Coating of an Anti-Fas Antibody on Silicone: First In Vivo Results

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Research

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Abstract

Background: Although the etiology of capsular contracture after breast augmentation has not yet been definitively clarified, the literature contains numerous reports placing the blame on a foreign body reaction. We have developed a procedure for covalently activating a silicone surface with an anti-Fas antibody, which might suppress the foreign body reaction on the silicone surface.

Objectives: The authors evaluate whether surrounding tissue might be influenced by anti-Fas antibody coating on silicone disks in comparison to untreated silicone disks in an in vivo model.

Methods: During this study, 4-mm anti–Fas-coated silicone disks were implanted subcutaneously in the paravertebral region of mice (C57/BL6). Silicone disks passing the activation coating process without anti-Fas antibody incubation were defined as the control group. Twelve weeks after implantation, the disks were removed and the surrounding tissue examined.

Results: The tissue surrounding the silicone disks in the experimental group showed significantly increased levels of collagen type 3, elevated levels of matrix metalloproteinase 9, markedly decreased levels of transforming growth factor β2, and a reduced CD68 expression in the pericapsular tissue.

Conclusions: The first in vivo data reveal that the tissue surrounding a silicone surface can be influenced by the vectored binding of an anti-Fas antibody.

Keywords

capsular contracture, breast implants, silicone, coating, plastic surgery, biocompatibility, research, anti-Fas

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In both aesthetic and reconstructive breast surgery, silicone implants have been used for decades and are part of a growing market. Implantation results in an immunological response to the foreign material polydimethylsiloxane, the most widely used synthetic polymer for surgical devices.[5-9] This immunological reaction results in the formation of a capsule surrounding the implant, which can constrict and harden. Capsular contracture (CC) is the complication most commonly associated with implant-based breast reconstruction and primary breast augmentation.[1-4] Capsular contracture leads to revision surgery and is associated with the increased complication risks of a second procedure.

Research has yielded various silicone surfaces and coatings such as polyurethane and titanium in attempts to improve implant biocompatibility. A study by Bassetto et al,[7] however, was unable to detect any differences in the histological morphology of capsules and the foreign body reaction between earlier models of breast implants and those coated with polyurethane. Similarly, Barr et al[10] were able to show only a slight advantage in biocompatibility for

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textured silicone surfaces of currently produced breast implants using nanostructure analysis. The existing literature contains diverse explanations for the etiology of CC such as physical implant properties, hematoma formation, bacterial prosthesis contamination, and seroma formation.[11] However, despite decades of research, the genesis of CC remains incompletely understood, and a multifactorial etiology seems to be involved.

We are convinced that immunological processes play a decisive role in the pathogenesis of CC, as also demonstrated by Wolfram et al,[9] who performed an immunohistological analysis in which they found a high level of T-cell activity in samples of the fibrotic capsules of explanted breast implants. Further studies have reported the primary role of many inflammatory cells such as macrophages, monocytes, and myofibroblasts in CC.[11,12] Based on this assumption, we set out to modify the immunological process involved in the identification of foreign materials by the body.

We hypothesized that the presence of an anti-Fas antibody located directly at the silicone surface would lead to the inhibition of the foreign body being identified through the induction of apoptosis in immune-competent Fas-sensitive cells.[13-16] This could potentially result in the prevention or minimization of CC development without systemic adverse reactions.

METHODS

Generating an Active Silicone Surface

The first step to functionalizing silicone (shell of a smooth silicone implant; DREAMXCELL GmbH, Mettmann, Germany) was the activation with APTES (3-aminopropyltriethoxysilane; Sigma-Aldrich Chemistry, Steinheim, Germany). We tested different concentrations of APTES (10%, 50%) in alcohol and in bi-distilled water. A concentration of 10% APTES in pure alcohol was effective in activating silicone, in contrast to APTES and bi-distilled water. We verified this result by ninhydrin testing to visualize free amino groups. To complete the silicone activation as a hybridization, we incubated the silicone in a 10% APTES/ethanol solution at 25°C (77°F) overnight while the incubation tubes were rotating in a hybridization incubator (Biometra OV4, Göttingen, Germany). The silicone was placed in the tubes as a sheet, with the coating surface arranged to face the lumen and the APTES/alcohol solution, to facilitate activation for the coating process.

After this activating process, the silicone was split into standardized silicone disks with a diameter of 4 mm using a biopsy punch (Stiefel, SFM, Waechtersbach, Germany) and carried in a 96-well plate. To perform an ester-activation, we generated an activation-buffer (AB): 0.1M MES (Sigma-Aldrich Chemistry) + 0.5M sodium chloride (J. T. Baker, Deventer, Netherlands), pH 6.0.

Table 1. Experimental Groups

Animals (n)	Native Silicone	Anti-Fas Antibody-Coated Silicone
1	+	+
2	+	+
3	+	+
4	+	+
5	-	+

+, silicone disks were explanted after 12 weeks without any complications during the time period; –, animal did not reach day of explantation.

Ester-activation was generated with 4.4 mg NHS (N-hydroxysulfosuccinimide sodium salt; Sigma-Aldrich Chemistry) and 1.6 mg EDC (N-3-dimethylaminopropyl-N-ethyl-carbodiimide hydrochloride (Fluka Chemie GmbH, Buchs, Switzerland) suspended in 4 mL of AB. Proteins could then be coated in different concentrations with the ester-activated AB (EAAB) on the surface of the activated silicone disks.

The silicone disks of the control group underwent the same activation process, particularly with the ester-activated activation buffer, but without incubation of the anti-Fas antibody.

Animals

The animals involved in the test were C57/BL6 mice (n = 10), all of which were female with an age of 4 to 12 weeks. The animals were housed and the testing performed in accordance with the guidelines set down in Paragraph 8 of the German Animal Protection Act (BGBI.IS.1206, 1313).

Each experimental group contained 5 animals. One animal in the control group treated with uncoated silicone disks was removed from the study prematurely (Table 1). The animals were initially anesthetized in a sealed container with isofluorane gas and oxygen. Continuation of anesthesia was performed with the same medications but with a mask.

In preparation for surgery, the animals were shaved and the operative field disinfected with Braunol (Braun Melsungen AG, Melsungen, Germany). Incisions were made horizontal to the long axis of the animal in the paravertebral regions. The silicone disks were implanted in the epifascial space with the coated side oriented toward the skin. The wounds were closed with 7-0 Prolene (Ethicon, Inc, Somerville, New Jersey) using interrupted sutures. A sterile bandage was then applied. The disks were removed after 12 weeks and prepared for histology. The animals were then sacrificed using the cervical dislocation technique.

Histology

The subcutaneous implant sites were sampled and dissected into 2 equal parts for routine histology and fresh

Table 2. Antibody Characteristics

Antibody	Clone	Supplier	Pretreatment	Dilution
Collagen type 3	III-53	Acris Antibodies, Herford, Germany Catalog No.: AF5810	HIER Tris-EDTA pH 9	1:50
MMP9	Polyclonal	Acris Antibodies, Herford, Germany Catalog No.: AP09001PU-N	HIER Tris-EDTA pH 9	1:200
TGF-β2	Polyclonal	Acris Antibodies, Herford, Germany Catalog No.: AP06351PU-N	HIER Citrat pH 6	1:50
CD68	KP1	Abcam, Cambridge, UK Catalog No.: ab955	HIER Citrat pH 6	1:100

HIER, heat-inactivated epitope retrieval; MMP9, matrix metalloproteinase 9; TGF-β2, transforming growth factor β2.

frozen storage. For routine histology, the samples were fixed in neutrally buffered 4% formalin for 36 hours and subsequently paraffin embedded. Immunohistochemistry was done using antibodies for collagen 3, matrix metalloproteinase 9 (MMP9), transforming growth factor β2 (TGF-β2), and CD68 (Table 2). All stainings were performed according to a standard ABC protocol using a DAB HRP detection kit (Zytomed Systems, Berlin, Germany).

Statistical Analysis

The immunohistochemical stains were double-blinded estimated. Scores from 0 to 4 were given, corresponding to the counted cells stained (Table 3). Significance was tested with χ^2 and Fisher's exact test. Significance was set to $P < .05$.

RESULTS

Collagen 3

Wound healing and the quality of a scar are primarily influenced by what type of collagen is involved. For this reason, we examined the interfaces between the silicone disks and the surrounding tissue to determine whether the coating with an anti-Fas antibody could effect changes leading to a rise in the levels of collagen type 3. A significant increase ($P = .048$) in the proportion of collagen type 3 with more parallel orientation of the type 3 fibers was observed in the group with anti-Fas antibody-coated silicone disks in comparison to the control group with untreated silicone (Figures 1 and 2).

MMP9

MMP9 is an essential matrix metalloproteinase, responsible for degradation of collagen type 4 and collagen type 1. The immunohistological examination of MMP9 was designed to determine whether a coating of functional anti-Fas antibody could result in changes in the extracellular matrix. MMP9 was present in both groups, but increased levels of MMP9 were observed in the coated silicone group (Figures 3 and 4).

TGF-β2

TGF-β2 is an immune-modulating cytokine regulating a large number of biological processes. It has been shown that periprosthetic breast capsules contain high levels of TGF-β2, similar to other fibrotic diseases.[17] We therefore examined whether the presence of TGF-β2 was influenced by the coating of silicone disks with anti-Fas antibody. A strong suppression of TGF-β2 cytokine secretion was demonstrated in the group with anti-Fas antibody-coated silicone disks (Figures 5 and 6).

CD68

CD68 is expressed by monocytes and macrophages and is primarily present as an intracytoplasmatic molecule associated with lysosomal granules.[18] Tissue macrophages and monocytes play a key role in tissue homeostasis and are important in innate and acquired immune responses. These cells efficiently recognize and phagocytose pathogenic particles and subsequently present antigens to T cells.[19] As a consequence of our aim to induce a local immune response at the borderline between coated silicone and its surrounding tissue, we expected to reduce

Table 3. Semiquantitative Analysis of Immunohistochemical Stains

	Score, %				
	0	1	2	3	4
Counted cells stained	No reaction	<10	<25	<50	>50

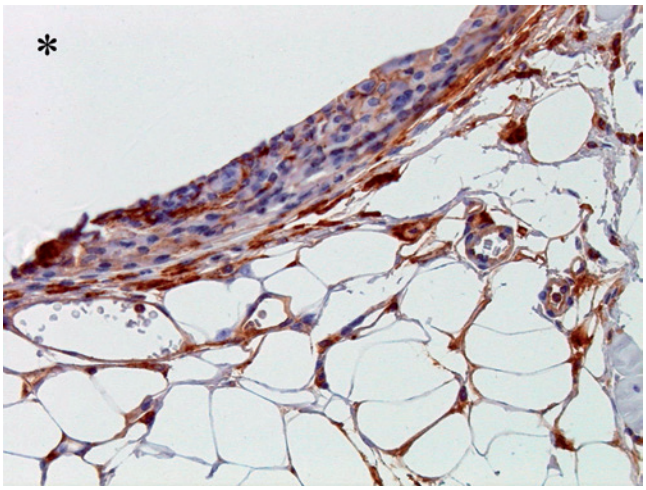

Figure 1. Collagen 3 at the interface between native silicone disks and surrounding tissue. The brown-colored tissue indicates collagen 3. *Asterisk displays the space of the silicone disk.

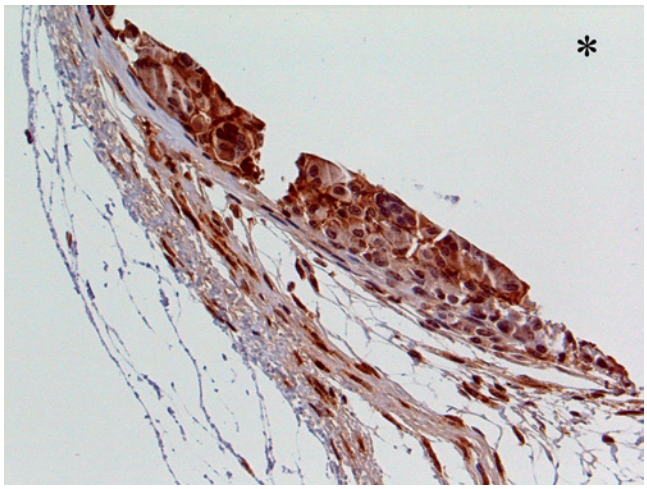

Figure 3. Matrix metalloproteinase 9 (MMP9) at the interface between native silicone disks and surrounding tissue. The brown-colored tissue indicates MMP9. *Asterisk displays the space of the silicone disk.

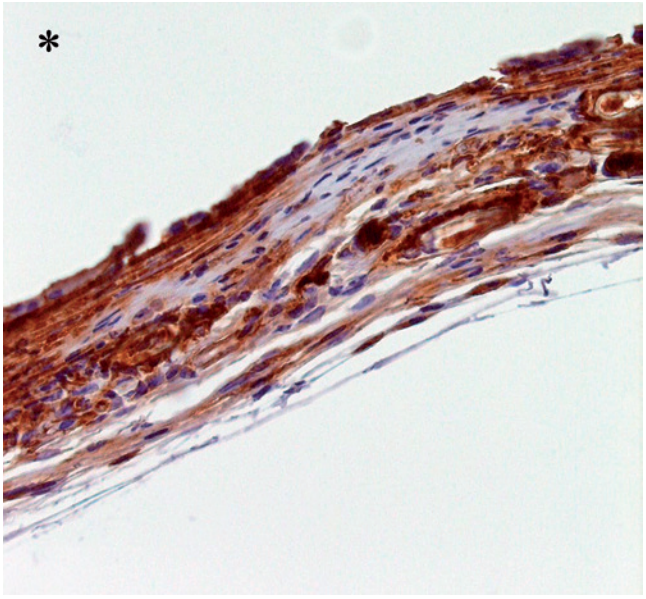

Figure 2. Significantly ($P = .048$) increased collagen 3 levels and more parallel orientation of the type 3 fibers at the interface between anti-Fas antibody-coated silicone disks and surrounding tissue. The brown-colored tissue indicates collagen 3. *Asterisk displays the space of the silicone disk.

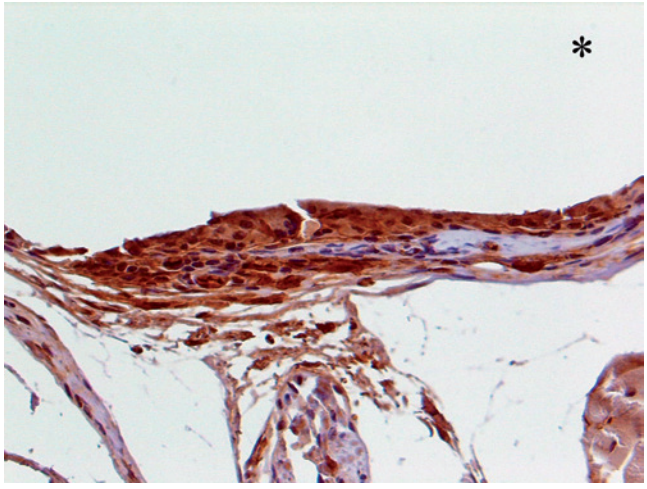

Figure 4. Increased matrix metalloproteinase 9 (MMP9) levels at the interface between anti-Fas antibody-coated silicone disks and surrounding tissue. The brown-colored tissue indicates MMP9. *Asterisk displays the space of the silicone disk.

CD68-positive cells in the pericapsular surrounding tissue. In our study, we observed a reduced expression of CD68-positive cells in the anti-Fas antibody-coated group (Figures 7 and 8).

Because of the small numbers in both experimental groups of this pilot study, the results of MMP9, TGF-β2, and CD68 were not statistically significant, but the histological findings are convincing.

DISCUSSION

For decades, materials research has strived to improve the integration and biocompatibility of silicone through implant modification. The goal has been to reduce, delay, or even eliminate the incidence of complications associated with the procedure. At the same time, the implant should maintain its stability of shape, at rest and during motion, and exhibit a natural feel or haptic quality. To date, research has focused almost exclusively on modifying physical properties such as textured or smooth surfaces or on the use of other materials such as silicone, tetanized

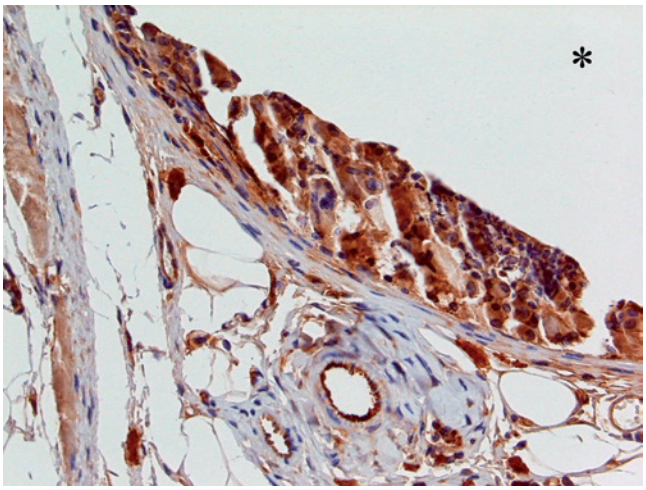

Figure 5. Transforming growth factor β2 (TGF-β2) at the interface between native silicone disks and surrounding tissue. The brown-colored tissue indicates TGF-β2. *Asterisk displays the space of the silicone disk.

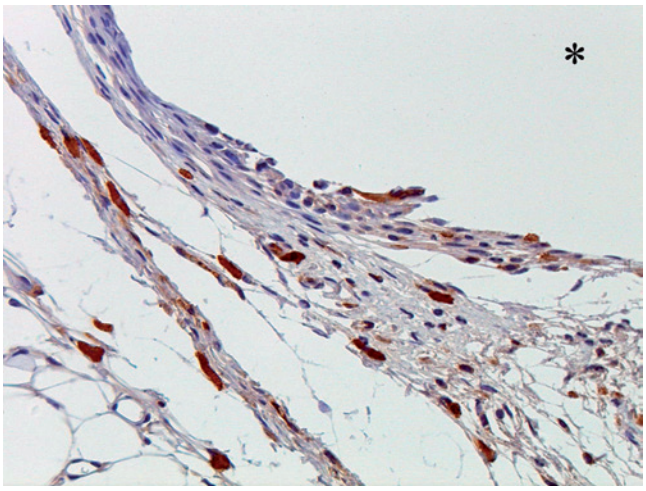

Figure 6. Decreased levels of transforming growth factor β2 (TGF-β2) at the interface between anti-Fas antibody-coated silicone disks and surrounding tissue. The brown-colored tissue indicates TGF-β2. *Asterisk displays the space of the silicone disk.

silicone, and polyurethane-coated silicone implants.[20,21] The novel approach of our study was to effect a change at the interface between the foreign body surface and the surrounding tissue by means of the covalently vectored functionalization of the surface with an antibody, as it is our opinion that immunological processes play the decisive role in CC formation.[5,6,9,22,23]

Recently, we developed a procedure for the vectored functionalization of silicone surfaces in order to realize the covalent binding of a functional synthetic anti-Fas antibody.[24] We have previously demonstrated the functionality of the bound protein on the silicone surface. The coating of the silicone surfaces with the synthetic anti-Fas antibody led to the apoptosis of Fas-sensitive Jurkat T cells in co-culture.[24]

Anti-Fas Antibody-Induced Local Immunosuppression

Dendritic cells (DC) play a central role in the recognition of foreign materials in the body. They belong to the mononuclear phagocyte system. Stimulated by chemotactic signals, they are capable of leaving the organ and infiltrating the interface between the foreign body and the surrounding tissue.[25]

Contact with the foreign body activates native DC, after which they travel to the neighboring lymph nodes as mature DC, where they then interact with T cells using their MHC class 1 or class 2 surface molecules. They thereby drive the T cells toward a Th1 or Th2 response, or they can induce T-regulatory cells to induce an immune response.

Immune competent cells are regulated by, among other things, the Fas/Fas-ligand system. This enables a self-regulation of the immune system, preventing an excessive immune response.[26] For this reason, anti-Fas antibody was selected in order to trigger apoptosis of immune-competent lymphocytes directly at the interface between the foreign body—in this case, silicone—and the surrounding tissue. These cells might otherwise be involved in the initial process by which the silicone is recognized as foreign. This would potentially have the consequence of eliciting local immune-suppressive effects directly at the implant surface without systemic adverse effects.

The aim of this in vivo study was to effect changes in the tissue surrounding silicone disks that had been coated with the immunosuppressive anti-Fas antibody in a covalent and vectored manner. The fibrotic processes in CC formation could be further analyzed by examining the levels of collagen type 3, MMP9, and TGF-β2, all of which assume central roles in the regenerative processes in connective tissue and in the development of fibrosis.

Collagen 3

A decisive process in regenerative wound healing lies in the conversion of collagen type 3 into collagen type 1. Studies on fetal wound healing have demonstrated that significantly higher levels of collagen type 3 are seen in fetal regeneration compared with postnatal scar formation.[27,28] During scar formation, collagen type 3 is converted to collagen type 1. In comparison to type 1 collagen, type 3 collagen displays smaller collagen fibers with a more parallel orientation of the fibers. Furthermore, type 3

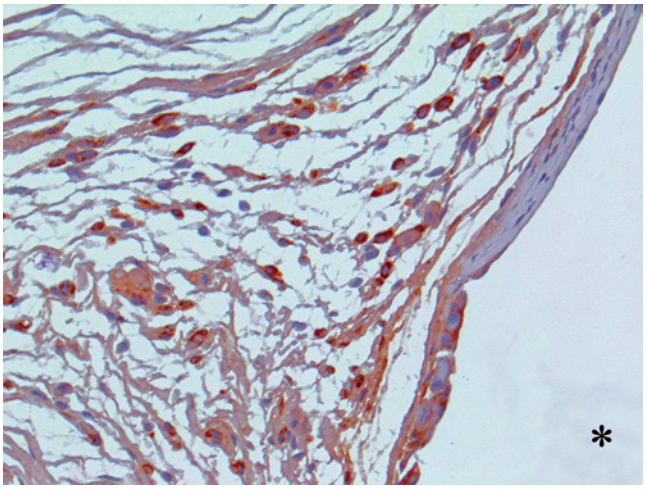

Figure 7. Staining of CD68-positive cells in the pericapsular tissue of the native silicone disks. The brown-colored tissue indicates CD68-positive cells. *Asterisk displays the space of the silicone disk.

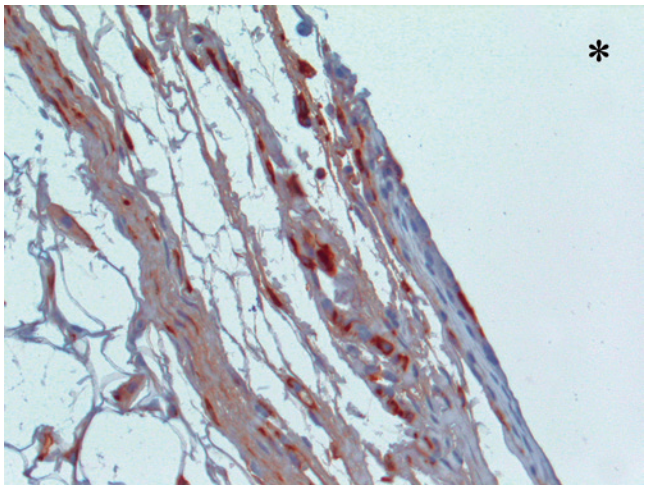

Figure 8. Decreased levels of CD68-positive cells in the capsular surrounding tissue of the anti-Fas antibody experimental group. The brown-colored tissue indicates CD68-positive cells. *Asterisk displays the space of the silicone disk.

fibers possess less crosslinking than type 1 fibers, resulting in a more elastic wound and scar.[27]

The etiology and the diverging progressions with regard to whether and to what degree CC arises have not been adequately explained to date. Examinations of Baker grade 3-4 capsules have shown that a substantial proportion of the CC is made up of collagen type 1.[5] Analogous to wound healing, the conversion of the elastic type 3 collagen into the more rigid type 1 collagen may also be involved in the development of CC. For this reason, we examined the expression of collagen type 3 in both experimental groups. We were able to demonstrate significant increased expression of type 3 collagen in the silicone disks coated with anti-Fas antibody with a more parallel fiber orientation (Figures 1 and 2).

MMP9

MMP9 is a gelatinase and a member of the essential regulatory proteinases of the extracellular matrix (ECM). Because of its ability to divide and degrade collagen type 1 as well as the ability to inhibit fibroblasts, MMP9 is of particular interest in the problem of CC formation.[29] This is due to the fact that myofibroblasts can differentiate from fibroblasts and that myofibroblasts are the cells centrally responsible for the deformation in capsular contracture.[30] The ECM regulates cell behavior by influencing cell proliferation, survival, shape, migration, and differentiation. Far from being a static structure, the ECM is constantly undergoing remodeling, particularly during the normal processes of development, differentiation, and wound healing.[3] MMP9 is particularly involved in the breakdown of collagen types 1 and 4 and can also cleave crosslinks in collagen type 1.[31] Moreover, high levels of MMP9 directly inhibit the biological behavior of fibroblasts and may be capable of inhibiting the differentiation of fibroblasts into myofibroblasts.[29] For this reason, elevated MMP9 levels in the ECM at the interface between the silicone surface and the capsule may be an indicator that antifibrotic processes could inhibit the development of CC. We observed an elevated proportion of MMP9 at the interface between the silicone disks and the surrounding tissue in the group of anti-Fas antibody-coated silicone disks compared with the control group (Figures 3 and 4).

TGF-β2

TGF-β2 is an immune-modulating cytokine that is involved in regulating fibrotic processes such as pulmonary fibrosis, scleroderma, and scarring disorders such as hypertrophic scars and keloids. TGF-β2 has also been detected in the capsules surrounding breast implants.[32,33] TGF-β2 is, however, absent in the desired scarless fetal wound healing.[28] As a regulatory cytokine involved in fibrotic processes, anti-Fas antibody at the surface of the implant could potentially reduce the production of TGF-β2 by means of an immunosuppressive effect at the cellular level.

Higher concentrations of TGF-β2 have also been demonstrated in breast capsules compared with normal surrounding breast tissue.[17] Working under the assumption that TGF-β2 is an important stimulator for fibrotic processes in the pathogenesis of CC, we examined whether the coating of the silicone surface with anti-Fas antibody would be capable of reducing the levels of TGF-β2. We showed decreased levels of TGF-β2 cytokine secretion in the experimental group with coated disks.

CD68

Human CD68 is a heavily glycosylated type 1 transmembrane protein that belongs to the lysosomal/endosomal-associated membrane glycoprotein (LAMP) family.[34] CD68 is widely regarded as a selective marker for human monocytes and macrophages, which are strongly involved in the innate and acquired immune response.[19] Under the assumption of reducing the immune response to silicone with an anti-Fas antibody coating on the silicone surface, we examined CD68 expression in the capsule surrounding tissue of explanted silicone disks. Overall, the marked differences in the explanted silicone surrounding tissue in the anti-Fas antibody-coated group in comparison to the native silicone disks matched with our hypothesis of the pathogenesis of CC.

As plastic surgeons, we not only strive for a perfect aesthetic result for our patients but also desire an implant material that can be placed at an exact location in the body, integrated into the body without losing its shape or natural feel, and has minimum complications.[35] Over the previous decades, research has focused much more on the modification of the silicone surface at a micro and nanometer scale.[36] Various studies—some of which were animal experiments—have attempted to compare the incidences of CC in implants with smooth or rough silicone surfaces.[20,21,37-39] In a recent and previously mentioned study by Barr et al,[10] the currently available implants were examined for fibroblast alignment with regard to the nanostructure of their surfaces. The authors observed improved ingrowth of fibroblasts on the textured implants and interpreted this as a supposed advantage compared with smooth implants. It is open to discussion whether this actually represents an advantage, as it is a well-established fact that fibroblasts are precursors to myofibroblasts. Myofibroblasts are known to play a key role in the deformation seen in advanced stages of CC.[30,40,41]

The development of materials for silicone implants and other silicone-coated foreign materials designed for use in the human body could be dramatically influenced by the development of a new surface technology. Our study presents the first in vivo results of a silicone surface activated with a vectored antibody. It is our view that the activation of the silicone surface with other molecules to achieve diverse desirable effects is conceivable.

CONCLUSIONS

Capsular contracture is the result of an immediate or chronic foreign body reaction of the human body against synthetic silicone implants. In our animal study, we demonstrated that the activation of a silicone surface with a vectored antibody influences the surrounding tissue. These first in vivo results create hope that future ground-laying research could enable the optimization of foreign material with regard to its functionality and tissue compatibility through specific activation of its surface. We are convinced that the coating with functional proteins can revolutionize the use of foreign materials in humans, improving compatibility and safety for patients.

Disclosures

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