Open Access

Cécile Boudot*, Sarah Burkhardt and Miriam Haerst

Long-term stable modifications of silicone elastomer for improved hemocompatibility

DOI 10.1515/cdbme-2016-0008

Abstract: Silicone elastomers are well established in medical engineering and particularly in blood-contacting applications such as catheters and medical tubing. Still, their intrinsic surface properties have potential for improvement. For example, hydrophobicity reduction can be a way to provide better hemocompatibility. In this study, several bulk and surface modifications of silicone elastomers using polyethylene glycol (PEG) were investigated. All modifications induced long-term (2 months), stable wettability of the surface. Moreover, cytotoxicity testing demonstrated their suitability as implant material. Hemocompatibility was investigated through a thrombin generation assay as well as a platelet adhesion study combining an enzymatic assay and a scanning electron microscope analysis. That the hemocompatibility of silicone was considerably improved thanks to the PEG modifications could be shown. The study introduces easily processable, cost-efficient, and long-term stable hydrophilic modifications of silicone elastomer for improved hemocompatibility.

Keywords: cytotoxicity; hemocompatibility; long-term stability; polyethylene glycol; Silicone elastomer; wettability.

1 Introduction

Silicone elastomers are of major interest for medical applications due to their high chemical- and physical-resistance properties combined with elasticity. Silicones in which the organic substitutes are methyl groups are the most widely used silicone elastomers [1]. These silicones are also known as PDMS (polydimethylsiloxanes). High chemical stability enables prolonged use with constant properties despite diverse influences such as hygienic reprocessing and watery environment. PDMS is furthermore referred to as nontoxic [1]. This is why it is for example used in medical tubing, catheters, sealing, or grips on medical devices.

Unmodified PDMS surfaces are hydrophobic and tend to generate uncontrolled protein adsorption, which could induce thrombogenic behavior under specific circumstances [2, 3]. Hydrophilic modifications of PDMS using passivating polyethylene glycols (PEGs) seem to be a promising strategy to improve silicone's biological surface properties. PEG is well known for its excellent antifouling and antithrombogenic properties [2–4]. However, the use of PEGs is limited since they are prone to thermal and oxidative degradation [5]. Moreover, PEG-modified silicones often suffer from so-called hydrophobic recovery [6]. Easily processable, stable, and cost-effective modifications of PDMS for biomedical applications are still lacking.

The following study aims at using PEG to hydrophilize silicone surfaces for long-term blood-contacting applications.

2 Material and methods

2.1 Silicone modification

Wacker Chemie AG, Germany, has provided medical-grade high-temperature-vulcanizing (HTV) silicone elastomer (Silpuran® UR 9030/60). This PDMS was used in this study as unmodified reference material and as substrate for the surface modifications. Reference samples are designated below as "Sil". Vulcanization was carried out in a heating press at 200°C, 50 bars for 10 min.

As an example of bulk modification, a commercially available compound containing a chemically connected polyethylene-glycol-siloxane copolymer was used (Elastosil® RT 629, Wacker Chemie AG, Germany). This room-temperature-vulcanizing (RTV) silicone was vulcanized at 120°C for 30 min and is designated below as "RTV+PEG." To demonstrate the effectiveness of this PDMS-PEG copolymer, additional samples of the reference silicone Silpuran® UR 9030/60 containing the

***Corresponding author: Cécile Boudot,** Institute of Medical and Polymer Engineering, Technical University of Munich, Boltzmannstr. 15, 85748 Garching, Germany, E-mail: cecile.boudot@tum.de
Sarah Burkhardt and Miriam Haerst: Institute of Medical and Polymer Engineering, Technical University of Munich, Boltzmannstr. 15, 85748 Garching, Germany

same copolymer as Elastosil® RT 629 have been provided by Wacker Chemie AG, Germany (designated below as "HTV+PEG").

After preparation, the samples were post cured (200°C, 4 h), thoroughly cleaned, and dried at room temperature for 24 h. The samples not used for silanization were sterilized with hot steam (121°C, 20 min, 2 bars).

Beside the previous bulk modifications, a coating using an organofunctional silane with PEG function (2-[methoxy (polyethylenoxy)6-9 propyl] trimethylsiloxane, ChemPur, Germany) was prepared. The silicone surface was first oxidized with atmospheric pressure plasma (PT-60, Plasmatreat GmbH, Germany). The silane was dissolved in ethanol and acetic acid at a volume ratio of 1:2:50 (acetic acid:silane:ethanol) and incubated on the oxidized silicone for 5 min at room temperature then heated for 1 h at 110°C. This procedure enables covalent binding of the PEG function on the silicone surface. This sample was designated as "Sil-PEG." In addition, a coating using amino-functional silane (APTES, Genosil® GF93, Wacker Chemie AG, Germany) was used as positive control for cell adhesion studies [7] and a coating with succinic acid functional silane (Genosil® GF20, Wacker Chemie AG, Germany) was used as positive control for the thrombin generation study [8]. The designations of those samples are "Sil-GF93" and "Sil-GF20."

After coating, the samples were washed for 20 min in an ultrasonic deionized-water bath, dried, and sterilized with hot steam (121°C, 20 min, 2 bars).

2.2 Suitability as implant material

2.2.1 Long-term stable wettability

Contact angle (CA) measurement gives information about the influence of modifications on the silicone samples' wetting behavior, which was determined with drops of 2 µl of distilled water at a dosing rate of 2 µl/s. The CA was recorded after drop spreading stabilized on the surface (Contact Angle System OCA and SCA20 software, Dataphysics GmbH, Germany).

According to ISO 10993-1, long-term implants remain in the patient's body for more than 30 days [9]. In this study, the stability of the modifications was tested through storage in simulated body fluid (SBF, produced according to [10]) at 37°C for 2 months. Finally, the wetting behavior of the stored samples was investigated (contact angle measurement) and compared with samples of the same materials that did not undergo SBF storage.

2.2.2 Cytotoxicity

Verifying that the modified silicones do not induce cytotoxic effects is necessary to investigate their suitability as implant material. Cytotoxicity was evaluated according to ISO 10993-5 [9] using human fibroblasts Hs27 (CRL-1643™, ATCC®, UK). Material samples were extracted in cell culture medium (DMEM, 5% FBS, 1% Pen/Strep, 1% Amphotericin B, Merk KGaA, Germany) for 72 h at 37°C at a surface-volume ratio of 1.5 cm^2/ml. Subconfluent fibroblasts (5000 cells/cm^2 in a 96-well microplate) were then incubated with 200 µl material extracts for 48 h at 37°C. Potential cytotoxic substances can reduce the number and vitality of cells during incubation. For the quantitative evaluation of cell vitality, the colometric assay CCK8 (Dojindo EU GmbH, Germany) was used and photometric absorption was recorded at 450 nm with an ELISA reader (Multiskan FC, Thermo Scientific, USA). Two additional samples (blank and copper) were used as negative and positive controls.

2.3 Hemocompatibility

2.3.1 Platelet adhesion

The adhesion of platelets on material surfaces is a key phenomenon in the formation of thrombi and a fundamental aspect of hemocompatibility. Platelets were isolated from freshly withdrawn blood through two-step centrifugation (6 min at 1750 g and 9 min at 2250 g). Citrate was used as the anticoagulant. The platelet suspension's concentration was adjusted to approximately 500×10^3/µl and incubated for an hour with the material samples in a flow chamber. Incubation under physiological flow conditions is essential for the investigation of platelets, because their adhesion is triggered by generated shear rates [11]. After incubation, the quantification of adhered platelets on the sample surface was carried out with the acid phosphatase (ACP) assay, as described by Bellavite et al. [12]. The colorimetric substrate (p-nitrophenyl phosphate) was purchased from Sigma Aldrich Chemie GmbH, Germany, and the photometric measurement was conducted with an ELISA reader (Multiskan FC, Thermo Scientific, USA).

A SEM analysis (JSM-6390, JEOL GmbH, Germany) was carried out after incubation of the platelet suspension under the same conditions as those for the ACP assay. The samples were fixed 24 h in 3% glutaraldehyde solution and water was removed with an ascending alcohol series (50% 15 min, 70% 15 min, 80% 15 min, 99% 12 h). The samples were then coated with a 7 nm gold layer.

2.3.2 Thrombin generation

Activation of the coagulation cascade after contact with a biomaterial is another important aspect of hemocompatibility. The thrombin generation assay (TGA) was carried out in this study. Unlike the platelet adhesion test, this experiment should be conducted under static flow conditions. It has indeed been shown that the coagulation cascade can only be initiated under static or low flow conditions [13] so that thrombin production is greatest in stagnant plasma [14]. Freshly withdrawn blood with citrate as anticoagulant was processed into platelet-poor plasma through two centrifugation steps (6 min at 1750 g and 9 min at 2250 g). The TGA test was carried out as described by van Oeveren et al. [15]. The colorimetric substrate (S2238™) was purchased from Haemochrom Diagnostica GmbH, Germany, and the photometric measurement was conducted with an ELISA reader (Multiskan FC, Thermo Scientific, USA).

3 Results

3.1 Suitability as implant material

3.1.1 Long-term stable wettability

Investigation of the wetting behavior showed that all the PEG-based modifications resulted in a strong diminution of the contact angle (under 90°; unmodified silicone 119°). Moreover, hydrophilic behavior was stable during the 2-month storage period in SBF (see Figure 1), showing that the modifications suffer neither from the so-called hydrophobic recovery nor were they degraded during storage.

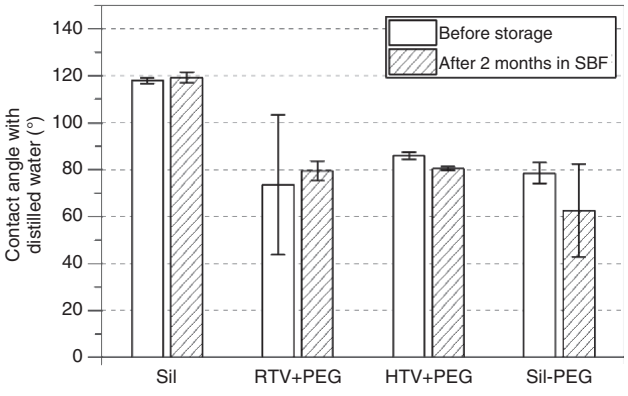

Figure 1: Contact angle with distilled water before and after storage of the sample in SBF (n = 10).

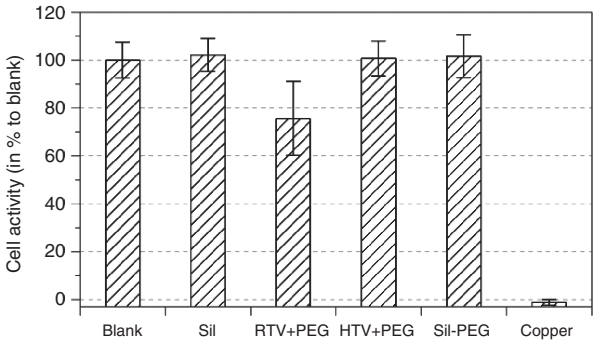

Figure 2: Cytotoxicity of the samples (n = 10).

3.1.2 Cytotoxicity

According to ISO 10993-5, a reduction of cell vitality under 70% of the value of the blank sample means that the materials exhibit a cytotoxic effect. Consequently, all tested samples could be considered noncytotoxic (see Figure 2).

3.2 Hemocompatibility

3.2.1 Platelet adhesion

Regarding this aspect of hemocompatibility, it could be shown that all PEG-based modifications caused a significant reduction of platelet adhesion onto the surface. The number of adhered platelets was reduced by more than 70% compared to the unmodified silicone samples (see Figure 3).

The SEM analysis confirmed those findings. Numerous adhered platelets in an advanced stage of adhesion (spreading of pseudopodia) were found on the unmodified silicone sample. Conversely, very few platelets were found on the PEG-modified surfaces (see Figure 4).

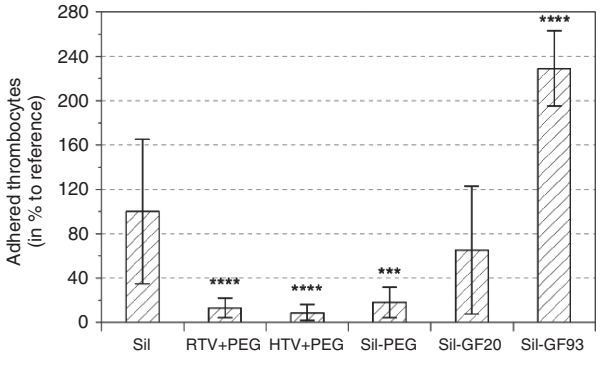

Figure 3: Platelet adhesion normalized to reference sample, ACP assay (n = 8). *p < 0.5, **p < 0.1, ***p < 0.05, ****p < 0.001 (Kruskal-Wallis ANOVA).

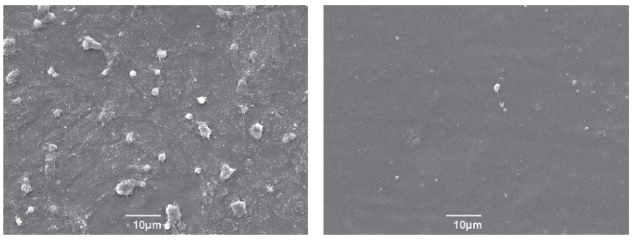

Figure 4: SEM images (×1500) of the pure silicone surface "Sil" (left) and the modified silicone surface "HTV+PEG" (right) after incubation with platelets. The white shapes on the surface are adhered platelets. The white bar corresponds to 10 µm.

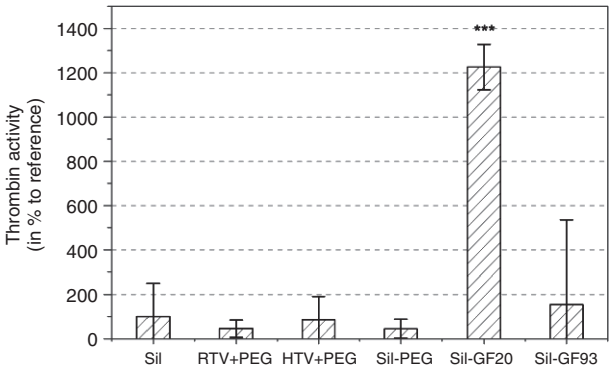

Figure 5: Thrombin generation normalized to reference sample, ACP assay (n = 7). *p < 0.5, **p < 0.1, ***p < 0.05, ****p < 0.001 (Kruskal-Wallis ANOVA).

3.2.2 Thrombin generation

Investigation of thrombin generation showed that the unmodified silicone reference did not exhibit pronounced activation of blood plasma (see Figure 5). As a consequence, it was necessary to verify that the modifications did not induce an additional activation. Only the positive control (surface coating with acid function) provoked very strong thrombin generation (12 times greater than that of the reference).

4 Conclusion

In this study, simple PEG-based modifications of silicone elastomer that exhibit long-term stable hydrophilic properties were introduced. Two modified silicones (one with an additive compound and the other with a surface coating) are well suited for use in the blood stream, because they do not induce cytotoxic effects and improve hemocompatibility. Moreover, those materials can be industrially processed as their properties were stable after post curing and sterilization. The additive compound is particularly interesting, because it does not require an additional processing step for the HTV silicone. For example, this new material could be easily extruded to form a catheter or tubing for dialysis and extracorporeal circulation.

Author's Statement

Research funding: The author state no funding involved. Conflict of interest: Authors state no conflict of interest. Material and Methods: Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

References

[1] Pachaly B, Achenbach F, Herzig C, et al. Silicone. In: R. Dittmeyer, W. Kein, G. Kreysa et al., editors. Chemische Technik, Wiley-VCH Verlag, Weinheim, 2005:1095–213.
[2] Li M, Neoh KG, Xu LQ, Wang R, Kang E-T, Lau T, et al. Surface modification of silicone for biomedical applications requiring long-term antibacterial, antifouling, and hemocompatible properties. Langmuir. 2012;28: 16408–22.
[3] Chen H, Zhang Z, Chen Y, Brook MA, Sheardown H. Protein repellant silicone surfaces by covalent immobilization of poly (ethylene oxide). Biomaterials. 2005;26:2391–9.
[4] Ross AM, Griffith CD, Anderson JR, Grieve D. Thromboembolic complications with silicone elastomer subclavian catheters. J Parenter Enteral Nutr. 1982;6:61–3.
[5] Arima Y, Toda M, Iwata H. Complement activation on surfaces modified with ethylene glycol units. Biomaterials. 2008;29:551–60.
[6] Pinto S, Alves P, Matos CM, Santos AC, Rodrigues LR, Teixeira JA, et al. Poly (dimethyl siloxane) surface modification by low pressure plasma to improve its characteristics towards biomedical applications. Colloids Surf B. 2010;81:20–6.
[7] Vogler EA, Graper JC, Harper GR, Sugg HW, Lander LM, Brittain WJ. Contact activation of the plasma coagulation cascade. I. Procoagulant surface chemistry and energy. J Biomed Mater Res. 1995;29:1005–16.
[8] Faucheux N, Schweiss R, Lützow K, Werner C, Groth T. Self-assembled monolayers with different terminating groups as model substrates for cell adhesion studies. Biomaterials. 2004;25:2721–30.
[9] DIN EN ISO 10993: Biological evaluation of medical devices. 2009
[10] Kokubo, T, Takadama, H, How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. 27, 2006, 2907–15.
[11] O'Brien JR. Shear-induced platelet aggregation. Lancet. 1990;335:711–3.

[12] Bellavite P, Andrioli G, Guzzo P, Arigliano P, Chirumbolo S, Manzato F, et al. A colorimetric method for the measurement of platelet adhesion in microtiter plates. Analyt Biochem. 1994;216:444–50.

[13] Kastrup CJ, Shen F, Runyon MK, Ismagilov RF. Characterization of the threshold response of initiation of blood clotting to stimulus patch size. Biophys J. 2007;93:2969–77.

[14] Shen F, Kastrup CJ, Liu Y, Ismagilov RF. Threshold response of initiation of blood coagulation by tissue factor in patterned microfluidic capillaries is controlled by shear rate. Arterioscl Throm Vas. 2008;28:2035–41.

[15] Van Oeveren W, Haan J, Lagerman P, Schoendd P. Comparison of coagulation activity tests in vitro for selected biomaterials. Artif Organs. 2002;26:506–1