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# Covalent vectored binding of functional proteins by bifunctional crosslinking at silicone interfaces

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Received 3 August 2011; revised 6 October 2011; accepted 27 October 2011
Published online 23 February 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34008

**Abstract:** In the daily clinical routine, numerous synthetic medical devices are implanted in the human body, either temporarily or permanently. The synthetic material most often implanted is polydimethylsiloxane (silicone). Numerous studies have demonstrated that silicone is encompassed in a connective tissue capsule by the body, preventing integration into the surrounding tissue. This can result in complications. The aim of our study was to develop a simple procedure to functionalize the silicone surface, thereby positively affecting the material's biocompatibility. By combining a silanization with the use of ester activation, a reactive amino group is generated, which can bind any free carboxyl group. Directional crosslinking of a near-infrared-conjugated fluorophore antibody to the activated silicone surface could be demonstrated on a dose-dependent basis. The redox reaction at a silicone surface coated with an HRP-conjugated antibody caused by the addition of NBT/BCIP could be shown. Covering the silicone discs with an anti-FAS-antibody coating followed by a coincubation with FAS-sensitive T-cells allowed highly significant detection of caspase-3. In summary, our crosslinking procedure enables the stable binding of proteins without the loss of biological function. Through this process, silicones could be endowed with new functions which could improve their biocompatibility.  J Biomed Mater Res Part A: 100A: 1248–1255, 2012.

**Key Words:** bioactive material, silicone, implants, biocompatibility/soft tissue, cell-material interactions, clinical

**How to cite this article:** Steiert A, Reimers K, Burke W, Zapf A, Vogt P. 2012. Covalent vectored binding of functional proteins by bifunctional crosslinking at silicone interfaces. J Biomed Mater Res Part A 2012:100A:1248–1255.

## INTRODUCTION

The engineering of interfaces using chemical, physical, and mechanical methods has become a major interest in materials science. Functionalizing surfaces on implant materials for use in humans has the potential of developing numerous novel treatment options in the future.

Materials typically employed are characterized by a high level of biocompatibility. Materials such as silicone (synonyms: polyorganosiloxanes, PDMS ∼ polydimethylsiloxane), titanium and polytetrafluoroethylene (PTFE, Gore -Tex®).[1–4] In addition to its chemical characteristics, the structure and morphology of an interface, such as surface roughness, are crucial parameters in determining its overall properties.[5–7] Further developments in the materials research for silicones involves changing the surface structure, in order to improve the integration into the surrounding tissues.[8,9] Furthermore, micro and nanoscale roughness are known to enhance interfacial phenomena such as cell adhesion or protein adsorption.[10] Silicone is an approved and established material routinely used in the practice of human medicine. Polymers of silicone, are widely used in medical devices and supplies such as heart valves, shunt tubing, arthroplasty prostheses, intravenous tubing, vial stoppers as well as facial and breast implants. Currently, very few synthetic materials whose surfaces have been functionalized with proteins have been used in the human body. Heparin-coated tubing systems used in extracorporeal heart-lung machines, for instance, are an established example.[11,12]

For several years now there has been an ongoing interest in modifying implant surfaces using biomimetic coatings such as matrix protein or growth factor coatings to improve cell adhesion and proliferation.[13] Most materials are covered by conventional dip coating techniques, hot plasma spraying or by using chemical coupling agents.

However, there are problems associated with many of the applied techniques. Dip-coating techniques can only be applied on surfaces with adequate roughness and hydrophilicity and, due to temperatures of over 2000°C, hot plasma spraying techniques cannot be applied on heat-sensitive materials or for heat-sensitive coatings. Another method of

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achieving good binding properties at the material's surface is the use of chemical coupling agents. Most of these agents, however, are rather cytotoxic and, consequently, their application is limited.[14]

The aim of our research was to develop a process of functionalizing a silicone surface. The covalent binding of functional proteins would be suitable for achieving this goal. To avoid losing the functionality of the protein and to avoid damage (for example due to heat), we conceptualized a crosslinking procedure with a reactive amino group at the silicone surface. This amino group would then be capable of covalently binding with a terminal carboxyl group.

For this, the silicone surface was treated with 3-aminopropyltriethoxysilane (APTES). APTES[15] for the covalent amide-bond formation, we chose between an amine-terminated silicone surface and protein target EDC-NHS crosslinking.[16]

Crosslinking by EDC-NHS is well-established in organic and bioconjugate chemistry, and has not only been shown to work in solution but also on surfaces EDC (1-Ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride) is a zero-length crosslinking agent used to couple carboxyl groups to primary amines, typically via the formation of amine-reactive NHS-esters.[17] EDC reacts with a carboxyl to form an amine-reactive O-acyl-isourea intermediate.

If this intermediate does not encounter an amine, it will hydrolyze and regenerate the carboxyl group. In the presence of *N*-hydroxy succinimide (NHS), EDC can be used to convert carboxylic acid groups to amine-reactive NHS esters. This is accomplished by mixing the EDC with an acid-containing protein and adding NHS. The addition of a sulfonic acid moiety on the NHS group increases the water solubility of both the activated ester and the leaving group, which facilitates cleaning of the substrates after activation.

## 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 and 50%) in alcohol and in bi-distilled water. A concentration of 10% APTES in pure alcohol was effective at activating silicone, in contrast to APTES and bidistilled 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 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 discs with a diameter of four millimeters using a biopsy punch (Stiefel®, SFM, Waechtersbach, Germany) and carried in a 96-well plate as a standardized procedure. To perform an ester-activation, we generated an activation-buffer (AB): 0.1*M* MES (2-(*N*-morpholino) ethanesulfonic acid; Sigma-Aldrich® Chemistry, Steinheim, Germany) + 0.5*M* sodium chloride (J.T.Baker®, Deventer, Netherlands), pH 6.0. Ester-activation was generated with 4.4 mg NHS (*N*-hydroxysulfosuccinimide sodium salt, Sigma-Aldrich® Chemistry, Steinheim, Germany) and 1.6 mg EDC (*N*-3-dimethylaminopropyl-*N*-ethyl-carbodiimide hydrochloride, (Fluka Chemie GmbH, Buchs, Switzerland) suspended in 4 mL of AB.

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### Crosslinking of a near-infrared conjugated fluorophore antibody (IRDye®-680CW) to the activated silicone surface

To demonstrate the presence of a bound antibody at the activated silicone surface, we performed our crosslinking procedure with a near-infrared conjugated fluorophore antibody (IRDye® 680CW donkey—anti-mouse, LICOR®, Biosciences, Lincoln, NE).

Following the activation of the silicone, the antibody IRDye® 680CW was suspended in EAAB, as described in Generating an active silicone surface, and transferred to the activated surfaces of the silicone discs at concentrations of 1.5, 0.3, 0.06, and 0 μg/mL (control) in a volume of 100 μL per well. The incubation was carried out for 90 min at room temperature with protection from light and agitation. 1.4 μL 2-mercaptoethanol (Fluka®, Buchs, Switzerland) was added per well to stop the crosslinking process.

The wells were then each washed three times with 200 μL TWEN 20 (v/v) (Sigma-Aldrich® Chemistry, Steinheim, Germany). The analysis was performed using an infrared imaging system ODYSSEY® (Li-COR®, Biosciences, Lincoln, NE).

To demonstrate any potential advantage of the crosslinking process in comparison to untreated silicone, the identical procedure was performed with untreated silicone, that is silicone without silanization was treated with a 10% APTES and ethanol solution at 25°C overnight and tested. For this, the near-infrared conjugated fluorophore antibody was suspended in AB without the ester activation.

The control in this experiment was carried out using activated silicone without the addition of the near-infrared conjugated fluorophore antibody.

### Detection of enzymatic activity of a vectored crosslinked antibody at a silicone surface

For showing functional crosslinking of a protein at the silicone surface we choose a horseradish peroxidase (HRP) conjugated IgG antibody (Sigma-Aldrich® Chemistry, Steinheim, Germany) to initiate a redox reaction with NBT / BCIP (NBT: Nitro blue tetrazolium chloride, BCIP: 5-Bromo-4-chloro-3-indolyl phosphate, toluidine salt, Roche®, Penzberg, Germany), which led to visible and quantifiable coloring.

A concentration of 15, 3.75, and 0 μg (control) HRP conjugated antibody per mL EAAB was chosen and placed on top of the activated silicone discs in a total volume of 100 μL per well. Native silicone control discs were incubated with EAAB without HRP conjungated antibody (control).

The incubation period was 90 min at room temperature; the 96-well plate was slightly rocked.

After the incubation period of 90 min the crosslinking process was discontinued by adding 1.4 μL 2-Mercaptoethanole per well. It was then washed three times with a magnesium buffer solution MBS: 0.1*M* TRIS (hydroxymethyl)-aminomethane hydrochloride, (Merck®, Darmstadt, Germany) + 0.1*M* Sodium Chloride (J.T.Baker®, Deventer, Netherlands) + 5 m*M* magnesium chloride (Merck®, Darmstadt, Germany). Totally, 100 μL of NBT/BCIP stock solution was suspended in 5 mL MBS. A total of 200 μL of this freshly prepared NBT/BCIP—magnesium buffer solution was then added to each well, on top of the HRP-conjungated antibody crosslinked silicone discs and control silicone discs. To quantify our results, we analyzed the intensity of the color reaction caused by different concentrations of crosslinked HRP conjungated antibody with an infrared imaging system (ODYSSEY®, Li-COR®).

### Proof of a bioactivated silicone surface by crosslinking an anti-FAS antibody on activated silicone discs

The silicone is first activated during the silanization process with 10% APTES in ethanol overnight, as described in Generating an active silicone surface. After cutting the activated silicone into silicone discs, these are placed into a 96-well plate. Totally, 15 μg/mL anti-FAS antibody (clone CH11, Millipore, Temecula, CA) in EAAB at a target volume of 100 μL/well were placed onto the surface of the crosslinked silicone discs (group A).

Control group B utilized silicone discs that had not undergone an activating crosslinking process but were incubated with anti-FAS-antibody. Control group C employed silicone discs that had undergone the crosslinking process but without incubation with the functional anti-FAS-antibody.

Control group D contained silicone discs that had undergone neither a crosslinking process nor incubation with anti-FAS-antibody.

After an incubation period of 1 h at room temperature, the reaction was stopped using 1.4 μL 2-mercaptoethanol per well. The silicone discs were then each washed three times using 200 μL/well PBS (Biochrom AG, Berlin, Germany). Subsequently, the 96-well plates were stored for 5 days at 4°C with slight shaking after which they were washed again with 200 μL/well PBS. On the 5th day, 5 × 104 FAS-receptor-sensitive Jurkat-T-cells were transferred to each well in 100 μL 10% FCS (fetal calf serum; Biochrom AG, Berlin, Germany) RPMI 1640 medium (Biochrom AG, Berlin, Germany) and incubated overnight at 37°C. Jurkat T-cells were consistent with acute T-cell leukemia cells and appeared morphologically as lymphoblasts. Jurkat T-cells (ATCC, American Type Culture Collection, Manassas, VA 20108, order number: CRT-2063) were incubated in RPMI 1640 (Biochrom AG, Berlin, Germany) with 10% FCS (fetal calf serum; Biochrom AG, Berlin, Germany), 10 μg/mL streptomycin (Biochrom AG, Berlin, Germany), 100 IU/mL penicillin (Biochrom AG, Berlin, Germany) and 1 m*M* sodium pyruvate (Biochrom AG, Berlin, Germany). The following day, the samples were analyzed using caspase-3 detection (APO-ONE-Kit: Promega, Madison, WV) to determine whether the crosslinking process had resulted in directional binding of the anti-Fas antibody to the activated silicone surface and whether this could induce apoptosis in FAS-sensitive Jurkat T-cells.

For this, the stock solution of the APO-ONE kit was diluted 1:8.3 with distilled water and then suspended 1:100 in the APO-ONE buffer solution. Totally, 100 μL was then transferred into each well to a total volume of 200 μL per well. This was then incubated for one hour at 37°C. Measurement and analysis were carried out using a microplate reader (TECAN US, Durham, NC 27703).

### Statistical methods

Statistical analysis was performed using SAS 9.2 (Cary, NC). The graphics were prepared using Statistica 8 (Tulsa, OK).

For the descriptive analysis, dot plots were used, yielding median, range and the individual values. For the analysis of the first two experiments we used the *p*-values of the Kruskal Wallis test as a global test and the Mann Whitney *U* test for the pairwise comparisons. For the experiment "Proof of a bioactivated silicone surface by crosslinking an anti-FAS antibody on activated silicone discs," we used as the primary hypothesis in a first step a two-way ANOVA with the factors crosslinking ± and anti-FAS-antibody ± and with the interaction of these two factors. Because the interaction tends toward significance, we subsequently used the one-way ANOVA with the factors crosslinking yes/no and anti-FAS-antibody yes/no. A *p*-value smaller than 0.05 was considered significant.

## RESULTS

### Crosslinking of a near-infrared conjugated fluorophore antibody (IRDye®-680CW) on the activated silicone surface

The detection of the antibody was concentration-dependent and detectable as a linear increase in intensity of the infrared-conjugated secondary antibody's (IRDye®-680CW) color reaction at the silicone surface (Fig. 1). At a dose of 1.5 μg/mL IRDye®-680CW, a strong color reaction with a mean

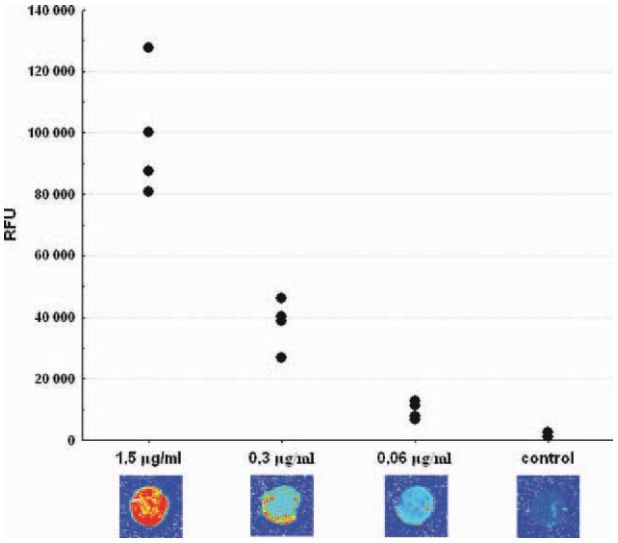


**FIGURE 1.** Quantitative analysis of the crosslinked, near-infrared-conjugated fluorophore antibody (IRDye®-680CW) on the activated silicone surface with the infrared imaging system (ODYSSEY®, Li-COR®). Additional statistical information: The *p*-values are descriptive. The *p*-values for all pair-comparisons are identical, as the various groups did not overlap. Because the Wilcoxon Mann-Whitney test is based on the ranks, the ranks for each pair comparison are identical. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

relative fluorescence unit (RFU) number of 93944 (range: min:80725 RFU, max: 127586 RFU) was detected. A reduction in the concentration to 0.3 µg/mL led to a considerable weakening in the color signal for the IRDye®-680CW to a median value of 39512 RFU (range: min: 26966 RFU, max: 46272 RFU). A further dose reduction to 0.06 µg/mL IRDye®-680CW resulted in a further drop in intensity of the color reaction during the infrared imaging system analysis (ODYSSEY® (Li-COR®), Median: 9522 RFU, (range: min:7771 RFU, max: 12738 RFU). The dose reduction was not only significant overall with a *p*-value of 0.0027, but also in the individual pair comparisons with a *p*-value in each case of 0.029.

To test whether our crosslinking process produces a stable linkage of antibodies to a silicone surface, we tested our crosslinking procedure with a near-infrared-conjugated fluorophore antibody (IRDye®-680CW, 1.5 µg/mL).

The silicone discs coated using our crosslinking procedure yielded a much higher IRDye®-680CW antibody density (mean 94508 RFU; range, 80,697–127,565 RFU) than uncoated plates without ester activation (mean: 32,407; range, 27,479–77,621), even though both procedures were carried out using identical concentrations of antibody (1.5 µg/mL). Both the global comparison ($p = 0.007$) and the paired comparisons are significant ($p = 0.029$ each) (Fig. 2).

### Detection of enzymatic activity of a vectored, crosslinked antibody on a silicone surface

To prove our concept of a bifunctional crosslinking procedure for the coupling of proteins to silicone surfaces while maintaining their functionality, we next chose an HRP conjugated antibody. Functional HRP bound to a surface has the advantage of being easily and reliably detectable and the ability of being used in other applications including directed polymerization reactions.[18]

The crosslinking of the HRP-conjugated antibody allowed a redox reaction to be generated by the addition of NBT/BCIP, the reaction product of which produced a discoloration of the silicone surface, allowing immediate macroscopic comparison with the control group (Figs. 3 and 4).

The silicone discs in the crosslinking group were prepared as described in Generating an active silicone surface, after which the HRP-conjugated antibodies were added at concentrations of 15 µg/mL and 3.75 µg/mL, followed by

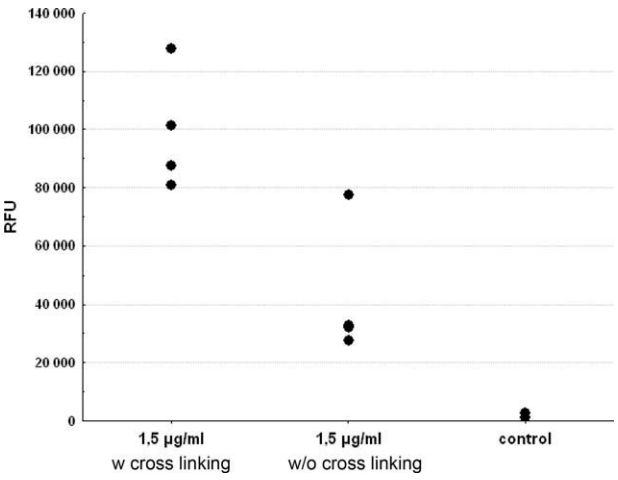


**FIGURE 2.** Quantitative analysis of the crosslinked near-infrared conjugated fluorophore antibody (IRDye®-680CW) on the activated silicone surface in comparison to application of IRDye®-680CW without the crosslinking process on native silicone surfaces. Control without IRDye®-680CW antibody. Additional statistical information see Figure 1.

**FIGURE 3.** Silicone crosslinked with HRP-conjungated antibody plus NBT/BCIP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

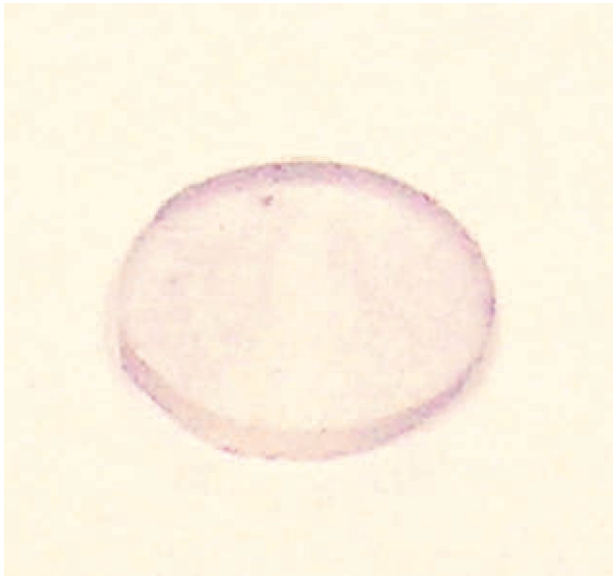

**FIGURE 4.** Native silicone disc w/o HRP-conjungated antibody plus EDC-NHS solution and NBT/BCIP, control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ester activation using EDC-NHS. They were then incubated with the activated silicone. Untreated silicone discs, disinfected with alcohol were incubated in activation buffer along with ester activation (EAAB) without the addition of HRP-conjugated antibody, in order to rule out binding effects with the silicone surface by EDC-NHS. In all four cases, an immediate discoloration of the treated discs was visible following the addition of NBT/BCIP, in comparison to the control discs (Figs. 3 and 4).

The higher dose of 15 µg yielded a measurable color shift from the redox reaction from a mean of 5262 RFU (range, 5895 RFU–4883 RFU), with the considerably lower dose showing a mean of 4372 RFU (range, 4025 RFU–4956 RFU). The measurable increase in intensity of the redox reactions between the two selected doses of 15 µg and 3.75 µg is not significant, with a *p*-value of $p = 0.057$. The quantitative analysis of the results is summarized in Figure 5.

### Proof of a bioactivated silicone surface by crosslinking an anti-FAS antibody on activated silicone discs

To demonstrate the functionality of our directional crosslinking procedure at the silicone surface, an anti-FAS antibody was coated onto the surface of the silicone so as to induce the apoptosis of FAS-sensitive Jurkat T-cells during coincubation.

If the absolute measured absolute values of the five identical experimental batches are considered, the median values of group B (+ anti-FAS-antibody, −crosslinking process; median 35465 RFU, range, 34,089 RFU–36,305 RFU) and group C (−anti-FAS-antibody, + crosslinking process; median 35,175 RFU, range, 33,813 RFU–36656 RFU) are only marginally and not significantly elevated compared to group D (−anti-FAS-antibody, −crosslinking process; median 33,997, range, 27,673 RFU–37,674 RFU). Only group A (+anti-FAS-antibody, + crosslinking process) displayed

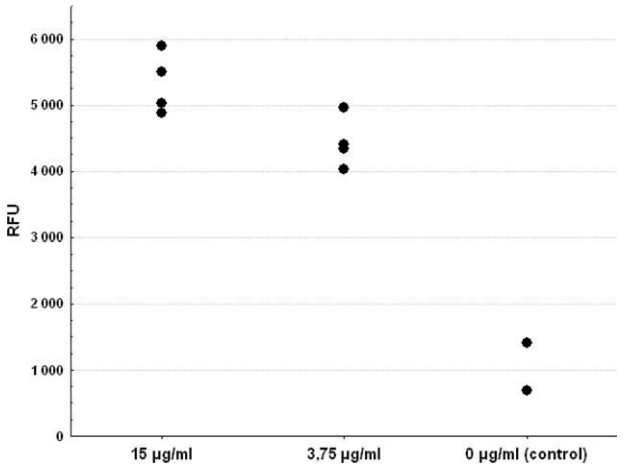


**FIGURE 5.** Quantitative analysis of the redox reaction generated with different concentrations of alkaline phosphatase conjungated anti-goat IgG with NBT/BCIP and infrared imaging system (ODYSSEY®, Li-COR®) analysis.

higher values (median 40767; range, 38,055 RFU–45,969 RFU). In the two-way ANOVA, the interaction between crosslinking and anti-FAS antibody tends toward significance ($p = 0.055$). For this reason, we used one-way ANOVA for the analysis of the effects of crosslinking, separately for anti-FAS antibody + and −, respectively.

The results showed no significant effect of crosslinking in the groups without anti-FAS antibody ($p = 0.507$) as expected, and a significant effect of crosslinking in the group with anti-FAS antibody ($p = 0.003$). Figure 6 summarizes the measured results of the caspase-3 assay.

## DISCUSSION

The industrial production of materials that can be sustainably integrated into the body has occupied the field of

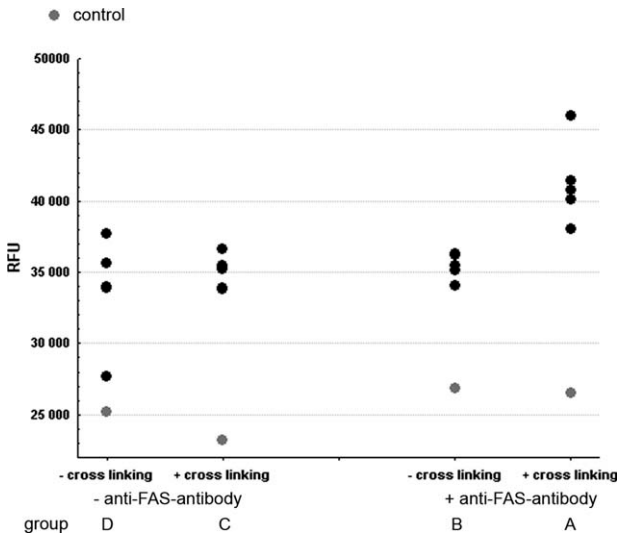


**FIGURE 6.** Our directional crosslinking procedure using anti-FAS-antibody maintains its activity following 5 days of washing with 200 l PBS per well. The measured relative fluorescence units (RFU) correspond to the detection of caspase-3 following incubation of the silicone discs with FAS-sensitive Jurkat T-cells.

materials research for decades and will continue to play a decisive role in the treatment of disease for the foreseeable future. Silicone is the foreign material most often used in human beings on both temporary and permanent bases. It is also the material for which the most scientific data are available. Silicone is a synthetic polymer with hybrid properties, which is currently unsurpassed by any other synthetic material with respect to its biocompatibility.[1]

That having been said, silicone does elicit a foreign body reaction in the human body, which can cause complications such as infection, acute rejection or painful encapsulation and deformation.[8,13,19,20] There is also evidence that subclinical bacterial infection of silicone breast implants increases the risk of capsular fibrosis.[21–24]

For this reason, numerous developments have been made to reduce the need using synthetic materials. Among these are acellular tissue scaffolds based on animal collagen, which display excellent biocompatibility characteristics;[25–27] however, the currently approved products are prohibitively expensive. The individual solutions and innovations of some research centers are only partially applicable to the clinic and are only accessible for a limited number of patients. A synthetically produced material with a level of biocompatibility comparable to acellular products would, therefore, be highly desirable for economic reasons. For certain applications, it would also be conceivable to outfit a synthetic product with biological functions. The goal of this study was to modify the most well-established foreign material, silicone, for use in implantation in human beings by modifying its surface to endow it with biocompatible properties favorably comparable to acellular materials.

To that end, we have developed a procedure for stably attaching functional proteins to the surface of silicone without damaging their biological activity. Because of its chemical and physical surface properties, the coating of silicone implant material is a challenging endeavor. The high hydrophobicity, inadequate wetting behavior and surface conditions of silicone all present extreme adhesion problems.[28] Ksander et al., who examined the incidence of capsule contracture of collagen-coated silicone implants in animals, described a significantly reduced capsule formation in the group of collagen-coated implants. The collagen coating described in the study of Ksander et al. was crosslinked with formaldehyde and glutaraldehyde.[29] In addition to the fact that these chemical additives are problematic in terms of cytotoxicity and their carcinogenic properties, the authors were faced with the fact that the coatings were imperfect. The authors described the coatings as rather thick, inflexible and showing defects due to adhesion problems.[30] In their study, they hypothesize that the development of better, more persistent coatings will permit prolonged inhibition of capsule formation. Other coating procedures utilize high temperatures or a combination of high temperature and pressure to achieve their goal.[31] Proteins, including antibodies, enzymes, and structural proteins possess long amino acid sequences with complex tertiary and quaternary structures and usually perform highly specialized tasks in cells. Regarding their functions, the high specificity and strength of the antibody-antigen interaction can be applied to target nanoscale building blocks onto desired locations for the construction of complex nano-architectures.

High temperatures, particularly under pressure undeniably lead to changes in the three-dimensional structure of proteins, damaging their functionality.[32]

Our goal, therefore, was to develop an effective, stable procedure for coating a silicone surface without the need for elevated temperatures above 37°C and without the need for excessive pressures, both of which can damage or destroy the functionality of a protein. We first performed a silanization of the silicone surface in pure alcohol using 10% APTES. We combined the sialinization with ester activation (EDC/NHS), which had the effect of directionally placing reactive amino groups onto the surface of the silicone. These can then stably bond to any free carboxyl group. This has the effect of producing a directional crosslinking, as the Fc-fragment of the antibody can then bind to the reactive amino group on the silicone surface.

After the silicone is sterilized, this crosslinking procedure can be performed under sterile conditions, so that the functional proteins are not damaged by the sterilization of the silicone itself. In our first experiment Crosslinking of a near-infrared-conjugated fluorophore antibody (IRDye®-680CW) on the activated silicone surface, we were able to demonstrate dose-dependent bonding of the IRDye®-680CW antibody using an infrared imaging system (Fig. 1). Furthermore, antibody was detected at a much higher density on the silicone surface in comparison to identical tests without the crosslinking process (Fig. 2).

To demonstrate the enzymatic activity of the protein following the crosslinking procedures, we employed an HRP-conjugated antibody together with NBT/BCIP to initiate a redox reaction. The instantly visible color shift was both macroscopically and quantitatively (infrared imaging system) detectable (Figs. 3–5). This experiment not only allowed us to demonstrate that the enzymatic activity of the antibody was still present, but also that the crosslinking was directional. Numerous studies have been carried out examining the capsule fibrosis of breast implants.

They have suggested the possible cause to be found at the interface between the silicone and the surrounding tissue.[33–35]

The immunological cell interaction of the tissues and the foreign material at this interface seems to play a pivotal role.[36] The tissue response to a biocompatible material follows a well-defined course of events following the injury itself. Tissue destruction is followed by acute inflammation, formation of granulation tissue, and finally repairs in the form of collagen deposition. The extent of this response depends on various material characteristics as well as the presence of various cytokines, growth factors and proteins.[37]

Variable numbers of inflammatory cells have been noted in the peri-implant fibrous tissue around silicone implants.[38] Histological studies by Wolfram et al. on explanted breast implants found a strong presence of CD4+ cells, macrophages, as well Langerhans-cell-like dendritic cells (DCs).[39] The development of a fibrous barrier between

the foreign material and the surrounding tissue is the morphological consequence of insufficient biocompatibility. As summarized in the current review by Wick et al., fibrosis is always accompanied by an activation of the immune system. Inflammation mediated by Th2 cells leads to the formation of fibrosis.[40] It is therefore possible, that a T-cell specific cellular interaction at the interface between the silicone and the surrounding tissue plays a key role in the foreign body reaction.[41–43] For this reason, we coated the silicone surface with an anti-FAS antibody in our experiment Proof of a bioactivated silicone surface by crosslinking an anti-FAS antibody on activated silicone discs. To further test the stability of the crosslinking process, following the initiation of the stop reaction with 2-mercptoethanol, the silicone discs used in the experiments were then subjected to three rounds of rinsing with 200 μL PBS per well and then gently agitated for 5 days at 4°C. The discs were additionally rinsed twice daily with 200 μL PBS per well. On the fifth day an overnight coincubation with the FAS-sensitive Jurkat-T cells was performed.

The following an apoptosis assay using the detection of caspase-3 was performed. The apoptosis was triggered by the FAS/FAS-ligand interaction.[41,42] Group A (silicone discs + crosslinking, + FAS-antibody + Jurkat-T cells; showed an increase in caspase-3 levels in comparison to the control group exclusively ($p = 0.003$). This demonstrated the continued biological function of the FAS-antibody directionally connected to the silicone surface. It also proved the functionality of the method itself.

It may be possible to initiate an immunosuppressive effect on the silicone surface by vectored coating of an anti-Fas antibody. A further advantage could be the directional coating of antimicrobial substances such as cathelicidin peptides[44] which could lead to a suppression of subclinical bacterial infection. This subclinical bacterial infection have been regarded as an important pathogenetic factor for capsular contracture of breast implants.[22,23]

Numerous studies have examined the influence of the surface morphology of breast implants. In a study by Barr et al.[5], the surfaces of common implants were compared. It revealed a slight advantage for fibroblast alignment in terms of textured implants compared with smooth implants. In our experiments we started our coating procedure with a vectored binding on a shell of a smooth silicone implant. Further studies are warranted also using textured silicone implants.

Numerous medical devices are routinely either temporarily or permanently implanted in humans. We are convinced that the further development of the materials research will result in new possibilities for placing foreign materials in the body. This will open man novel avenues of treatment.

It is not only induction of apoptosis that stands to profit from the functionalization of silicone surfaces: numerous other functions cab be conceived to increase biocompatibility, such as the development of synthetic vascular prostheses that are endowed with anticoagulative properties along with trigger substances that can effect the local differentiation of tissues into native vascular endothelium. Animal studies are planned and will investigate whether the surface activation of silicone with functional proteins can improve the biocompatibility of this material *in vivo*.

## CONCLUSION

In summary, our crosslinking procedure results in the stable bonding of functional proteins. The linked antibodies are directed such that their Fc-fragment is bound to the silicone surface, thereby preserving their biological functionality. Our crosslinking process did not influence the biological function of the antibody tested in our study.

We consider our crosslinking process for the coating of silicone surfaces to be a simple, easily reproducible procedure for functionalizing silicone surfaces with the potential of improving the biocompatibility of this synthetic material.

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