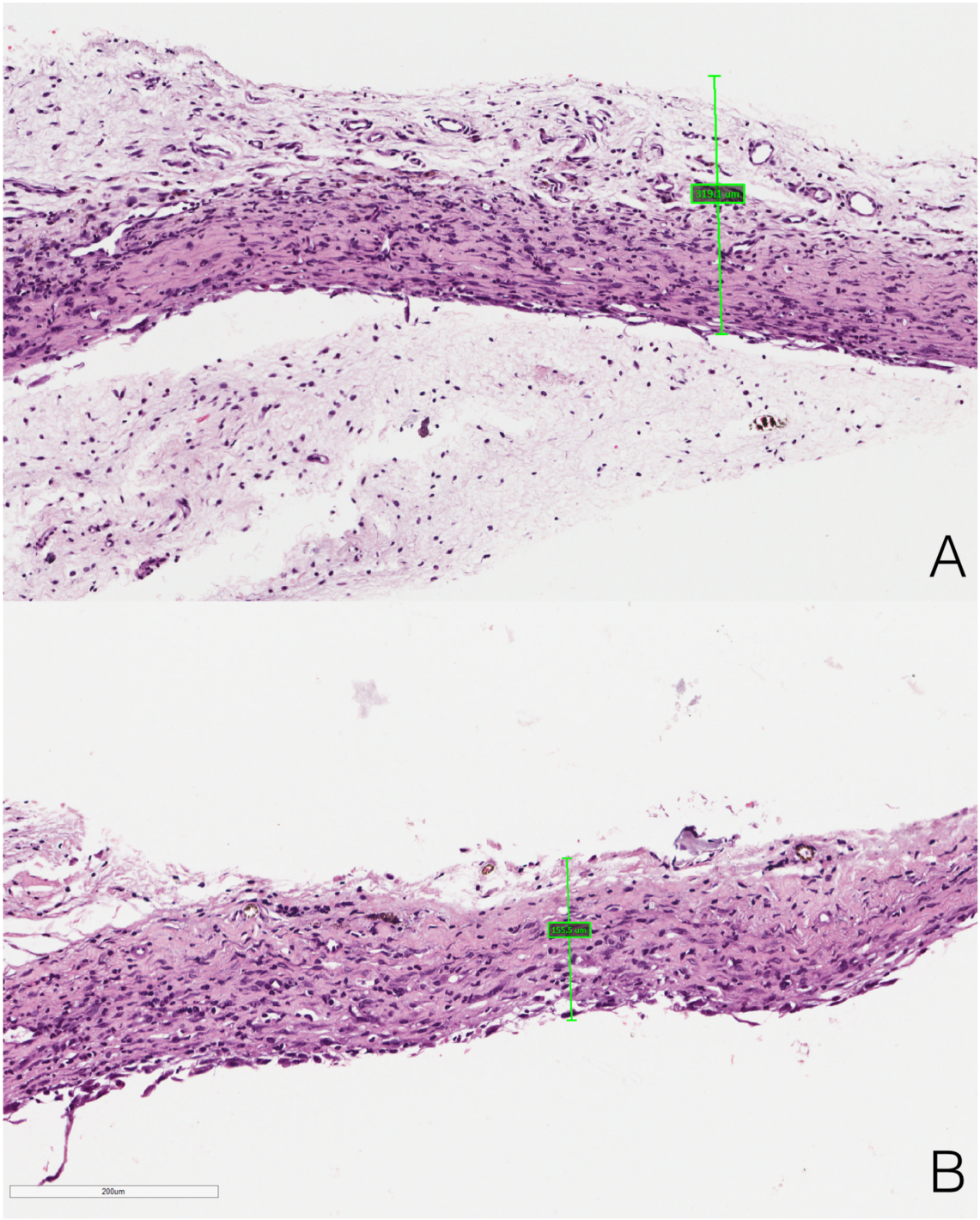

Fig 5. The capsular thickness was thinner under magnification. the omega 3 group shows less fibrosis with less deposition of collagen and less vascularization.

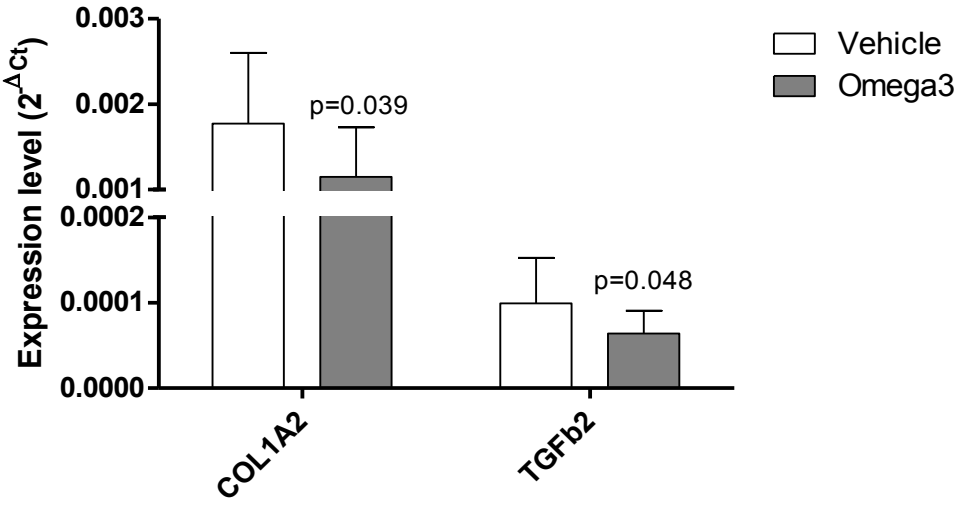

Fig.6 TGF-β2 and COL1A2 gene expression decreased significantly in the omega 3 group compared to the control group.

Discussion

Causes of the Capsular Contracture.

In the last forty years, capsular contracture has been the most common complication in aesthetic and reconstructive breast surgery.

This pathologic process occurs in response to breast implants and it is one of the most common causes of reoperation following implantation [Adams 2006, Spear 2003, McLaughlin JK 2007].

A certain amount of connective tissue physiologically develops around any foreign body, but its progression into a thick and firm capsule, as it usually occurs around breast implants, is responsible for varying degrees of local

inflammation, dislocation, and deformation of the prosthesis. This inevitably affects the surgeon's efforts, who attempts to achieve both aesthetically pleasing and good functional results. [Marangi 2010]

Several hypotheses have been forwarded to explain why capsules form and why they pathologically progress around some breast implants. There are not definitive evidences concerning the causes of capsular contracture. Current understanding of capsular contracture can be viewed as a balance of multiple related factors that affect peri-prosthetic inflammation. The authors define these factors as potentiators and suppressors. [Adams 2009]. (FIG.)

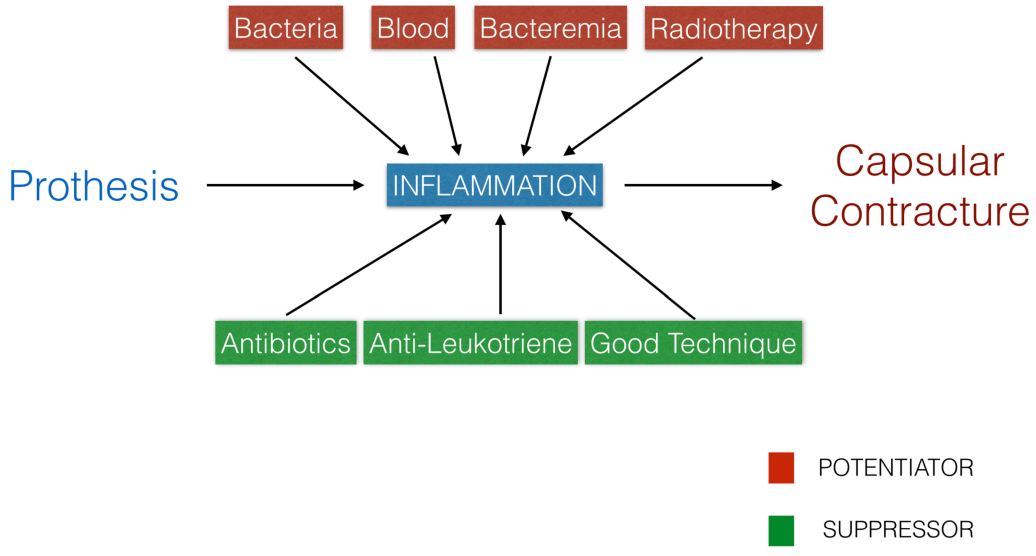

Fig 7. Etiology of capsular contracture. The relative influence of potentiating and suppressing factors influencing formation of capsular contracture.

Historically the authors correctly guessed that the cause of capsular contracture was a continuative and excessive inflammation around the prosthesis. In 1974 Peterson et al. showed how 60 mg of triamcinalone diacetate placed in the pocket of a silicone gel implant resulted in a softer augmented breast compared to the contralateral one used as control; since then, many surgeons recurred to corticosteroids.[Peterson 1974, Perrin 1976].

Nevertheless, later on this procedure was shown to be quite useless in managing long-term complications and it was thus abandoned. [Price 1976, Cohen 1980].

Successively Shan et al. defined the first theories about the correlation between capsular contracture and bacteria contamination [Shan 1981], evolving after few years in the concept of subclinical infection [Burkhardt 1985].

Detection of this type of infection may be difficult: Microorganisms are "hidden." Bacteria easily adhere to the implant silicone and produce extracellular polysaccharides and glycoprotein, which form a slime layer. Protected by this biofilm, they stay in a dormant, viable state without multiplying. They may not be readily accessible to nutrients from culture media or to treatment with antibiotics. Indeed, the persistent nature of foreign body

infections is due to the ability of bacteria to adhere to biomaterial surfaces and to develop biofilms. [Virden 1992]

The subclinical infection was proven in vivo using a pig living model, which allowed the authors to successfully demonstrate their hypothesis of subclinical infection as a cause of capsular contracture following augmentation mammaplasty. Although they did not assume that subclinical infection is the only cause of capsular contracture, eliminating the risk of infection may prevent or reduce its incidence [Tamboto 2010]. Besides, the same group studied, on a porcine living model, the biofilm adherence on the implant surfaces comparing textured and smooth implants; although textured implants may confer better tissue ingrowth, they also have been shown to potentiate the early and rapid formation of S. epidermidis biofilm in vivo and in vitro compared with smooth implants. Thus, they highlighted the need of a stringent intraoperative attention in case of use of textured implants, in order to prevent bacterial contamination and to reduce the risk of biofilm formation with subsequent capsular contracture [Jacombs2014].

Although in vivo models seem to demonstrate a pathophysiological correlation between subclinical infection and capsular contracture, the levels of evidences of these studies are low and some authors published controversial results.

Indeed, *Marangi et al* reported data which do not support any correlation between subclinical infection and degree of capsular contracture. In their opinion, capsular contracture must be considered a multifactorial disorder, comprising known, unknown and, as mentioned, not clearly defined risk factors.

According to their results, seems to be a determinant factor in promoting staphylococcal infection. Although subclinical infection alone does not, in their opinion, appear to determine capsular contracture, under certain local and systemic conditions favouring bacterial growth, such as radiotherapy, subclinical infections seem to have a negative effect, at a later stage.

Prevention.

Capsular contracture's prevention is historically based on two essential points:

1. Bacteria contamination reduction during implants insertion.

2. Inflammation reduction.

Considering the evidences about the correlation between capsular contracture and subclinical infection, many authors proposed treatments aimed at reducing bacteria contamination, during the preoperative, intraoperative and

postoperative time. For instance, Wixtrom et al used Tegaderm (3 M, Two Harbors, MN) nipple shield to reduce implant contamination from endogenous breast flora [Wixtrom RN 2012]. In spite of that, after a 6 months follow-up, three of the 32 patients developed CC.

Breast pocket irrigation with antibiotics and/or anti- bacterial agents has been practiced and recommended for many years. Since polymicrobial infections are usually implied with CC, finding the optimal broad- spectrum irrigation remains unsettled. In 2000, Adams et al conducted an in vitro study comparing the breast pocket irrigations which are most commonly used.[Adams 2000] At a lower concentration, betadine, gentamicin, and cefazolin solutions were 100% effective against bacteria. In 2000, due to concerns that betadine-caused implant deflation, the US Food and Drug Administration banned immersion of breast implants in betadine solution. This prompted testing for alternative broad-spectrum solutions. Adams et al reported use of bacitracin, cefazolin, and gentamicin solution.[Adams 2001]. After a mean follow-up of 14 months (range 6 to 75 months), incidence of CC was 4 to 5-fold less for breast augmentation compared to manufacturer pre-market approval data. [Adams 2001].

Prophylactic intravenous (IV) antibiotics have also been studied. *Arad et al* conducted an animal study using rats and IV vancomycin.[Arad 2013] Although this treatment showed a quite good efficacy against immature biofilms and soft-tissue infection, it had limited results against mature biofilm. Clinically, preoperative antibiotics have also been evaluated in prevention of CC. [Gylbert 1990] After a 12-month follow-up, there were no statistical differences between control and antibiotic groups regarding prevalence of CC (47% and 53%, respectively), and the biofilm formation was not assessed. These data suggest that local treatment with antibiotic irrigation is more effective in prevention of bacterial colonization and initial biofilm formation compared to systemic perioperative antibiotics. Additionally, irrigation may decrease selection of antibiotic-resistant bacteria compared to IV prophylaxis.

Jacombs et al used a porcine model to examine the effectiveness of antibiotic impregnated mesh in the prevention of biofilm formation and CC. Researchers implanted a total of 28 prostheses into 5 pigs. All 28 implants and their pockets were inoculated with S. epidermidis isolated from a human patient with CC. Fourteen implants were inserted with antibiotic mesh (treatment) and the other 14 were untreated (control). All untreated implants developed Baker grade III/IV CC. In contrast, all treated implants were Grade I/II after 16 weeks, ($P < 0.001$). Specimens with CC had at least 10-fold higher bacterial counts.

Bacterial colonization of mesh-covered implants was typically single-layered, if present. In contrast, multilayered biofilms were detected by electronic microscope in all untreated implants. [Jacombs 2012] This study highlights that prevention of biofilm formation in its early stage using antibiotic coated implants, rather than treating biofilm related infections, would be more desirable in clinical settings. However, due to the rise of antibiotic resistance, additional studies and approaches are needed.[Chen 2013, van Heerden2009]

Moyer et al conducted a cadaver study to assess the amount of skin contact, and thus skin and breast parenchyma contamination, with standard implantation compared to delivery via the Keller Funnel (Keller Medical Inc., Stuart, FL).[Moyer 2012] The funnel is composed of rip-stop nylon and a hydrophilic inner coating and is designed to facilitate implant placement without skin contact. Bacterial transfer from the breast parenchyma to implant surface with the funnel appeared to be 37.5%, while it raised up to 62.5% with the standard implantation technique. No long-term data regarding CC could be determined, considering that it was a cadaver study.

Surgical technique may also affect implant contamination with microorganisms. A retrospective study by Wiener demonstrated the effect of the incision on the development of CC in over 400 patients. Patients who had an inframammary

incision had a 0.59% incidence of CC compared with 9.5% incidence reached in patients where a peri-nipple-areolar incision had been performed. The explication could be that peri-nipple-areola approach implies ducts transection near the nipple, which is an area particularly reach in bacteria. These ducts can continue to release bacteria until the healing process is over. Thus this approach potentially increases bacterial contamination and biofilm formation. Contrariwise, the inframammary incision resides in a deeper plane, hence with a minor risk of exposure to endogenous bacteria.[Wiener 2017]

Implant location also seems to impact CC development, since a higher incidence of CC was reported when implants had been placed in the subglandular vs subpectoral plane.[Stevens 2013, Namnoum 2013] As mentioned before, this is also likely due to proximity to bacteria harboured in mammary ducts.

Adams postulated that this contamination and chronic subclinical infection finally leads to a perpetual inflammation, fibrosis and thus it clinically manifests as a capsular contracture.[Adams 2009]

Although many researches on capsular contracture have been carried out, it appears clear that inflammation reduction around the prosthesis remains a major concern. This is true especially in patients who underwent breast

reconstruction following cancer treatment, considering that systemic therapy with steroids or other anti-inflammatory drugs are forbidden for oncological issues.

In 1974 Peterson et al. attempted to irrigate the pocket with steroids solution [Peterson 1974]. Nevertheless, this procedure was successively abandoned for an increased complications rate. [Cohen 1980] Besides, the steroid solution is absorbed so rapidly that the role of one bolus of prednisone into the pocket appears to be quite poor. For this concern, Moreira et al proposed a liposome-delivered prednisolone device inserted into the pocket. They actually showed how a local depot of liposomal prednisolone was effective in decreasing fibrous capsule thickness around textured silicone breast implants.[Moreira 2010].

In addition, leukotriene inhibitors were used to attempt the inflammation reduction. As well known, leukotrienes are derivatives of arachidonic acid, physiologically produced by the 5-lipoxygenase enzyme in response to allergens or other stimulus that result in cell activation. Their action leads to inflammation, smooth muscle cells contraction [Gryskiewicz 2003], increased neutrophils and influx of lymphocytes through promotion of increased vascular permeability.[Riccioni 1997] This inflammatory condition plays an important

role in the pathophysiology of asthma, and it seems to be also implicated in capsular contracture pathogenesis.[Reid 2005]

Several studies reported specific leukotriene receptor antagonists, such as zafirlukast and montelukast, as a possible option for capsular contracture prevention and/or treatment. [Adams 2009] Indeed, they inhibit the cysteinyl leukotrienes (leukotriene C 4, leukotriene D 4, and leukotriene E 4), and they presumably also suppress the myofibroblasts contraction. Thus, it is the alteration of the inflammatory cascade which should lead to prevention of the severe fibrotic reaction associated with capsular contracture.[Scuderi 2006]

Both zafirlukast and montelukast appear to effectively reverse capsular contracture in a time-related manner. Reid et al. demonstrated how zafirlukast led to a decrease in capsular contracture of 75.7% at 3 months, 81.1 % at 6 months and 82.9% at 16.9 months follow-up, with no relevant side effects. [Reid 2005]

Similarly, Huang and Handel obtained promising results in the prevention and treatment of capsular contracture with the administration of montelukast. Over 17 patients reporting capsular contracture following a different breast implantation procedures, 11% worsened, 16% had no change, 26% improved, and 37% achieved complete resolution. [Huang 2010]

Zafirlukast is orally administered, twice a day, and it undergoes to liver metabolism, causing a CYP450 inhibition at therapeutic concentrations. Some possible side effects can be reported, such as headache (12.9 %), nausea (3.1 %), and, rarely, liver disease. [Scuderi 2006] Since elevation of alanine transaminase and aspartate transaminase was not significant compared with placebo,[Riccioni 1997] the liver biochemistry monitoring is not routinely indicated. However, Gryskiewicz [Gryskiewicz 2003] collected data on this concern from November 1997 to October 2002 which revealed 66 cases of hepatitis or liver failure in patients who received normal doses of this drug (13 of these patients were not taking any other medication). Of those patients, two required liver transplantation, 23 died (including eight patients who did not use any other medication), and 12 deaths were preceded by liver failure.[Gryskiewicz 2003] In contrast to zafirlukast, the indicated dose of montelukast is 10 mg, once a day. The most common adverse effects of montelukast are headache, flu, abdominal pain, cough, and dyspepsia. Similarly to zafirlukast, elevation of liver enzymes alanine transaminase and aspartate transaminase didn't show to be significant, since it was similar to what was found in the placebo group. Moreover, therapeutic doses of montelukast do not lead to cytochrome P450 inhibition. [Riccioni 2007] It is noteworthy, however, that some cases of montelukast-induced hepatitis have been reported.

Furthermore, moderate to severe asthma, eosinophilia, pulmonary infiltrations, cardiomyopathy, and other signs of vasculitis (Churg-Strauss syndrome) may be developed in many patients treated with either of these leukotriene receptor antagonists.[Guilpain 2007].

In conclusion, in consideration of the possible significant adverse effects (mostly hepatotoxicity-related) related to these drugs, and due to the lack of confirmed scientific basis demonstrating their efficacy, the off-label use of these medications, particularly Accolate,a leukotriene inhibitor, is not recommended [Adams 2009].

Other non surgical options have been considered in past studies, such as the use, in capsular contracture of breast cancer patients, of pentoxifylline (Trental) and vitamin E. Vitamin E seemed to induce a regression of the radiation-induced fibrosis, through its capacity of collagen inhibition and antioxidant properties. [Delanian 2007] However, vitamin E alone didn't show to induce any significant variation neither in SOMA scores (a radio-induced damage skin score) nor in decreasing the radiation-induced fibrosis surface, compared with their placebo counterparts. [Delanian 2003]

Vitamin E was proposed to help reverse radiation-induced fibrosis because of its antioxidant properties [Delanian 2007] and inhibition of collagen,

transforming growth factor (TGF)-beta1, and fibronectin mRNA production.[Chojkier 1998 Hamama 2012]

Patients treated with vitamin E alone did not have significantly different SOMA scores or mean radiation-induced fibrosis surface area when compared with their placebo counterparts. [Delanian 2003]

Since the previous studies showed Trental's efficiency in decreasing fibrosis through its anti-TNF-a, anti-inflammatory properties, and the ability to increase vasodilation and erythrocyte flexibility, it was proposed as well as a possible treatment in capsular contraction.

Nevertheless, according to a randomized study comparing Trental plus placebo vs double placebo, no difference in mean radiation-induced fibrosis surface area, mean volume regression, or SOMA scores was identified. [Hamama 2012] On the other hand, several studies reported a successful reduction in radiation-induced fibrosis using a combination between vitamin E and Trental. [Chiao 2005, Haddad 2005, Hamama 2012 Delanian 2003].

Many authors have proposed several therapies to achieve the inflammation decrease, but without finding a definitive and unique evidence based therapy to follow.

The Role of Omega 3 in Inflammation.

The beneficial effects of n-3 polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are well-known since 1960s, when some epidemiological evidences underlined how populations with a diet particularly rich in n-3 PUFA reported a lower incidence of myocardial infarction [Mozaffarian 2011].

The role of N-3 PUFAs in inflammation reduction could be relevant at several steps of inflammatory pathways, for instance decreasing the eicosanoids levels in plasma and urine (i.e. leukotrienes), as well as other biomarkers, such as interleukin-1b (IL-1b), IL-6, and tumor necrosis factor-alpha (TNF-alpha). This could be achieved either acting as second messengers or altering the transcription of specific genes involved in inflammation (Wall 2010).

Moreover, n-3 PUFAs have a notable capacity in increasing prostaglandins and other molecules with local anti-inflammatory effects, like resolvins, protectins, and other mediators (Mozaffarian & Wu, 2011). The beneficial effects of omega-3 PUFAs are commonly attributed to the prevention of conversion of the omega-6 PUFA arachidonic acid (AA) into pro-inflammatory prostaglandins (PGs) and leukotrienes (LTs), and, on the other side, its role as an alternative

substrate, producing weaker mediators, such as 3-series PGs, thromboxanes (TXs) and 5-series LTs.

Besides, the omega-3 PUFAs have several anti-thrombotic roles, reducing the formation of the pro-thrombotic prostanoid TXA2 from AA, being metabolized to PGI3, which possesses anti-platelet effects, and to TXA3, which impairs platelet aggregation [Dyerberg1978]

Different pro-resolving mediators derived from omega-3 PUFA, such as resolvins, protectins, and maresins, have been identified through liquid chromatography–mass spectrometry (LC–MS)-based lipidomic analyses of murine inflammatory exudates or activated cell supernatants. [Serhan 2014] They apply their anti-inflammatory effects in a stereospecific manner thanks to their distinct chemical structures. In literature, authors usually refer to these mediators with the term “specialized pro-resolving mediators” (SPMs). Particularly, Resolvin E originates from EPA through the conversion of 18-hydroxyeicosapentaenoic acid (18-HEPE) operated by aspirin-acetylated COX2 or CYP450 monooxygenase. RvE1 main role is to switch off leukocyte migration to the inflamed site, to promote the clearance of inflammatory cells and debris, and to suppress cytokine production, thereby leading to resolution of acute inflammation [Serhan 2014]. In addition, RvE1 can achieve anti-thrombotic

function, inhibiting platelet aggregation by ADP or TXA2 receptor activation [Serhan 2014].

DHA-derived mediators, such as protectins, resolvin D-series, and maresins, are generated by 15-LOX in humans or by 12/15- LOX in mice. Serhan et al. showed how PD1 exhibits has a protective role against brain ischemia, retinal oxidative injury, and against renal ischemia/reperfusion injury [Serhan 2014]. Similarly, RvD1 has protective effects in various disease models, such as insulin resistance, atherosclerosis, ischemia reperfusion, and others [Serhan 2014]. In addition, the same authors reported a reduction in damage, and subsequent scarring of kidneys, after ischemia/reperfusion RvD2-mediated.

Both PD1 and RvD1 reduce pathogenic neovascularization in murine oxygen-induced retinopathy. Furthermore, administration of PD1 isomer (PDX) improved insulin sensitivity in obese diabetic db/db mice, as showed in a recent paper, suggesting a possible therapeutic activity of this SPM. [Serhan 2014]

In contrast with these findings, Some authors reported an increased synthesis of proinflammatory eicosanoids derived from AA, such as prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, and a decreased synthesis of anti-inflammatory eicosanoids from EPA and DHA, following high intake of dietary n-6 PUFAs. [Simopoulos, 2008]

Additional studies on animal models accurately described these phenomena, reporting an increased expression of genes involved in lipid metabolism, proteins and obesity-linked pro-inflammatory cytokines, related to a low n-3:n-6 PUFAs ratio. [Duan 2014; Heerwagen 2013; Liu 2013]

EPA and DHA inhibit the NF-kB signalling either by blocking IkB phosphorylation [Zhao 2014], or through the nuclear receptor PPARa/g [Gani 2008], thus down-regulating the expression of several inflammation-related genes

In addition, omega-3 PUFA exert different roles as a ligand of the GPR120, a member of the GPCR family, highly expressed on macrophages and mature adipocytes. Through this pathways, omega-3 PUFA attenuate both toll-like receptor 4- and TNF-a-mediated pro-inflammatory signalling in macrophages, and augment GLUT4 glucose transporter expression to promote glucose uptake in mature adipocytes [Oh 2010]. Indeed, as widely known, chronic tissue inflammation can cause insulin resistance. In a mouse obesity model, it has been demonstrated that anti-inflammatory and insulin-sensitizing effects of omega-3 PUFAs were related to GPR120 expression, and that insulin resistance improved through administration of a selective agonist [Oh 2014].

It has been shown how pyrin domain-containing 3 (NLRP3), belonging to NOD-like receptor family, senses non-microbial danger signals and forms an

inflammasome, trigging a sterile inflammatory response in myocardial infarction, ischemia reperfusion injury, and pressure overload-induced cardiac remodelling. Omega-3 PUFAs enabled NLRP3 inflammasome-dependent inflammation and metabolic disorder in HFD-induced diabetic mice [Yan 2013]. Moreover, Chen et al reported a direct activity of EPA and DHA on cardiac fibrosis reduction under pressure overload, by inhibiting TGF-b1-induced smad2/3 nuclear translocation, a major pathway involved in the development of cardiac remodelling [Chen 2013].Indeed, EPA and DHA were able to increase nitric oxide production, thus promoting activation of the cyclic GMP/PKG pathway in cardiac fibroblasts. According to all these findings, omega-3 PUFAs may have both anti-inflammatory and anti-fibrotic properties.

Omega 3 and Capsular Contracture, Our Results and future perspectives

How can Omega 3 reduce the fibrosis around the prosthesis? Most of the studies about Omega 3 fatty acids concern heart disease, since epidemiological evidence, in the late 1960s, highlighted how the Inuit population, characterized by a diet rich in n-3 PUFA, had a lower incidence of myocardial infarction [Mozaffarian 2011].

The numerous studies on myocardial infarction allowed us to know the several pathways implied by PUFA's prevention of arachidonic acid conversion into pro-inflammatory eicosanoids, acting as an alternative substrate for cyclooxygenase or lipoxygenase or decreasing the fibrosis process through numerous cascades [Endo 2015].

As Adams postulated, inflammation is the final pathway prior to capsular contracture. So, chronic inflammation apparently finally leads to fibrosis. If on one hand anti-inflammatory action of Omega 3 fatty acids is nowadays widely known, their action on the fibrosis process is yet based on new evidences, mainly discovered studying the cardiac remodelling following a heart attack. Indeed -3 PUFAs suppress cardiac fibroblast proliferation, transformation, and collagen production, thus leading to inhibition of the cardiac fibrotic response and prevention of cardiac dysfunction progression

These anti-fibrotic effects are exerted through the cGMP/PKG pathway activation, which blocks the TGF-1–stimulated nuclear translocation of phosphorylated Smads.[Chen 2011]

In a recent study Kim et al demonstrated that botulinum toxin type A suppress TGF-β1 signalling, thus inhibits the syntheses of collagen types 1 and 3 and activates matrix metalloproteinases, finally leading to prevention of capsule formation around silicone implants. Consequently, the authors supposed that botulinum toxin type A could help in reducing capsular formation and that TGF-β1 signalling is an important target of capsule formation induced by silicone implants.[Kim 2016]

When considering the deposition of collagen and fibrosis that occur during foreign body response, TGF-β plays definitely a relevant role in this process [Kondo 2001]. Shah et al. demonstrated that by suppressing the TGF-β expression, hypertrophic scars or keloid were reduced [Shah 1994]. Based on the results of the present study, TGF-β2 levels were significantly reduced in the area surrounding the prosthesis, in the experimental group. This decrease reflected the lower incidence of capsular formation. Therefore, targeting the TGF-β pathway might be effective for controlling collagen synthesis and capsular formation.

In our findings the level of COL1-A2 in the capsule was significantly lower.

Furthermore the capsule showed a minor thickness n the treatment group, with a mean value of x, compared to the control group where thickness measured in average X.

In the present study, we attempted to demonstrate the efficiency of Omega 3 supplement in preventing capsular contracture occurrence. To increase the clinical relevance of this work, we resorted to a miniaturized version of the prosthesis that was specifically designed using the current protocol for breast augmentation and breast reconstruction. Results were evaluated at 8 weeks after surgery, according to previous experimental studies that have shown how capsules around breast implants are typically evident within 4 to 6 weeks after implantation [Adams 2006].

This study's results suggest that Omega 3 supplement seems to be effective in reducing capsular formation occurrence.

The main role in Omega 3 fatty acids in inflammation and fibrosis reduction around the implant is their inhibition of TGF-β pathway and thus impairment of collagen deposit.

We believe that omega 3 supplement is a simple and promising method that could be used to prevent capsular contracture after silicone implant surgery.

This therapy could have a high impact considering the number of patients who every year undergoes to breast reconstruction or to aesthetic mammoplasty.

Furthermore Omega fatty acids 3 are dietary supplement with minimal side effects, normally used worldwide for multiple purposes.

Nevertheless, further clinical studies are warranted to examine their therapeutic applicability and additional studies should be conducted to attempt a decrease in capsular contracture occurrence.

Bibliography

Adams WP Jr, Rios JL, Smith SJ. Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study. Plast Reconstr Surg 2006;117:30.

Adams WP Jr., Conner WC, Barton FE Jr., Rohrich RJ. Optimizing breast pocket irrigation: an in vitro study and clinical implications. Plast Reconstr Surg. 2000;105 (1):334-338. discussion 339-343

Adams WP Jr., Conner WC, Barton FE Jr., Rohrich RJ. Optimizing breast-pocket irrigation: the post-betadine era. Plast Reconstr Surg. 2001;107(6):1596-1601

Adams WPJ. Capsular contracture: what is it? What causes it? How can it be prevented and managed? Clin Plast Surg 2009;36(1):119–26.

Anderson JM, Rodriguez A, Chang DT. Foreign body reac- tion to biomaterials. Semin Immunol. 2008;20:86–100.

Araco A, Caruso R, Araco F, Overton J, Gravante G. Capsular contractures: A systematic review. Plast Reconstr Surg. 2009;124:1808–1819.

Arad E, Navon-Venezia S, Gur E, et al. Novel rat model of methicillin-resistant Staphylococcus aureus-infected silicone breast implants: a study of biofilm pathogenesis. Plast Reconstr Surg. 2013;131(2):205-214

Bartsich S, Ascherman JA, Whittier S, et al. The breast: a clean-contaminated surgical site. Aesthet Surg J 2011;31(7):802–6.

Borriello G, Werner E, Roe F, et al. Oxygen limitation contributes to antibiotic tolerance of Pseudomonas aeruginosa in biofilms. Antimicrob Agents Chemother 2004;48(7):2659–64.

Burkhardt B: Fibrous capsular contracture around breast implants: the role of subclinical infection. Infect Surg 4:469, 1985

Burkhardt BR, Fried M, Schnur PL, et al. Capsules, infection, and intraluminal antibiotics. Plast Reconstr Surg 1981;68(1):43–9.

Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie 2009; 91: 791–95.

Chen M, Yu Q, Sun H. Novel strategies for the preven- tion and treatment of biofilm related infections. Int J Mol Sci 2013;14(9):18488-18501.

Chiao TB, Lee AJ. Role of pentoxifylline and vitamin E in attenuation of radiation-induced fibrosis. Ann Pharmacother 2005;39: 516–22.

Chojkier M, Houglum K, Lee KS, et al. Long- and short-term D-alpha- tocopherol supplementation inhibits liver collagen alpha1(I) gene expression. Am J Physiol 1998;275:G1480–5.

Cohen 1980 Capsular Contracture and Steroid-related Complications in Augmentation Mammoplasty

Collis N, Sharpe DT. Recurrence of subglandular breast implant capsular contracture: Anterior versus total capsulec- tomy. Plast Reconstr Surg. 2000;106:792–797

Delanian S, Lefaix JL. The radiation-induced fibroatrophic process: therapeutic perspective via the antioxidant pathway. Radiother Oncol 2004;73:119–31

Delanian S, Porcher R, Balla-Mekias S, et al. Randomized, placebo- controlled trial of combined pentoxifylline and tocopherol for regres- sion of superficial radiation-induced fibrosis. J Clin Oncol 2003;21: 2545–50.

Deva AK, Adams WP Jr, Vickery K. The role of bac- terial biofilms in device-associated infection. Plast Reconstr Surg 2013;132(5):1319–28.

Deva AK, Chang IC. Bacterial biofilms: a cause for accelerated capsular contracture? Aesthet Surg J 1999;19(2):130–3.

Escudero FJ, Guarch R, Lozano JA. Tissue reaction to breast prostheses. Periprosthetic capsular contracture. An Sist Sanit Navar 2005;28(suppl 2):41–53.

Fux CA, Costerton JW, Stewart PS, et al. Survival strategies of infectious biofilms. Trends Microbiol 2005;13(1):34–40.

Fux CA, Wilson S, Stoodley P. Detachment charac- teristics and oxacillin resistance of Staphyloccocus aureus biofilm emboli in an in vitro catheter infection model. J Bacteriol 2004;186(14):4486–91.

Gryskiewicz JM. Investigation of accolate and singulair for treatment of capsular contracture yields safety concerns. Aesthet Surg J. 2003;23:98–101

Guilpain P, Pagnoux C, Lhote F, Mouthon L, Guillevin L. Antileukotrienes and Churg-Strauss syndrome (in French). Presse Med. 2007;36:890–894

Gurunluoglu R, Sacak B, Arton J. Outcomes analysis of patients undergoing autoaugmentation after breast implant removal. Plast Reconstr Surg. 2013;132:304–315.

Gylbert L, Asplund O, Berggren A, Jurell G, Ransjo U, Ostrup L. Preoperative antibiotics and capsular contracture in augmentation mammaplasty. Plast Reconstr Surg. 1990;86(2):260-267. discussion 268-269

Haddad P, Kalaghchi B, Amouzegar-Hashemi F. Pentoxifylline and vitamin E combination for superficial radiation-induced fibrosis: a phase II clinical trial. Radiother Oncol 2005;77:324–6.

Hamama S, Gilbert-Sirieix M, Vozenin MC, et al. Radiation-induced enteropathy: molecular basis of pentoxifylline-vitamin E anti-fibrotic effect involved TGF-b1 cascade inhibition. Radiother Oncol 2012; 105:305–12.

Handel N, Cordray T, Gutierrez J, Jensen JA. A long-term study of outcomes, complications, and patient satisfaction with breast implants. Plast Reconstr Surg. 2006;117:757–767; discussion 768.

Huang CK, Handel N. Effects of Singulair (montelu- kast) treatment for capsular contracture. Aesthet Surg J. 2010;30:404–408

Jacombs A, Allan J, Hu H, et al. Prevention of biofilm- induced capsular contracture with antibiotic-impregnated mesh in a porcine model. Aesthet Surg J. 2012;32(7): 886-891

Jesaitis AJ, Franklin MJ, Berglund D, et al. Compro- mised host defense on Pseudomonas aeruginosa biofilms: characterization of neutrophil and biofilm interactions. J Immunol 2003;171(8):4329–39.

Leid JG, Shirtliff ME, Costerton JW, et al. Human leu- kocytes adhere to, penetrate, and respond to Staph- ylococcus aureus biofilms. Infect Immun 2002; 70(11):6339–45.

Mah TF, O'Toole GA. Mechanisms of biofilm resis- tance to antimicrobial agents. Trends Microbiol 2001;9(1):34–9.

Mah TF, Pitts B, Pellock B, et al. A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resis- tance. Nature 2003;426(6964):306–10.

McCoy BJ, Person P, Cohen IK. Collagen production and types in fibrous capsules around breast implant. Plast Reconstr Surg 1984;73:924–7.

McLaughlin JK, Lipworth L, Murphy DK, et al. The safety of silicone gel-filled breast implants: a review of the epidemiologic evidence. Ann Plast Surg 2007; 59:569.

Moyer HR, Ghazi B, Saunders N, Losken A. Contami- nation in smooth gel breast implant placement: testing a funnel versus digital insertion technique in a cadaver model. Aesthet Surg J. 2012;32(2):194-199

Namnoum JD, Largent J, Kaplan HM, Oefelein MG, Brown MH. Primary breast augmentation clinical trial outcomes stratified by surgical incision, anatomical placement and implant device type. J Plast Reconstr Aesthet Surg. 2013;66(9):1165-1172.

Netscher DT. Subclinical infection as a possible cause of significant breast capsules. Plast Reconstr Surg 2004;113(7):2229–30.

Pajkos A, Deva AK, Vickery K, et al. Detection of sub- clinical infection in significant breast implant cap- sules. Plast Reconstr Surg 2003;111(5):1605–11.

Perrin ER: The use of soluble steroids within inflatable breast prostheses. Plast Reconstr Surg 57:163, 1976

Peterson HD, Burr GB: The role of steroids in prevention of circumferential capsular scarring in augmentation mammoplasty. Plast Reconstr Surg 54:28, 1974

Price RIM: Failure of steroid instillation to prevent capsular contracture after augmentation mammoplasty. Plast Reconstr Surg 57:371, 1976

Rees D, Miles EA, Banerjee T. Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men, Am J Clin Nutr 2006; 83: 331–42.

Reid RR, Greve SD, Casas LA. The effect of zafirlukast (Accolate) on early capsular contracture in the primary aug- mentation patient: A pilot study. Aesthet Surg J. 2005;25:26–30.

Riccioni G, Bucciarelli T, Mancini B, Di Ilio C, D'Orazio N. Antileukotriene drugs: Clinical application, effectiveness and safety. Curr Med Chem. 2007;14:1966–1977

Schlesinger SL, Ellenbogen R, Desvigne MN, Svehlak S, Heck R: Zafirlukast (Accolate): A new treatment of a difficult problem. Aesth Surg J 22:329336, 2002

ScuderiN,MazzocchiM,FioramontiP,BistoniG.Theeffects of zafirlukast on capsular contracture: Preliminary report. Aesthetic Plast Surg. 2006;30:513–520

Spear SL, Low M, Ducic I. Revision augmentation mastopexy: indications, operations, and outcomes. Ann Plast Surg 2003;51:540.

Stevens WG, Nahabedian MY, Calobrace MB, Harrington JL, Capizzi PJ, Cohen R, d'Incelli RC, Beckstrand M. Factor analysis for capsular contracture: a 5-year

Sientra study analysis using round, smooth, and textured implants for breast augmentation. Plast Reconstr Surg. 2013;132(5):1115-1123.

Tamboto H, Vickery K, Deva AK. Subclinical (biofilm) infection causes capsular contracture in a porcine model following augmentation mammaplasty. Plast Reconstr Surg 2010;126(3):835–42.

Thornton JW, Argenta LC, McClatchey KD, et al. Studies on the endogenous flora of the human breast. Ann Plast Surg 1988;20(1):39–42.

van Heerden J, Turner M, Hoffmann D, Moolman J. Antimicrobial coating agents: can biofilm formation on a breast implant be prevented? J Plast Reconstr Aesthet Surg. 2009;62(5):610-617.

Wick G, Backovic A, Rabensteiner E, Plank N, Schwentner C, Sgonc R. The immunology of fibrosis: Innate and adaptive responses. Trends Immunol. 2010;31:110–119

Wiener TC. Relationship of incision choice to capsular contracture. Aesthetic Plast Surg. 2008;32(2):303-306.

Wixtrom RN, Stutman RL, Burke RM, Mahoney AK, Codner MA. Risk of breast implant bacterial contamination from endogenous breast flora, prevention with nipple shields, and implications for biofilm formation. Aesthet Surg J. 2012;32(8):956-963

Young VL. Guidelines and indications for breast implant capsulectomy. Plast Reconstr Surg. 1998;102:884–891; discussion 892–894.