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ORIGINAL ARTICLE

Breast Implant Texturization Does Not Affect the Crosstalk Between MSC and ALCL Cells

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Abstract— In the last decade, there has been a growing interest about the possible association between anaplastic large cell lymphoma (ALCL) and breast implants (BIA-ALCL). Many variables, such as breast implants texturization, have been investigated. Breast implants often lead to the formation of a periprosthetic capsule, characterized by inflammation. The presence of the inflamed capsule has been found in the majority of patients with BIA-ALCL. Inflammation may be sustained or counteracted by mesenchymal stem cells (MSCs) by the secretion of pro- or anti-inflammatory cytokines. MSCs were isolated from three capsules surrounding micro-textured (micro-MSCs) and from three capsules surrounding macro-textured (macro-MSCs) implants; after characterization, MSCs were co-cultured with KI-JK cells (a cell line derived from the cutaneous form of ALCL). The secretion of cytokines related to inflammation, the proliferation rate, and the expression of genes referred to pro-tumoral mechanisms were evaluated. Co-cultures of KI-JK cells with micro- or macro-MSCs gave the same results about the secretion of cytokines (increase of IL10, G-CSF, and TGF-β1 and decrease of IL4, IL5, IL12, IL13, IL17A, IFN-γ ($p < 0.05$) with respect to mock sample), expression of selected genes (increase for ACVR1, VEGF, TGF-βR2, CXCL12, and MKi67 ($p < 0.05$) with respect to control sample), and the proliferation rate (no variation between mock and co-cultured samples). Our results suggest that MSCs derived from capsules surrounding micro- and macro-textured implants display the same effects on the ALCL cells.

KEY WORDS: breast implants; mesenchymal stem cells; inflammation; texturization; ALCL.

Monia Orciani and Miriam Caffarini contributed equally to this work.

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INTRODUCTION

Breast implants have been often associated to anaplastic large cell lymphoma onset (Breast Implants Associated ALCL, BIA-ALCL); despite the large amount of literature about this, especially systemic reviews, no firm conclusions have been reached [1–6].

With the aim to answer to this issue, many variables have been investigated: age of the patients, presence of seroma and/or palpable mass, years between implants positioning and ALCL development [7] and the presence of chronic infection on the biofilm around breast implants [8]. Among others, an increasing interest has been devoted to the formation of the periprosthetic fibrotic capsule that acts as a

source of inflammation that is in turn implicated in the onset of many tumors; in particular, the presence of capsules was found in the majority of patients with BIA-ALCL [9]. Capsular contracture is the most common complication following implant-based breast surgery with an overall incidence of 10.6% [10]. It is a condition with multifactorial origins: filler material, surgical methods, infection of mammary implants with bacterial biofilm, and implant surface texture are risk factors in its development [10, 11]. In detail, the results of systematic reviews indicate that the risk of capsular contracture is lower with silicone than with saline filler material and with a submuscular than subglandular placement of the implant [10].

The role of the type of texturization in capsular formation has been already investigated with different results: some researchers found that women with textured implants displayed a lower risk of contracture than women with smooth implants while others reported a higher degree of inflammation for capsules around textured implants [10].

In addition, the type of texturization of the breast implants deserves attention since the different levels of porosity have been associated with different risk of the onset of BIA-ALCL but, as stated by the FDA update of 2017, details on breast implant surface are limited [12].

During inflammation, a plethora of cytokines is secreted by different cells [13]; among the others, mesenchymal stem cells (MSCs) organize and superintend inflammation and immune system [14–16]. The ability of MSCs in secreting factors is now well established and has been the subject of an entire field of research named immunobiology of MSCs [17]. Previous works reported as MSCs can differently affect the tumor fate: in some models, MSCs enhance tumor development while in others, they counteract tumor survival. This apparent contradiction depends on the different tumor model, the different source of MSCs, and other still unknown aspects [18]. In previous works [19–21], we isolated and characterized MSCs from periprosthetic capsules, with evident markers of inflammation (inflamed-MSCs, I-MSCs) and from the contralateral breast (control-MSCs, C-MSCs); subsequently, the immunobiology of C- and I-MSCs was evaluated and we found that I-MSCs secrete higher amounts of cytokines related to chronic inflammation than C-MSCs. Finally, C- and I-MSCs were co-cultured with an ALCL cell line and the analysis of the secreted cytokines was repeated. The release of some cytokines was changed after co-cultures.

Here, we deepened our research on the role of texturization. Considering that in Italy, the use of smooth implants is very limited and the sample collection is very hard, MSCs were isolated from periprosthetic capsules of three women with Mentor implants (micro-textured) and from three women with Allergan implants (macro-textured). RT-PCR, ELISA, and cell proliferation experiments were performed before and after co-cultures with the ALCL cell line to evaluate if texturization affects the degree of inflammation and if in turn, this influences the MSCs immunobiology and the ALCL cell fate.

MATERIALS AND METHODS

Sample Collection

In this study, approved by Università Politecnica delle Marche Ethical Committee and conducted in accordance with the Declaration of Helsinki, six women, undergoing implant replacement and capsulectomy due to capsular contracture, were enrolled. The collection of periprosthetic capsular tissue was performed at the Departments of Plastic and Reconstructive Surgery of Università Politecnica delle Marche, Ancona, and of Università degli Studi di Udine. Three patients had Mentor Siltex® implants (Mentor Corporation, Santa Barbara, Calif), characterized by nodules with an approximate height of between 40 and 100 μm and a diameter of 50–150 μm (micro-texturization).

The other three patients had Natrelle® implants (Allergan Medical Corporation, Santa Barbara, Calif), with a surface pitted with cuboid-shaped wells with a depth of 100–200 μm and a diameter of approximately 200 to 500 μm (macro-texturization).

All patients underwent implant replacement and capsulectomy due to Baker grade III capsular contracture, and all of them reported a history of mastectomy and immediate implant-based breast reconstruction (Table 1).

The following inclusion criteria were used for patient selection: patients who underwent breast implant placement at least 10 years earlier were enrolled, as well as women with Baker grade III capsular contracture. Patients who received radiotherapy were excluded from the current study, as well as women with implant rupture, implant exposure/infection, or smooth/polyurethane implants. Data regarding implant shape (round/anatomical), volume, and projection were not considered as exclusion criteria, as well as the reason of implant placement (esthetic/reconstructive purposes) and the implant pocket placement (subglandular/submuscular). The presence of periprosthetic seroma was not considered as exclusion criteria as well.

Inflammation at the capsular site was confirmed by the presence of focal calcification, pus corpuscles, and fibrin and by the expression of CD43 (known as a marker of

Table 1. Clinical Details of the Enrolled Patients

Patients	Type of implant		Biofilm infection	Implant placement	Implant rupture	Date of implant
1	Mentor Siltex®	Silicone	No	Submuscular	No	10 years
2	Mentor Siltex®	Silicone	No	Submuscular	No	15 years
3	Mentor Siltex®	Silicone	No	Submuscular	No	12 years
4	Natrelle®	Silicone	No	Submuscular	No	10 years
5	Natrelle®	Silicone	No	Submuscular	No	11 years
6	Natrelle®	Silicone	No	Submuscular	No	10 years

inflammation), tested by immunohistochemistry (Fig. 1); the presence of cells CD43+ was detected in all the six capsules, with a percentage ranging between 40 and 50%.

These inflamed tissues were used for the isolation of MSCs. MSCs derived from the three samples of capsules surrounding micro-textured implants were named micro-MSCs; the MSCs, isolated from the three capsules surrounding macro-textured implants, were called macro-MSCs.

MSCs Isolation and Cell Culture

As already described [19–21], MSCs were isolated from tissue biopsies then cultured with Mesenchymal Stem Cell Growth Medium bullet kit (MSCGM, Lonza Group® Ltd).

All the subsequent experiments were performed individually on each of the six samples of MSCs derived from capsular inflamed tissues (three micro-MSCs cultures and three macro-MSCs cultures). As a model for ALCL, the cell line KI-JK (ACC-695 from Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ) was chosen since it is reported [7] that the form of lymphoma, eventually associated with breast implants, behaves more like the less aggressive cutaneous form rather than the more aggressive systemic form. The cell line was cultured in RPMI 1640 medium (Invitrogen) + 20% FBS (Invitrogen).

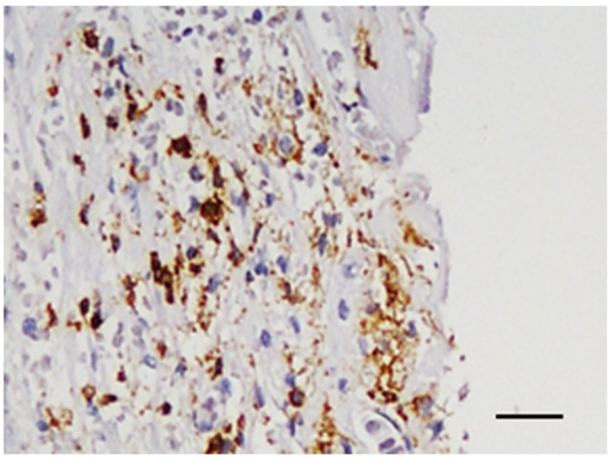


Fig. 1. Representative images of the immunohistochemical expression of CD43. The expression of CD43, known as a marker of inflammation, was tested by immunohistochemistry in tissue biopsies derived from periprosthetic capsule; in all samples, many cells were positive for CD43 (Immunoperoxidase × 200 original magnification). Scale bar 150 μm.

Stemness Characterization

As previously reported [19–21], the stemness of the isolated cells was analyzed according to the minimal criteria for MSCs identification by Dominici [22]. The immunophenotype was assayed by cytofluorimetric analysis and the differentiative potential towards osteogenic, chondrogenic, and adipogenic lineages was tested. For immunophenotyping, 2.5×10^5 cells were stained for 45 min with fluorescein isothiocyanate (FITC)-conjugated antibodies (Becton Dickinson) against: HLA-DR, CD14, CD19, CD34, CD45, CD73, CD90, and CD105. Since it is reported [23] that many of the mesenchymal markers are also found in fibroblasts, we analyzed the level of CD9 (Becton Dickinson), which is differently expressed by the two cellular subsets. Differentiation into osteocytes, chondrocytes, and adipocytes was assessed using STEMPRO® Osteogenesis, Chondrogenesis, and Adipogenesis Kits (GIBCO, Invitrogen,) respectively. Cells cultured in MSCGM alone were used as a negative control.

Osteogenic differentiation was assessed by Von Kossa and Alkaline phosphatase (ALP) staining; adipogenic differentiation was tested by Oil Red staining; for chondrogenesis, cells were cultured in pellet culture system and the sections were exposed to a solution of Safranin-O.

MSCs/KI-JK Co-culture in Transwell and Cell Proliferation

KI-JK cells were co-cultured with micro- and macro-MSCs to evaluate their paracrine effect. 2×10^5 of each sample of MSCs cells were individually seeded at the lower surface and the day after, 2×10^6 KI-JK cells were added at the upper surface of a polycarbonate transmembrane filter in a Transwell filter system in a 6-well plate (pore size 0.4 μm; BD Falcon). Cells were co-cultured for

72 h (micro-MSCs/KI-JK; macro-MSCs/KI-JK), then tumor cells were recovered and cell proliferation was assessed by XTT test (Trevigen, Gaithesbrug MD, USA). The data of absorbance (mean ± SD) about co-cultured KI-JK cells are reported as percentages of the values measured in parallel in KI-JK cells cultured alone (referred as 100%), over three independent experiments.

Analysis of the Secretion of Selected Cytokines by ELISA Test

The secretion of cytokines referred to inflammatory processes, such as IL6, IL12, IFN-γ, TNF-α, IL2, IL4, IL5, IL13, IL10, TGF-β1, IL17A, and G-CSF, was investigated by ELISA test (Multi-Analyte ELISArray kit, Qiagen). Briefly, medium conditioned by each sample of micro- and macro-MSCs was used for the test. Samples were dispensed into a 96-well microtiter plate and incubated for 2 h at room temperature. After washing, avidin-HRP-conjugated antibody was added to the plate and incubated for 30 min. Finally, captured cytokines were detected by the addition of a substrate solution. The OD at 450 nm was determined using a microtiter plate reader (Multiskan GO Microplate reader, Thermo Scientific). Tests were performed as triplicates. Data are reported as mean ± SD for micro- and macro-MSCs from three independent experiments. Subsequently, each sample of MSCs was co-cultured with KI-JK cells for 72 h and the ELISA test was repeated. The level of each cytokine detected in each co-cultured sample of MSCs is calculated as % of its level in the same MSCs cultured alone. After, mean ± SD from three independent experiments in triplicates was calculated for co-cultured micro- and macro-MSCs and displayed.

Analysis of the Expression of Selected Genes by RT-PCR

The expression of selected genes involved in specific cellular mechanisms, such as proliferation (MKi67), EMT induction (ACVR1, TGF-βR2), invasion and migration (CXCL12), and angiogenesis (VEGF) was tested by real-time PCR (RT-PCR) in KI-JK cells. Total RNA was isolated from KI-JK cells before and after co-cultures with each sample of micro- and macro-MSCs by using 5 PRIME PerfectPure RNA Purification (5 PRIME, Hamburg, Germany) and retrotranscribed in cDNA (GoScript™ Reverse Transcription System, Promega, Italy). The primer sequences are reported in Table 2.

All samples were tested in triplicate with the housekeeping genes β-ACTIN and GAPDH for data normalization. Of the two, GAPDH was the most stable and was used for subsequent normalization. After amplification, melting curves were acquired. Direct detection of PCR products was monitored by measuring the fluorescence produced by SYBR Green I dye (EVA Green PCR Master Mix, Bio-rad) binding to double strand DNA after every cycle. These measurements were then plotted against cycle numbers. The parameter threshold cycle (Ct) was defined as the cycle number at which the first detectable increase above the threshold in fluorescence was observed. The amount of mRNA detected in KI-JK cells after co-cultures was calculated as x-fold with respect to control sample (KI-JK cultured alone, expressed as 1) by the $2^{-\Delta\Delta Ct}$ method [24], where $\Delta Ct = Ct$ (gene of interest)—Ct (control gene) and $\Delta(\Delta Ct) = \Delta Ct$ (KI-JK co-cultured)—ΔCt (KI-JK mock). X-fold was calculated for the selected genes in all the co-cultures with the six samples MSCs. Subsequently, mean ± SD from three independent experiments in triplicates was calculated for KI-JK cells co-cultured with micro-MSCs and for Ki-JK cells co-cultured with macro-MSCs and displayed.

Statistical Analysis

Independent sample *t* test was used to evaluate statistically significant differences between samples. Statistical significance was analyzed for data from at least three independent experiments. *p* values less than 0.05 were considered significant.

RESULTS

Isolation and Characterization of MSCs

Cells were isolated from the six biopsies (three derived from periprosthetic capsules surrounding micro-textured implants—micro-MSCs and three from periprosthetic capsules surrounding macro-textured implants—macro-MSCs) without differences in morphology and proliferation rate. To confirm the mesenchymal nature, cells were tested for the immunophenotype and for the differentiative potential. All the six cultures were strongly positive for CD73, CD90, and CD105, negative for HLA-DR, CD11, CD14, CD34, CD45, and CD9 (Supplementary Fig. 1) as well as able to differentiate towards osteo-, chondro-, and adipogenic cells (Supplementary Fig. 2).

Proliferation of KI-JK Cells after Co-Cultures with Micro- and Macro-MSCs

KI-JK cells were recovered after co-cultures (72 h) and cell proliferation assessed by MTT test. As a control,

Table 2. Sequence of the Primers Used in Real-Time PCR

Gene symbol	Forward	Reverse
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
B-ACTIN	ACCAACTGGGACGATATGGAGAAGA	TACGACCAGAGGCATACAGGGACAA
IGF1R	TTAAAATGG CCAGAACCTGAG	ATTATAACCAAGCCTCCCAC
MKi67	ACAGAAAAATCGAACTGGGAAA	GTTTATGAAGCCGATTCAGACC
ACVR1	TTCCTCACTGAGCATCAACG	TAATGAGGCCAACCTCCAAG
TGFBR2	CTAACCTGCTGCCTGTGTGA	TCGGTCTGCTTGAAGGACTC
CXCL12	GAGCCAACGTCAAGCATCTG	CGGGTCAATGCACACTTGTC
VEGF	CCTCCGAAACCATGAACTTT	ATGATTCTGCCCTCCTCCTTCT

KI-JK cells were cultured alone for 72 h. Co-cultured KI-JK cells, both with micro- or macro-MSCs, did not show a significantly different proliferation rate compared to that of mock sample (Fig. 2).

Cytokines Secretion by MSCs

The secretion of cytokines related to inflammation was individually analyzed in three micro- and three macro-MSCs samples. Since the three samples of micro-MSCs, as well as the three samples of macro-MSCs, gave results with no significant differences among each other, the average value for micro-MSCs and for macro-MSCs was calculated and used for the elaboration of data by ELISA test. Both micro-MSCs and macro-MSCs show a very similar profile of secreted cytokines where the mostly secreted were IL2, IL4, IL5, L6, and TGF-β1. No significative differences were noted among the six samples (three micro- and three macro-MSCs) (Fig. 3a). Subsequently, MSCs were co-cultured for 72 h with KI-JK cells and the ELISA test was repeated. The level of each cytokine detected in each co-cultured sample of MSCs was calculated as % of its level in the same MSCs cultured alone.

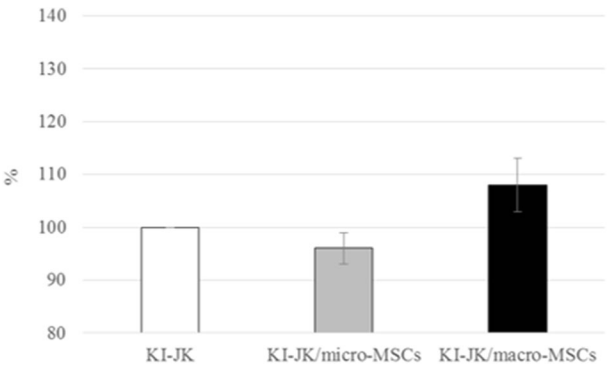


Fig. 2. Proliferation of KI-JK cells after co-cultures with MSCs. The histogram depicts the number of KI-JK cells assessed by an automated cell counter before and after co-cultures (72 h) with micro- and macro-MSCs. Data are reported as mean ± SD from three independent experiments in triplicates. KI-JK: cells cultured alone as a control sample; KI-JK/micro-MSCs: KI-JK cells co-cultured for 72 h with MSCs isolated from inflamed periprosthetic capsule around micro-textured implant; KI-JK/macro-MSCs: KI-JK cells co-cultured for 72 h with MSCs isolated from inflamed periprosthetic capsule around macro-textured implant.

With respect to mock samples, the most relevant change was an increased secretion of IL10 and TGF-β1. These variations were found both in micro- and macro-MSCs after co-cultures (Fig. 3b) with no statistically relevant differences.

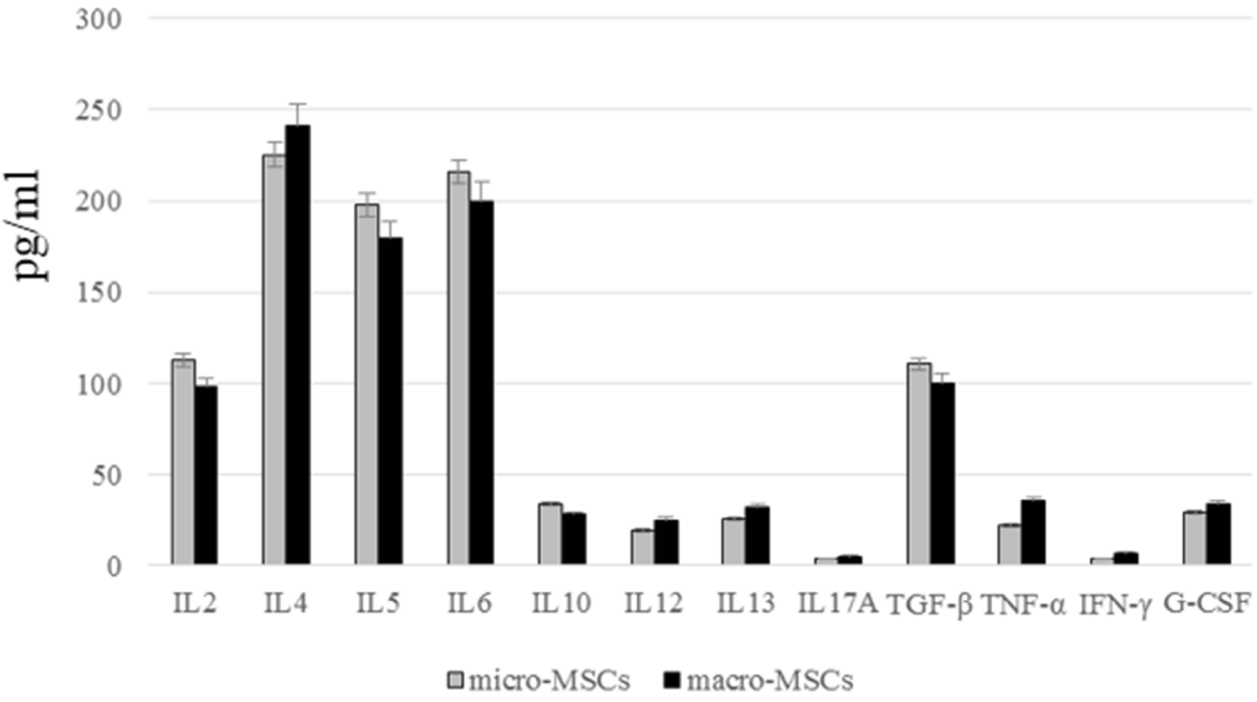
Gene Expression in Co-cultured KI-JK Cells

To evaluate if the paracrine effect exerted by micro- and macro-MSCs could influence the KI-JK cell behavior, the expression of genes implicated in mechanisms amenable to tumor development was analyzed in KI-JK cells before and after co-cultures with MSCs. Data derived from co-cultures of KI-JK cells with each of the six sample of MSCs have been elaborated as relative expression (X-fold) towards mock (KI-JK cells cultured alone, referred as 1). Since the results obtained from the three co-cultures with micro-MSCs, as well as from the three co-cultures with macro-MSCs, gave results with no significant differences among each other, the average value for co-cultures with micro-MSCs and with macro-MSCs was calculated and displayed. Results from gene expression analysis confirm that texturization does not influence the effects of mesenchymal stem cells on KI-JK cells. In detail, the expression of ACVR1, VEGF, TGF-βR2, CXCL12, and MKi67 was higher ($p < 0.05$) in mock samples than in co-cultured KI-JK cells and no significant differences were noted between co-cultures with micro- or macro-MSCs (Fig. 4).

DISCUSSION

Since the first report of BIA-ALCL in 1997 [25], there has been a growing interest about the possible association between this uncommon non-Hodgkin lymphoma

a


300
250
200
150
100
50
0
pg/ml
IL2
IL4
IL5
IL6
IL10
IL12
IL13
IL17A
TGF-β
TNF-α
IFN-γ
G-CSF
micro-MSCs
macro-MSCs

b

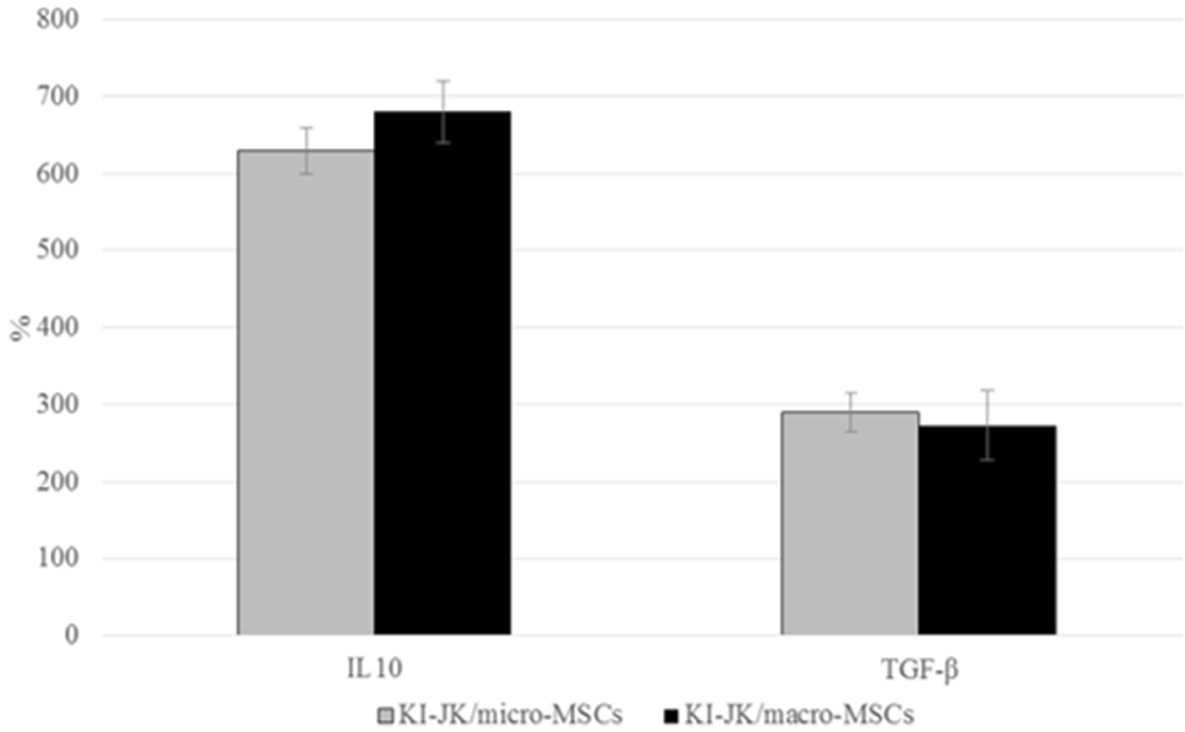

800
700
600
500
400
300
200
100
0
%
IL10
TGF-β
KI-JK/micro-MSCs
KI-JK/macro-MSCs

Fig. 3. Detection of secreted cytokines by ELISA before (**a**) and after co-cultures with KI-JK cells (**b**). The histograms display the level of secretion of selected cytokines refereed to inflammation detected in micro- and macro-MSCs (**a**). Data are reported as mean ± SD from three independent experiments in triplicates. Histograms display only cytokines whose level was changed after co-cultures with KI-KJ cells for 72 h (**b**). The level of each cytokine detected in each co-cultured sample of MSCs was calculated as % of its level in the same MSCs cultured alone. Subsequently, mean ± SD from three independent experiments in triplicates was calculated for co-cultured micro- and macro-MSCs and displayed. Micro-MSCs/KI-JK: MSCs isolated from inflamed periprosthetic capsule around micro-textured implant co-cultured with KI-JK cells; macro-MSCs/KI-JK: MSCs isolated from inflamed periprosthetic capsule around macro-textured implant co-cultured with KI-JK cells.

subtype and breast implants. Therefore, in January 2011, the US Food and Drug Administration (FDA) issued an alert describing a risk of developing ALCL in the breasts of women with implants [26]. The subsequent updates of 2016 and 2017 [12] detailed the risk factors about BIA-ALCL, paying attention also to the surface and the fill type of breast implants and indicating a list of medical recommendations [27, 28]. Although this risk is exceedingly low, an adequate knowledge of the clinical behavior of ALCL is mandatory and several hypotheses about its pathogenesis have been proposed throughout the literature [29–31].

Breast implants are widely used in the field of plastic surgery both for esthetic and reconstructive purposes, and

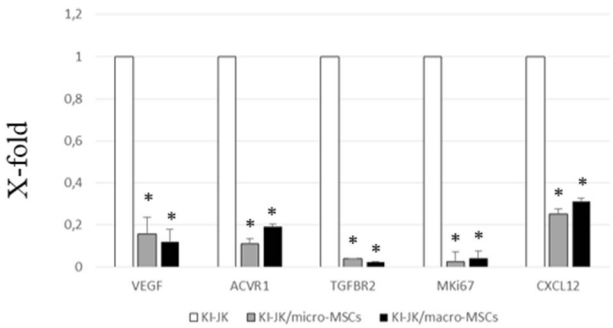


Fig. 4. Expression of selected genes by real-time PCR. The histogram displays the expression of selected genes related to specific cellular mechanisms, such as proliferation (MKi67), anti-apoptosis (IGF1R), EMT induction (ACVR1, TGF-βR2), invasion and migration (CXCL12), and angiogenesis (VEGF) in KI-JK cells after co-cultures with the six samples of MSCs (three micro- and three macro). Data derived from co-cultures of KI-JK cells with each of the six samples of MSCs have been elaborated as relative expression (X-fold) towards mock (KI-JK cells cultured alone, referred as 1). Subsequently, mean ± SD from three independent experiments in triplicates was calculated for KI-JK cells co-cultured with micro-MSCs and for KI-JK cells co-cultured with macro-MSCs and displayed. *$p < 0.05$ KI-JK cells *versus* co-cultured KI-JK cells. KI-JK: cells cultured alone; KI-JK/micro-MSCs: KI-JK cells co-cultured with MSCs isolated from inflamed periprosthetic capsule around micro-textured implant; KI-JK/macro-MSCs: KI-JK cells co-cultured with MSCs isolated from inflamed periprosthetic capsule around macro-textured implant.

they are usually associated with mild to severe scarring of the surrounding tissues, finally leading to capsule development. A significant potentiator of capsule contracture is surely the infection of mammary implants by bacterial film [8]. Basing on the observations that chronic inflammation has been implicated in the development of lymphomas, several authors supposed that capsular fibrosis should not be merely considered as the result of a foreign-body reaction. On the contrary, the chronic inflammatory response in the fibrous capsule, along with the production of specific cytokines, may play a possible role in neoplastic transformation [32, 33].

The relationship between texturization of the implants and the presence of capsular contracture as well as the correlation between texturization and BIA-ALCL has been partially investigated. About the capsular contracture, it has been established that textured implants are associated with a lower risk of clinically significant capsular contracture than smooth ones [10, 34–37]; this result may due to the particular arrangement of the connective fibers in the capsule that disrupt the contractile forces around the implant. Conversely, a statistically significant higher inflammation in the capsule has been described for textured implants than for smooth implants, characterized by T cell infiltration [11]. About the risk of BIA-ALCL, the role of texturization was investigated by Hu et al. [8]; they firstly evaluated the correlation between chronic biofilm infection and grade of capsular contracture, then if the type of texturization influenced the formation of the biofilm. They found that there was a positive correlation between the bacterial numbers and the grade of capsular contracture and, even if all capsules were positive for biofilm, the T lymphocytes number (recruited as a consequence of the number of detected bacteria) was higher for textured implants than for the smooth ones.

Nevertheless, as stated by the FDA update of 2017, details on breast implant surface are limited [12].

None of the above-listed studies put the attention on the involvement of mesenchymal stem cells as a promoter of both inflammation and capsular contracture that in turn enhance the risk of BIA-ALCL neither if this potential involvement is linked to the texturization of the implants.

In our previous studies [19–21], we investigated if an inflamed microenvironment (as the periprosthetic capsule) can modify both the immunobiology and the paracrine effect exerted by MSCs on an ALCL cell line, due to the described possible association linking MSCs—inflammation—tumor development. Our findings showed that even if inflammation affects the immunobiology of MSCs, it does not change the effects of MSCs on ALCL cells.

In the current study, we selectively investigated the role of implant texturization isolating MSCs from periprosthetic capsules surrounding Mentor implants (micro-textured) and Allergan implants (macro-textured).

To address if texturization affects the degree of inflammation and in turn the tumoral cell fate, the proliferation rate, the levels of cytokines referred to inflammatory processes, and the expression of selected genes related to pro-tumoral mechanisms were analyzed in MSCs derived from capsules surrounding micro-textured implants (micro-MSCs) and from capsules surrounding macro-textured implants (macro-MSCs), before (mock) and after co-cultures with KI-JK cells. Our data indicate that the type of texturization of the implants did not produce different effects on MSCs immunobiology neither on KI-JK cell fate. ELISA test on the supernatant of micro- and macro-MSCs displays as the amount of secreted cytokines is almost the same in the two cell groups (micro- and macro-MSCs). After co-cultures with KI-KJ cells, both micro- and macro-MSCs showed a strong increase of TGF-β1 and, in particular, of IL10. These observations are partially in contrast with the results obtained by Hu et al. that found as textured implants caused a deeper chronic biofilm infection and consequent higher inflammation than the smooth implants. Nevertheless, they compared smooth and textured implants and not micro- and macro-textured implants.

The expression of IL10 in ALCLs has been largely studied in lymphomas and it has been displayed as in 73% ALCLs exhibited a large number of IL10-expressing cells; further studies clarify that IL10-expressing cells were tumor cells. To exclude an involvement of MSCs in IL10 overexpression, it was measured in KI-JK cells and it confirmed a strong secretion of IL10 directly by tumor cells (data not shown) [38]. In addition, IL10 seems to in turn affect the expression of TGF-β [39].

After finding that texturization did not promote dysregulation in cytokines secretion, the expression of genes involved in other pro-tumorigenic mechanisms, such as proliferation (mKI67), EMT induction (ACVR1, TGF-βR2), invasion and migration (CXCL12), and angiogenesis (VEGF) was considered. Both co-cultures produce a decrease in the expression of VEGF, ACVR1, TGFβR2, CXCL12, and MKi67 with respect to mock sample.

ACVR1 and TGF-βR2 are involved in the epithelial-to-mesenchymal transition (EMT) and in the metastatic process; these genes are directly related to CXCL12 that influences invasion and migration; with regard to the gene expression of VEGF, it plays an important role both in angiogenesis and in the initiation of EMT [40]. PCR results show a decrease in the expression of all these genes, indicating that the crosstalk between MSCs and tumor cells do not enhance tumor growth. This hypothesis is also confirmed by the decrease of MKi67, a gene of proliferation. Nevertheless, since no modification on the proliferation is observed even if the expression of Mi-67 is reduced, it is possible to hypothesize a post-transcriptional control of this gene. In conclusion, our data suggest that breast implants texturization (a) does not affect the cytokines secretion by MSCs, (b) does not affect the cytokines secretion by MSCs after co-cultures with ALCL cells, (c) does not influence the proliferation rate of tumor cells, and (d) does not affect the expression of genes related to pro-tumoral mechanisms in KI-JK cells after co-cultures. Finally, the variations in cytokine secretion observed both for micro- and macro-MSCs do not correlate with an evident enhanced risk of BIA-ALCL. To better understand the underlying processes that may induce BIA-ALCL, further studies will be necessary and the major limitations of this work, such as the restricted number of the samples and the lack of smooth implants, will have to be overcome.

COMPLIANCE WITH ETHICAL STANDARDS

This study was approved by the Università Politecnica delle Marche Ethical Commitee and was conducted in accordance with the Declaration of Helsinki.

Conflict of Interest. The authors declare that they have no conflict of interest.

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