Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Silicone grafted bioactive peptides and their applications

Julie Martin[1], Jean Martinez[1], Ahmad Mehdi[2] and Gilles Subra[1]

As bioinert material, silicone has to be modified to display suitable biological properties. Peptides are attractive additives for such a purpose. Most of the time, several steps are needed for the synthesis of the peptide-modified silicone: first the silicone surface is modified to display a reactive function, then a bifunctional spacer can be grafted, and finally, the peptide is attached on the reactive group. However, some other alternatives exist to reduce the number of steps, involving either the synthesis of modified silicones by copolymerization, or the use of silylated peptides. This review present the different pathways and the conjugation chemistries developed so far to afford peptide silicones conjugates.

Addresses
[1] IBMM, Univ Montpellier, CNRS, ENSCM, Montpellier, France
[2] ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier, France

Corresponding author: Subra, Gilles (gilles.subra@umontpellier.fr)

Current Opinion in Chemical Biology 2019, **52**:125–135

This review comes from a themed issue on **Synthetic biomolecules**

Edited by **Johan Winne** and **Annemieke Madder**

For a complete overview see the Issue and the Editorial

Available online 3rd August 2019

https://doi.org/10.1016/j.cbpa.2019.06.012

Introduction

Polydimethylsiloxane (PDMS) is a synthetic organic/inorganic polymer. Thanks to its interesting mechanical and chemical properties, such as high flexibility, thermal, electrical and chemical stability [1], optical transparency and oxygen permeability [2], it is widely used in industrial applications [3]. Besides, being a bio-compatible synthetic polymer [4], it is one of the most used polymers for bio-applications especially for implants and other medical devices. Compared to other FDA-approved polymers such as polyethylene glycol (PEG) or polylactic acid (PLA), the flexibility and the transparency of PDMS make it particularly suitable for the design of soft devices for ophthalmologic applications (contact lenses, artificial cornea) or blood contacting devices such as catheters [5].

PDMS can be used as silicone oil or can be cross-linked to yield a solid material. In the latter case, it requires functionalization of the silicone oils with vinyl ($Si–CH=CH_2$) and silane (Si–H) groups, which may react by hydrosilylation to create bridges between the PDMS polymer chains [6].

Despite its huge popularity, PDMS suffers from some drawbacks. First, it is highly hydrophobic and this may lead to non-specific adhesion of biomolecules, in particular lipids and proteins [7]. The fouling of a PMDS device can be problematic by provoking unwanted biological reactions triggered by the adsorbed biomolecules [8]. It is thus desirable to improve the hydrophilicity of the surface, resulting in a reduced protein adsorption and improved biocompatibility.

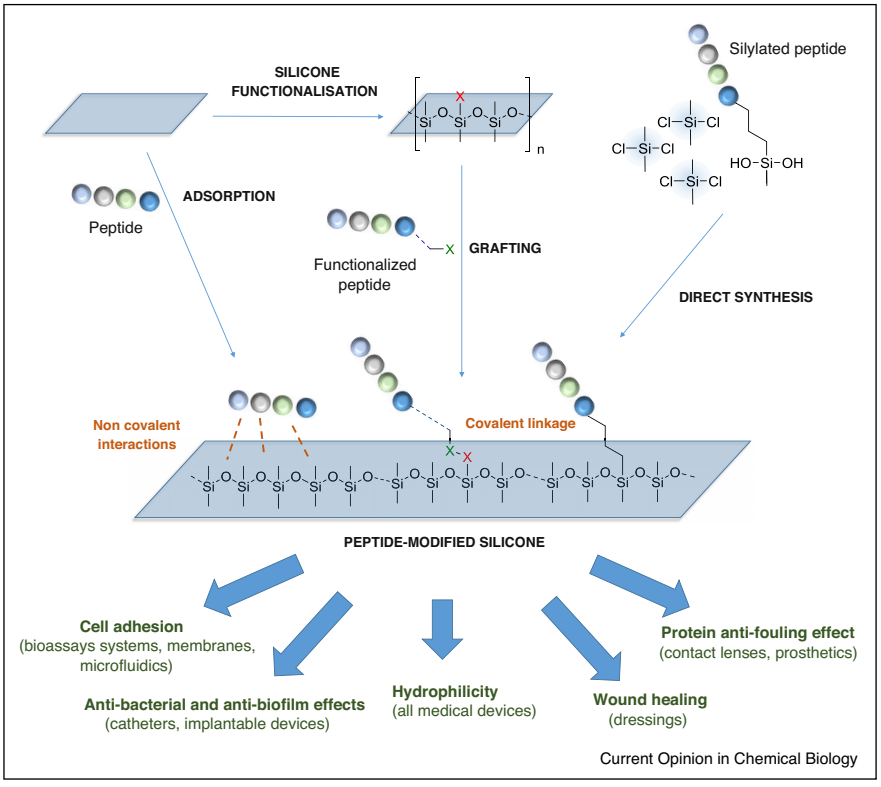
Then, like most synthetic polymers, PDMS is bio-inert. Therefore, it has to be functionalized to reach the requirements of biological and therapeutic applications. PDMS surface can be functionalized by simple adsorption of relevant (bio)molecules or modified covalently generally using an activation step (e.g. plasma, UV) followed by multistep conjugation chemistries. These post-functionalization approaches were already performed with a wide range of compounds including simple organic molecules (e.g. fluorescein to study attachment of bacteria on silicone) [9], polymers such as PEG to confer hydrophilic properties [10] and polysaccharides (e.g. oxidized dextran or hyaluronic acid) [11,12] which limit non-specific adsorption and enhance biocompatibility. Proteins were also immobilized on silicone surface. Collagen was used to get PDMS surfaces suitable for fibroblasts culture [13] and, trypsin-functionalized PDMS microchannels were used as enzymatic microreactors for MS analyses [14•].

Peptides are good candidates for bio-functionalization of PDMS. Indeed, they exert a wide range of biological activities which improve the properties of silicone devices notably changing their hydrophilicity, cell-compatibility or adding antifouling behavior. Moreover, their small size and their ease of synthesis, comparatively to proteins or biopolymers, allow the introduction of chemical functions enabling selective conjugation to the silicone material.

Peptide adsorption on silicone surface

It is possible to obtain bioactive PDMS without any chemical modification, by non-covalent adsorption of the biomolecule on the silicone surface. This simple process, generally performed by dip coating, is sufficient to change the PDMS properties. For example, hydrophobicity and consequently non-specific adsorption yield can be increased by adsorption of amino acid homo oligomers. Indeed, cationic

Figure 1



Peptide-modified silicone and its applications.

PEG-DOPA-PolyLysine (DOPA: L-3,4-dihydroxyphenylalanine) conjugate was coated on untreated PDMS leading to a material with improved lubrication properties [15]. Because of numerous non-covalent interactions, macromolecules (e.g. biopolymers) can be adsorbed efficiently on bare PDMS surface. This is not the case with smaller molecules such as peptides, which do not bind strongly on silicone surface and are quickly desorbed in biological media. In that case, the PDMS has to be previously modified to generate additional interactions. Oxidation is the most common modification of PDMS. Operated by air plasma, oxidation generates mainly Si–OH moieties from siloxane chain breakage or Si-CH_3 modification. Using this process, the peptide H-[(Ala-Glu-Ala-Glu-Ala-Arg-Ala-Arg)$_2$]-OH (EAR16-II) was adsorbed by dip coating, with the help of ionic interactions between the guanidine groups of Arginine side chains and silanolates (Si–O^-) on the PDMS surface. Adopting a β-sheet conformation, EAR16-II peptides self-assemble into parallel or anti-parallel fibers onto the PDMS surface because of the presence of both the positive as well as the negative charges from arginine and glutamic acid side chains respectively. Such peptide-modified silicone was used to prepare microfluidic devices with low non-specific adsorption of proteins [16].

Plasma oxidation is also the first step of covalent modification of PDMS surfaces required for peptide adsorption. For instance, perfluorinated triethoxysilylalkanes are covalently grafted onto the silanols of PDMS plasma activated surface, by SiOSi condensation [17,18]. This new perfluorinated layer of allows the selective and strong adsorption of fluorine-tagged-peptides, thanks to the hydrophobic nature of the perfluorinated alkyls moieties. In contrast, non-fluorinated peptides and proteins (e.g. insulin, ubiquitin) are much less adsorbed on these treated PDMS surfaces.

UV light activation of PDMS surfaces, generate radicals on the methyl groups (Si–$CH_2^{\bullet}$). This activation enables the immobilization of acrylic acid (AA) by radical coupling. The resulting anionic surface (PDMS-COO^-) enables the self-assembly of a monolayer of poly (diallyldimethylammoniumchloride) (PDDA) by ionic interactions. Quaternary ammoniums from the PDDA layer are also used to create ionic interactions with the anionic groups of proteins. Trypsin, a serine protease, was immobilized on such PDDA-modified PDMS and used as active microfluidic device for on-line protein digestion and analysis [14•].

Alternatively, instead of coating a biomolecule on the PDMS surface, it is possible to load it into porous PDMS. This is an attractive strategy for controlled drug delivery

applications, the biomolecule diffusing in a passive way through the porous PDMS layer. Porous PDMS nanoparticles (NPs) can be obtained by infiltration of the polymer into sacrificial silica porous NPs. Subsequently, the silica phase is dissolved by HF treatment. To date, only doxorubicin has been loaded in such nanoparticles, which could also accommodate peptides [19].

To favor a long-term biological effect and to avoid the release of the active moiety, the linkage between the peptides and the PDMS has to be covalent. While the simple coating of drugs and bioactive molecules on PDMS can be quite straightforward, their covalent anchoring is much more challenging.

Peptide grafted silicone

PDMS does not display any suitable function for further covalent modification. Consequently, the establishment of a covalent bond between PDMS and any other compound, including peptides, requires either generation of a reactive function on non-functionalized PDMS (e.g. Si–OH like in the case of non-covalent coatings), or the preparation of functional PDMS by copolymerization of functional monomers with non-functionalized monomers such as dimethyldichlorosilane and hexamethyl(cyclotrisiloxane) [6].

Theoretically, a peptide could be directly conjugated onto such functionalized PDMS, but all examples reported so far use an additional spacer, which can also be a polymer, to link the peptide to the PDMS surface. This spacer (Y-spacer-Z) is first coupled to the PDMS backbone and still presents a suitable organic (Z) function that allows the conjugation with the peptide.

First, we will present the methods to obtain PDMS displaying reactive functions by post-modification (Section 'PDMS activation to generate functions'), and by direct preparation of functional-PDMS by copolymerization (Section 'Direct synthesis of functional PDMS by copolymerization'). Then, the functionalization by spacers adapted to each type of modified silicone will be presented (Section 'Post-functionalization of activated silicone by a spacer'). Finally, peptide-conjugation chemistries that are accessible to the spacer-modified PDMS will be described (Section 'Grafting of peptides on functionalized silicones').

PDMS activation to generate functions

Generation of Si–OH functions

As already stated, oxygen [11,16–18,20–22,23••,24] and water plasma [25,26] or H_2O_2 [27] oxidation lead to the formation of Si–OH groups at the surface of the PDMS, either by replacing one or two Si–Me group on the same silicon atom, or by cleaving a Si–O–Si bond of the silicone backbone [28]. This type of activation is fast (i.e. 30 s to a few minutes) and efficient, and may double the oxygen content of the silicone [29]. Si–OH functions may react readily with a range of organosilane reagents to fix a desired function on the surface by further condensation with organosilane derivatives, yielding a Si–O–Si bonds. Aminopropyltriethoxy or trimethoxysilane or (APTES or APTMS) are commonly used to generate a primary amine function at the surface of the PDMS (see Section 'Post-functionalization of activated silicone by a spacer').

Generation of Si-H functions

Si-H functions can be generated by triflic acid (CF_3SO_3H) treatment of PDMS surfaces. This function may react with unsaturated compounds by hydrosilylation using Karstedt's Pt catalyst yielding Si-alkyl covalent bonds. Bifunctional PEGs bearing both an allyl and a *N*-hydroxysuccinimide (NHS) activated carboxylic acid, was used to obtain NHS-functionalized PDMS able to anchor amino functionalized peptides [12,30–32].

Generation of radicals (Si–X=. Si–$CH_2^•$ or Si–$O^•$)

Radicals can be generated on the backbone and surface PDMS, to react, for example, with allyl-containing compounds initiating free radical polymerization (FRP) [33]. Free radicals can be generated either by direct argon plasma activation or with a photo-initiator.

Si–$CH_2^•$ radical generation by UV irradiation and photo-initiation

Acrylic acid was successfully polymerized by FRP on the surface of PDMS using benzophenone as radical initiator, yielding Si–$CH_2^•$ radicals [34•,35]. The resulting polyacrylic acid (PAA) modified PDMS was further functionalized with peptides, thanks to the activation of carboxylic acids (cf. Section 'Active ester functionalized PDMS. Z = CO-NHS (or CO-Act, Act being electron attractor and leaving group), W = NH_2'). Noteworthy, Si–$CH_2^•$ radicals can be generated on PDMS directly by UV irradiation [14•,36••]. In this case, the PDMS was immersed into a solution containing acrylic acid, for example, and then UV-irradiated to react with allyl monomers.

Si–$CH_2^•$ radical generation by argon plasma activation

Alternatively, argon plasma generates free radicals at the PDMS surface, which are converted into hydroxyl, carboxylic acid, C=O function or Si–$O^•$ radical when in contact with air [33]. It is also possible to use ionic argon plasma to activate the surface in order to gain more stability over time thanks to the ion-beam [13,37]. Then hydroxyl and radical modified PDMS can be functionalized by grafting allyl-containing monomers such as allyl glycidyl ether (AGE) [38], which can also be polymerized on the activated surface by FRP [39]. In the latest case, the epoxy groups were useful for subsequent peptide immobilization (cf. Section 'Alcohol functionalized PDMS, Z = OH, W = NH_2').

Direct synthesis of functional PDMS by copolymerization

Copolymerization of dichlorodimethylsilane with methylsilane bearing a functional group yields PDMS chains displaying organic functions. Strictly speaking, these copolymers are not PDMS but functionalized polysiloxanes. Several functions have been introduced in this way including azide for click-chemistry reactions [40]. However, only thiol-modified and vinyl-modified PDMS obtained by copolymerization have been used for subsequent modification with peptides.

Copolymerization of dichloromethylmercaptopropylsilane with dichlorodimethylsilane affords poly[(3-mercaptopropyl)methylsiloxane-*co*-dimethylsiloxane] [9]. This SH-containing polymer may undergo any thiol-ene reaction (either Michael or free radical addition). As example, glycidyl methacrylate (GMA), along with other acrylate-based monomers, has been polymerized on SH-modified PDMS by UV-catalyzed free radical thiol-ene polymerization. Therefore obtaining a vinyl-modified PDMS such as polystyrene-block-poly(dimethylsiloxane-vinylmethylsiloxane) (PS-*b*-P(DMS-VMS) [41••] by copolymerization is also described. This polymer was further functionalized by thiol-ene addition with SH-containing peptides (e.g. Fmoc-[(Lys(PEG_3)-Lys(octanoate)]$_3$-Cys-OMe or Fmoc-[Lys(PEG_3)]$_3$-[Lys(octanoate)]$_3$-Cys-OMe, amphiphilic oligopeptides).

Post-functionalization of activated silicone by a spacer

Starting from a primary functional group (see Section 'PDMS activation to generate functions', X = OH, H or radical), bifunctional spacers (Y-spacer-Z) of different lengths can be introduced. One function (Y) reacts with the silicone surface, while the other one (Z) constitutes the conjugation point for the peptides bearing a complementary reactive group (i.e. W-peptide, see Figure 2)).

Reaction between Si–X = Si–OH and Y = Si(OR)

As already stated, silanols (Si–OH) react readily with functional alkoxysilanes such as sulfobetaine silane [22] or perfluorooctyltriethoxysilane [17,18]. The resulting functionalized PDMS material are popular for non-covalent binding of perfluorinated peptides. To achieve covalent modification of PDMS with biomolecules and biopolymers, the APTES spacer was preferred [11,20,25,26]. It affords primary amino groups on the silicone surface, which can react with an activated carboxylic acid (e.g. NHS-ester) to form an amide bond, and with epoxides to yield secondary amines. However, no peptide grafting have been described do far on amine-modified silicone, although peptides have been straightforwardly grafted this way on silicon wafers [42] and silica NPs (Figure 3, pathway a) [43,44].

Reaction between Si–X = Si–H and Y = vinyl

Allyl spacers can be grafted on Si–H containing PDMS by Karstedt's catalyst-mediated hydrosilylation. In most cases, a bifunctional vinyl PEG is bound to the PDMS surface to bring hydrophilicity, and to present a suitable functional group (Z) on the other end to enable further peptide coupling. For example, grafting of allyl-PEG-OH afforded PDMS-PEG-OH, which in turn can be further converted into PDMS-PEG-OCONHS by *N*,*N*-disuccinimidyl carbonate (NHSCONHS) treatment. These *N*-hydroxysuccinimidylcarbamate functions are suitable for peptide anchoring (Figure 3, pathway b) [31,32]. Noteworthy, direct grafting of allyl-PEG-O CO-NHS on PDMS was also performed to give the same NHS-activated material [30]. Allyl-PEG-OH-Tosyl was also used to get PEG modified PDMS, and after Tosyl substitution by diethylenetriamine, PDMS-PEG-NH-$(CH_2)_2$-NH-$(CH_2)_2$-NH_2 is obtained [12]. The amine-functionalized PDMS was used to covalently immobilize hyaluronic acid after carboxylic acid activation, but no peptide was coupled this way so far.

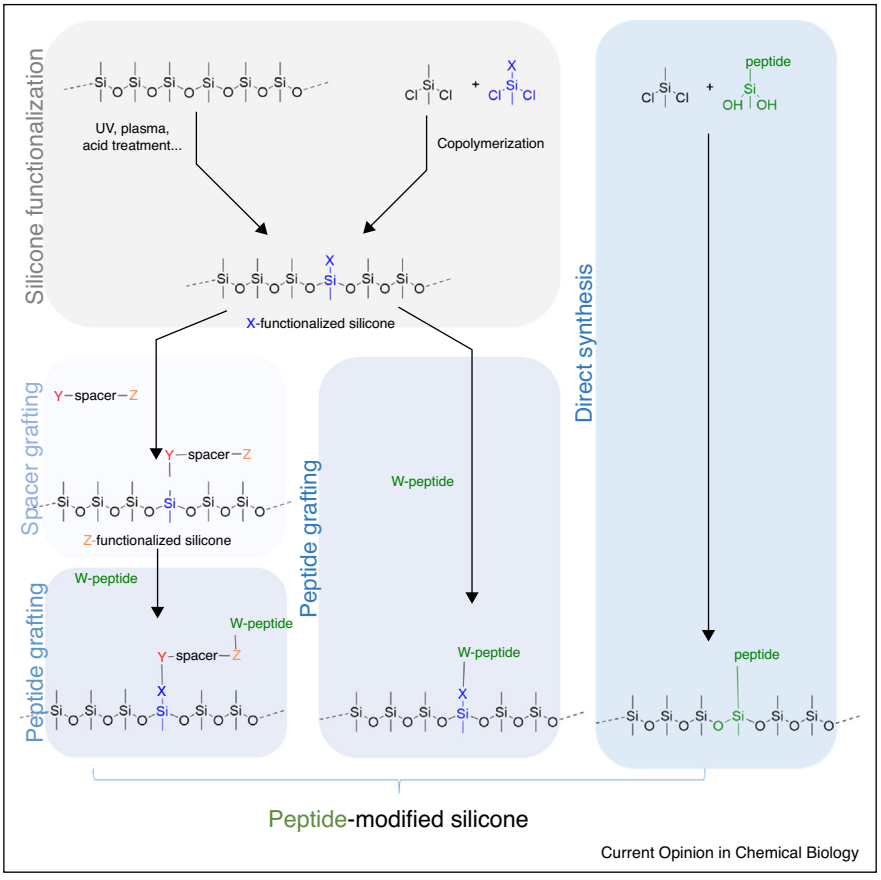
Reaction with Si–X = Si–$CH_2^•$ radicals and Y = vinyl or Y = epoxy

Free radical polymerization of vinyl-containing monomers can be performed activated PDMS. Acrylic acid (AA) is polymerized on radical-activated PDMS to give a carboxylic acid (Z = CO_2H) functionalized PDMS [30–32,34•] that can be further converted into a primary amine (Z = NH_2) functionalized PDMS by reacting with NH_2-PEG-NH_2 through carbodiimide activation (Figure 3, pathway c) [13,37]. Another example shows a way to obtain HO-PDMS. Directly after the argon plasma treatment of the PDMS, a graft polymerization of allyl alcohol by microwave irradiation is operated [45•]. Initiated by radicals, AGE polymerization afforded pendant epoxy groups on PDMS. Starting from that epoxy, two ways of introduction of a maleimide moiety on the PDMS were explored. It can be (i) a one-step addition of NH_2-PEG-Maleimide or (ii) a two-step modification using first diaminopropane, then reacting the resulting amino function with NHS-PEG-Maleimide [39] to finally obtain a Maleimide-PDMS, in both case (Figure 3, pathway d).

Reaction of unmodified PDMS with radicals, Y = N_3

Bifunctional azido-containing spacers can be advantageously grafted on unmodified PDMS by UV activation at 320 to 350 nm. For example, the azido function of sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino)hexanoate (Sulfo-SANPAH) [36••] turns into a nitrene upon UV treatment, which is able to react with PDMS. Finally, it gives a sulfoNHS-ester activated PDMS, which may react with any nucleophile function of peptides (Figure 3, pathway e). It is worth noting that NHS and sulfoNHS esters present the same reactivity, the later displaying a better water-solubility. In the same way, 4-azido-2,3,5,6-tetrafluoro-benzoic acid (AFB) was grafted on PDMS, and its carboxylic acid function was reacted with ethyl(dimethylaminopropyl)carbodiimide/NHS (EDC/NHS) to obtain another type of NHS ester functionalized PDMS [34•].

Figure 2



Different ways to obtain a peptide-modified PDMS. Most of the time, several steps are needed for the synthesis of the peptide-modified silicone: (i) silicone surface functionalization, (ii) spacer grafting, (iii) peptide attachment. Noteworthy, the peptide can also be grafted directly of the functionalized silicone. Advantageously, a peptide monomer bearing a methyldihydroxysilyl moiety could be copolymerized with dimethyldichlorosilane to obtain peptide-functionalized PDMS material in one step.

Grafting of peptides on functionalized silicones

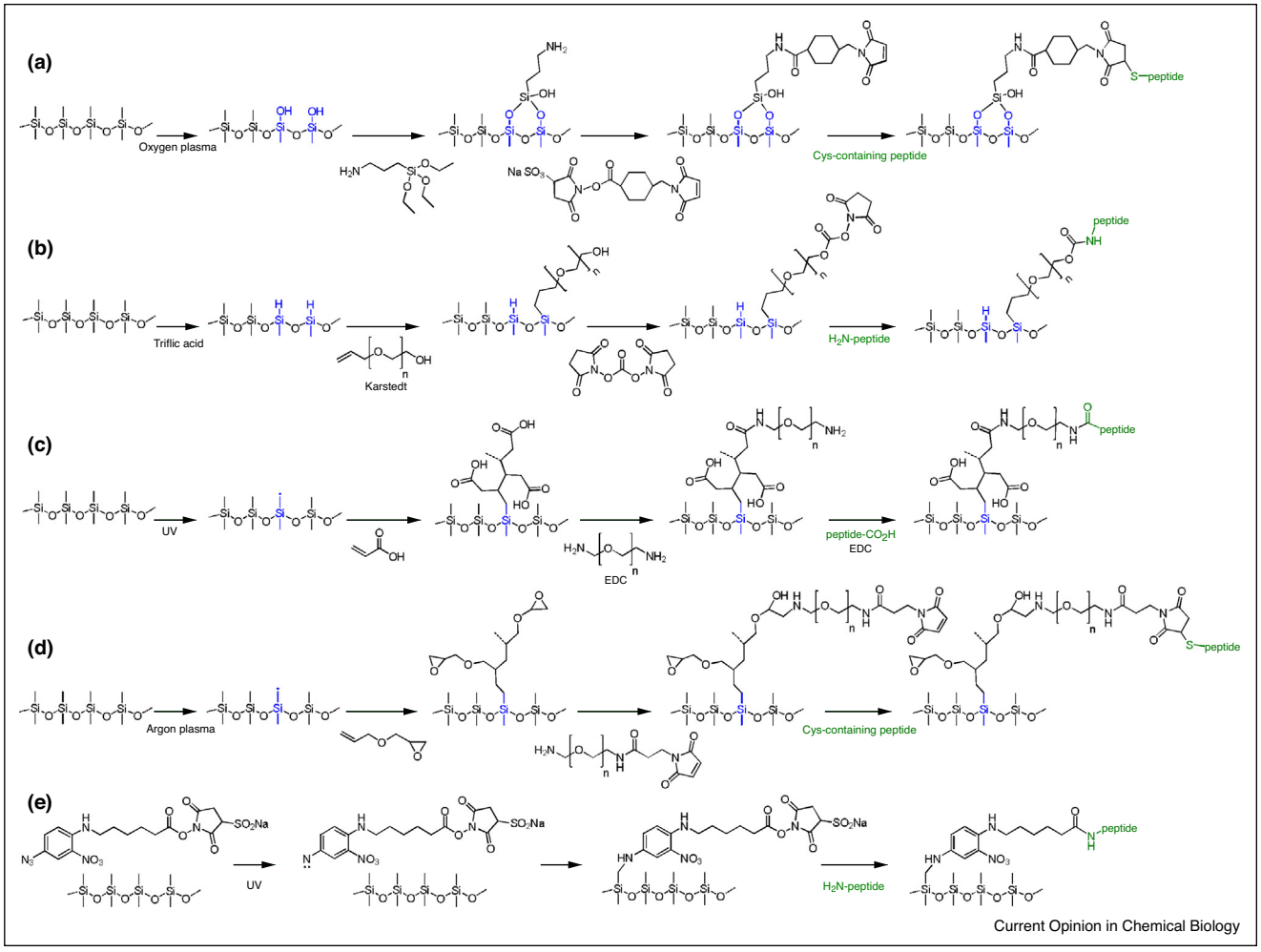
Ideally, covalent immobilization of a peptide on functionalized PDMS should proceed in a controlled manner to guarantee the suitable orientation of the peptide on the material, and to avoid undesired reactions with peptide side chains, which could affect its bioactivity. This can be achieved in two ways: either by using chemoselective reactions involving two mutually reactive moieties present on the peptide and on the PDMS (i.e. chemoselective ligation between Z and W), or by using a peptide presenting a single unprotected reactive function. While the use of a temporary protection (e.g. on Lys side chains) is theoretically possible during the grafting step to control the regioselectivity of the covalent bond, in practice chemists prefer using unprotected peptides displaying a single reactive function (e.g. the N-terminus amine, the thiol of cysteine). Indeed, removal of protecting groups on a PDMS-grafted peptide would probably raise coating and silicone stability issues depending on the pH, the solvents, and the reagents required.

Amide forming chemistries, whatever the amine or the carboxylic acid is present on the polymer (e.g. Z = NH_2 or Z = CO-NHS), fall in the first category and are mostly used when a single reactive function is present on the peptide. If any 'click reaction' may theoretically be used for chemoselective immobilization of peptides, only Michael addition was employed so far to obtain peptide-grafted PDMSs. The last step of peptide grafting is preferentially performed in water, avoiding the used of any organic solvent, which could be detrimental to cell survival, but also facilitates the handling of unprotected water-soluble peptides.

Active ester functionalized PDMS. Z = CO-NHS (or CO-Act, Act being electron attractor and leaving group), W = NH_2

Any suitable nucleophile, including alcohols and phenols, may react with activated carboxylic acids such as NHS-esters. However, amines are much more reactive at pH close to neutrality resulting in a rather good chemoselectivity. Consequently, NHS ester-modified PDMS materials were reacted with a variety of primary amine-containing peptides

Figure 3



Examples of multistep preparation of peptide-grafted PDMS. **(a)** PDMS activation by oxygen plasma followed by APTES grafting. Peptide is immobilized through one of its carboxylic acid after activation (Section 'Reaction between Si–X = Si–OH and Y = Si(OR)'); **(b)** Si–H are generated on PDMS by triflic acid treatment. Hydrosilylation of allyl-PEG yields an alcohol function. Peptide is grafted through carbamate bond with one of its primary amine (Section 'Reaction between Si–X = Si–H and Y = vinyl') using carbonyldimidazole; **(c)** Radicals generated by UV irradiation reacted with acrylic acid. Peptide is grafted with one of its amino group via a diamino spacer using EDC activation; **(d)** Radicals generated by plasma initiated the polymerization of allyl glycydyl ester. Pendant epoxy function reacted with amino maleimide bifunctional spacer which can handle a Cys-containing peptide by a Michael addition (Section 'Reaction with Si–X = Si–$CH_2^{\bullet}$ radicals and Y = vinyl or Y = epoxy'); **(e)** Radicals generated by UV irradiation on azido function of a functional molecule reacted with methyl group of PDMS. Pendant NHS function reacted with primary amine function of peptide (Section 'Reaction of unmodified PDMS with radicals, $Y = N_3$').

(i.e. $W = NH_2$). Peptides derived from fibronectin promoting cell adhesion by interacting with integrin receptors are commonly used to enhance the cell-interacting properties of PDMS. For example, H-RGDS-OH and H-YIGDS-OH were coupled to PDMS-PEG-OCONHS [30]. H-RGDS-OH and H-GYRGDS-OH were reacted with PDMS displaying a sulfoNHS ester on PEG [31,32] and H-RGD-OH or H-GRGDSP-OH were reacted with sulfo-SANPAH treated PDMS [36••]. Other antimicrobial peptides, Histatin 5 (H-DSHAKRHHGYKRKFHEKHHSHRGY-OH) and two of its derivatives Dhvar 4 (H-KRLFKKLLFSLRKY-OH) and Dhvar 5 (H-LLLFLLKKRKKRKY-OH), as well as polyLeu, polyHis and polyArg, were also grafted on PDMS-CO-NHS creating then an anti-biofilm surface used especially in Robbins device [34•].

Instead of using a pre-activated PDMS-CONHS, PDMS-COOH can be activated by a coupling reagent to generate *in situ* an active ester suitable for peptide coupling.

In the case of an amino acid modified PDMS surface, the grafting of an amino peptide is performed using a coupling reagent such as EDC, and an activating functional NHS group. This coupling chemistry has been applied

using Histatin 5 and other derivatives on amino acid or AFB modified PDMS [34•]. The final peptide silicone obtained can be used for the design of Robbins modified microfluidic devices, in order to avoid the formation of bacteria biofilm.

All these reactions have been performed by incubation of NHS-ester PDMS using peptides dissolved in aqueous buffers, for several hours at room temperature. Noteworthy, the authors have used peptides bearing a single primary amino group (i.e. the N-terminus). This trick allows to avoid the use of temporary protections, for example, on lysine side chains, which should have been removed after the grafting step.

Amine functionalized PDMS, Z = NH_2 W = carboxylic acid

This reaction is similar to the one described in the previous paragraph (i.e. Section 'Active ester functionalized PDMS. Z = CO-NHS (or CO-Act, Act being electron attractor and leaving group), W = NH_2'), the reactive functions being switched between the partners. Here, PMDS bears the amino group instead of the peptide. This situation is not as straightforward as the previous one. Indeed, carboxylic acids present on the peptides have to be activated. This leads to uncontrolled peptide intermolecular cross-linking between unprotected side chains (mainly Lys, Ser, Thr and Tyr). Despite these drawbacks, EDC was used to activate carboxylic acids of type 1 collagen to react with PDMS-PEG-NH_2 yielding biocompatible protein-grafted PDMS surface with improved adhesion of fibroblast cells [13].

Isocyanate functionalized PDMS, Z = N = C = O, W = NH_2

PDMS functionalized with amino groups can be readily converted into reactive PDMS-isocyanates by triphosgen. This reaction has been performed on PDMS previously treated with APTMS. Isocyanates are highly reactive, and by reaction with amines and alcohols lead to ureas and carbamates respectively, thus being non-chemoselective. Peptides bearing a single primary amine function (e.g. H-RGD-OH) [27] are reacted with isocyanate functions of PDMS to obtain silicone micro channels suitable for cell immobilization.

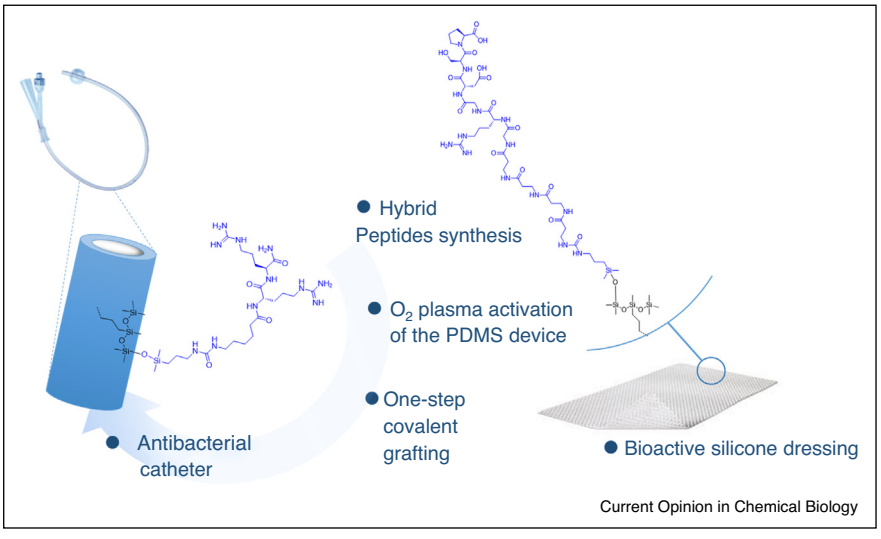
Alcohol functionalized PDMS, Z = OH, W = NH_2

In the case of PDMS modified by polymerization of allyl alcohols, the resulting functions available at the surface are primary alcohols. The hydroxyl group can be converted to a leaving group by 2,2,2-trifluoroethanesulfonyl chloride [45•]. Free amino N-terminus groups of H-YIGSR-OH, H-RGDS-OH, H-PDSGR-OH or H-PHSRN-OH, were then able to react with modified PDMS by nucleophilic substitution. The resulting material was designed for cornea replacement and the adhesion of corneal epithelial cells was improved by grafting of a combination of peptides ligands derived from laminin and fibronectin.

Alkene functionalized PDMS, Z = Maleimide or vinyl, W = SH

The previous methods of immobilization of peptides on PDMS are not chemoselective and have to be used carefully, preferring peptides having a single unprotected amine function. In contrast, thiol-ene reactions (e.g. free-radical or Michael additions) are biorthogonal reactions. When no other thiol is present (i.e. when the peptide carries only one Cys residue), cysteine-containing peptides can be grafted chemoselectively on alkene-modified PDMS. Among these reactions, Michael addition involving maleimides as α,β-unsaturated carbonyl acceptors, becomes most popular. As an example, the Ac-CGGEGYGEGRGDSPG-NH_2

Figure 4



Direct grafting of hybrid silylated peptides on PMDS activated by O_2 plasma. The oxygen plasma generates Si–OH function at the surface of the PDMS. This enables the Si condensation of Si(OH)Me_2 silylated peptide. This functionalization method was applied to both silicone dressing [24] and catheter [23••].

p-
e-
p-
ti-
de
w-
as
i-

Table 1

Summary of peptide sequences, functional groups on the activated PDMS (X), bifunctional linker (Y, Z), on peptides (W), and applications of peptide modified-silicones

Peptides	W	Z	Linkers	Y	X (*obtained by*)	Applications	Grafting density (pmol/cm^2)	Ref
Silylated-Ahx-Arg-Arg-NH_2	$Si(Me)_2$-OH	/	/	/	Si–OH (*oxygen plasma)*	Antimicrobial silicone catheter	~30	[23••]
Silylated peptides : H-(bAla)4-GRGDSP-OH, H-(bAla)4-EGLEPG-OH and Ac-Lys(H)-[Pro-Hyp-Gly]3-NH2	$Si(Me)_2$-OH	/	/	/	Si–OH (*oxygen plasma)*	Wound healing dressings	~60	[24]
Cystein-Oligopeptide modified by PEG or alkyl chain	SH	/	/	/	Vinyl *(functionalized PMDS obtained by copolymerization)*	Anti-fouling coating on glass slide	n.d.	[41••]
H-RGD-OH	H_2N	S=C=N– (obtained from amine)	-APTMS-	$Si(OEt)_3$	Si–OH (*oxidation)*	Micro channel for cell immobilization	n.d.	[27]
Ac-CGGEGYGEGRGDSPG-NH_2	SH	Maleimide	-APTES-grafted with maleimide spacer	$Si(OEt)_3$	Si–OH (*water plasma)*	Flexible silicone membrane for cardiac fibroblast adhesion study	~30	[25,26]
H-YIGSR-OH, H-RGDS-OH, H-PDSGR-OH or H-PHSRN-OH	H_2N	HO–	-Poly(Allyl alcohol)-	Allyl	Si–OH *(microwave irradiation)*	Artificial cornea	~1	[45•]
H-RGDS-OH, H-RDGS-OH and GYRGDS-OH	H_2N	NHS-CO	-PEG-	Allyl	Si–H (*triflic acid)*	Biomaterial for cell adhesion	~100	[31]
H-RGDS-OH and H-GYRGDS-OH	H_2N	NHS-CO–	-PEG-	Allyl	Si–H (*triflic acid)*	Biomaterial for cell adhesion	60	[32]
H-RGDS-OH, H-YIGSR-OH	H_2N	NHS-CO	-PEG-	Allyl	Si–H (*triflic acid)*	Biomaterial for cell adhesion	~30	[30]
H-RGD-OH	H_2N	sulfoNHS-CO–	-Azido hexanoic-	Azide	$Si–CH_2^•$ *(UV)*	Biomaterial for cell adhesion	~10	[36••]
Dhvar 4 and 5, Histatin 5, poly(L, H or R)	H_2N	NHS-CO–	-Poly(AFB)-	Azide	$Si–CH_2^•$ *(UV)*	Anti-biofilm surface used in Robbins device (bioassays)	n.d.	[34•]
Dhvar 4	H_2N	HOOC–	-PAA-	Allyl				
Collagen, type 1	COOH	H_2N–	-PEG-	Allyl	$Si–CH_2^•$ *(argon plasma)*	Biocompatible PEG-stabilized surface	n.d.	[13]
H-CVNWKKILGKIIKVVK-NH_2	SH	Maleimide	-PEG-	Allyl	$Si–CH_2^•$ *(argon plasma)*	Antimicrobial silicone catheter	~3500	[39]
H-CWFWKWWRRRRR-NH_2	SH	Maleimide	-PEG-	Allyl	$Si–CH_2^•$ *(argon plasma)*	Anti-biofilm surface	~400	[38]

n.d. non determined.

mmobilized on Maleimide-PDMS to obtain a surface suitable for cardiac fibroblasts adhesion and study [25,26]. Antibacterial peptides were also grafted on maleimide-PDMS: a catheter was functionalized with CysLasioIII peptide (H-CVNWKKILGKIIKVVK-NH_2) [39] and peptide CRW11 (H-CWFWKWWRRRRR-NH_2) [38] was used to construct an anti-biofilm PDMS surface. Interestingly, the same authors grafted CRW11 by nucleophilic addition on a polydopamine layer coated on PDMS [46].

Radical thiol-ene reaction has been used to immobilize cysteine-containing peptides on a vinyl modified PDMS [i.e. PS-b-P(DMS-VMS)]. A solution containing a short oligolysine whose side chains are modified with diethylene glycol moieties [i.e. Fmoc-[Lys($COCH_2$ (OCH_2CH_2)$_2$OMe)]$_6$Cys-OMe] [41••] was sprayed over the vinyl modified silicone. Upon heating, the peptide was covalently grafted, probably through the generation of thiyl radical $RS^{\bullet}$. As expected, the final peptide-modified silicone showed interesting anti-fouling and allowed to avoid non-specific protein adsorption.

Direct hybrid peptide grafting on PMDS functionalized with silanol, Z = Si–OH, W = Si(Me)$_2$OH

As already stated, the creation of silanol groups at the surface of PDMS can be achieved easily by oxygen plasma treatment. Thus, the most straightforward way to prepare peptide-grafted PDMS is to use hybrid silylated peptides, which may react by sol-gel hydrolysis and condensation on silanols to form Si–O–Si bonds at the silicone surface [23••,24]. The key point of the strategy is the synthesis of the hybrid peptide, in solution or on solid support, silylated at a suitable position [47,48]. However, once it is prepared, this strategy is straightforward as it does require neither successive chemoselective reaction, nor spacer addition. The modified PDMS is simply immersed into a solution containing the hybrid biomolecule for several hours at room temperature. Interestingly, this reaction proceeds chemoselectively allowing the use of any unprotected peptide sequences. Antimicrobial catheters were obtained by grafting short amphipathic peptides [i.e. [SiOH(Me)$_2$-(CH_2)$_3$NHCO-AhxArg-Arg-NH_2]. These devices have shown a superior efficiency compared to commercially available Ag-doped catheters [23••]. Using the same technique, wound-healing dressings were prepared, using silylated peptides whose sequences were derived from ECM proteins [24] (Figure 4).

Conclusion and future developments

If proteins can be adsorbed quite easily on bare PDMS surface, this is not the case with smaller peptides which are quickly removed once in contact with aqueous media. Therefore, to achieve a long-term effect, covalent grafting is required. Chemical modification yields a relatively homogenous repartition of the peptide on the surface, as a monolayer, and improves the stability over time. For that purpose, a wide range of organic functions can be introduced on PDMS in a few steps. However, only a small number of bioorthogonal ligation reactions have been used so far to conjugate peptides on the material surface. There are still a lot of possibilities to explore digging into the 'click' reactions tool-box, for peptide chemists.

Grafting densities obtained by covalent methods are much lower than those obtained by adsorption. They typically range from one to one hundred picomole per cm^2 depending on the anchoring chemistry and the peptide used (Table 1). Roughly, this corresponds to a monomolecular layer of peptide on the PDMS surface. Higher covalent grafting densities can be expected (e.g. above 500 pmol/cm^2), if the peptide is not grafted directly on PDMS but on a brush-like polymeric structure grafting from the PDMS [38,39]. Besides reported post-functionalization approaches, an alternative one-step strategy can be considered to produce peptide modified PDMS. The first requirement is the synthesis of silylated peptide monomers. Depending on the nature, the position, and the number of silyl groups within the peptide sequence, different peptide-polymer geometries have already been obtained [49,50], when silylated peptide are used as the only monomers. Moreover, methyldihydroxysilane-modified peptides could also be copolymerized with dimethyldichlorosilane (Me_2SiCl_2) to get peptide-functionalized silicone oils with a PDMS-like backbone (Figure 1, Direct Synthesis) [51]. Beyond a single bioactive peptide, copolymerization with other silylated polymers such as PEG, fluorophores, and drugs could be considered to afford multifunctional PDMS-based materials with unprecedented properties.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Madsen FB, Yu L, Daugaard AE, Hvilsted S, Sko AL: **A new soft dielectric silicone elastomer matrix with high mechanical integrity and low losses**. *RSC Adv* 2015, **5**:10254-10259.
2. Chekina NA, Pavlyuchenko VN, Danilichev VF, Ushakov NA, Novikov SA, Ivanchev SS: **A new polymeric silicone hydrogel for medical applications: synthesis and properties**. *Polym Adv Technol* 2006, **17**:872-877.
3. Owen MJ: **Properties and applications of silicones**. *Advances in Silicones and Silicone-Modified Materials*. American Chemical Society; 2010:13-18.
4. Lee JN, Jiang X, Ryan D, Whitesides GM: **Compatibility of mammalian cells on surfaces of poly(dimethylsiloxane)**. *Langmuir* 2004, **20**:11684-11691.
5. Bhatt S, Pulpytel J, Arefi-Khonsari F: **Low and atmospheric plasma polymerisation of nanocoatings for bio-applications**. *Surf Innov* 2015, **3**:63-83.

6. Moretto H-H, Schulze M, Wagner G: **Silicones**. In *Ullmann's Encyclopedia of Industrial Chemistry*. Edited by Wiley-VCH Verlag GmbH & Co. KGaA. Wiley-VCH Verlag GmbH & Co. KGaA; 2000.

7. Liu Y, Zhang L, Wu W, Zhao M, Wang W: **Restraining non-specific adsorption of protein using Parylene C-caulked polydimethylsiloxane**. *Biomicrofluidics* 2016, **10**.

8. Brash JL: **Exploiting the current paradigm of blood–material interactions for the rational design of blood-compatible materials**. *J Biomater Sci Polym Ed* 2000, **11**:1135-1146.

9. Jońca J, Tukaj C, Werel W, Mizerska U, Fortuniak W, Chojnowski J: **Bacterial membranes are the target for antimicrobial polysiloxane-methacrylate copolymer**. *J Mater Sci Mater Med* 2016, **27**:55.

10. Hu S, Ren X, Bachman M, Sims CE, Li GP, Allbritton N: **Cross-linked coatings for electrophoretic separations in poly (dimethylsiloxane) microchannels**. *Electrophoresis* 2003, **24**:3679-3688.

11. Feng J-J, Wang A-J, Fan J, Xu J-J, Chen H-Y: **Hydrophilic biopolymer grafted on poly(dimethylsiloxane) surface for microchip electrophoresis**. *Anal Chim Acta* 2010, **658**:75-80.

12. Alauzun JG, Young S, D'Souza R, Liu L, Brook MA, Sheardown HD: **Biocompatible, hyaluronic acid modified silicone elastomers**. *Biomaterials* 2010, **31**:3471-3478.

13. Keranov I, Vladkova T, Minchev M, Kostadinova A, Altankov G: **Preparation, characterization, and cellular interactions of collagen-immobilized PDMS surfaces**. *J Appl Polym Sci* 2008, **110**:321-330.

14. • Wu H, Zhai J, Tian Y, Lu H, Wang X, Jia W, Liu B, Yang P, Xu Y, Wang H: **Microfluidic enzymatic-reactors for peptide mapping: strategy, characterization, and performance**. *Lab Chip* 2004, **4**:588-597.

Two different ways of PDMS functionalization are presented, leading to either covalent or non-covalent attachment of an enzyme, the Trypsin. Acrylic acid is used as linker, grafted by UV activation and either activated by ECD/NHS chemistry or modified by PDDA (Poly(diallyldimethylammonium chloride)).

15. Chawla K, Lee S, Lee BP, Dalsin JL, Messersmith PB, Spencer ND: **A novel low-friction surface for biomedical applications: modification of poly(dimethylsiloxane) (PDMS) with polyethylene glycol(PEG)-DOPA-lysine**. *J Biomed Mater Res A* 2009, **90A**:742-749.

16. Yu X, Xiao J, Dang F: **Surface modification of poly (dimethylsiloxane) using ionic complementary peptides to minimize nonspecific protein adsorption**. *Langmuir* 2015, **31**:5891-5898.

17. Wang D, Oleschuk RD, Horton JH: **Surface modification of poly (dimethylsiloxane) with a perfluorinated alkoxysilane for selectivity toward fluorous tagged peptides**. *Langmuir* 2008, **24**:1080-1086.

18. Wang D, Douma M, Swift B, Oleschuk RD, Horton JH: **The adsorption of globular proteins onto a fluorinated PDMS surface**. *J Colloid Interface Sci* 2009, **331**:90-97.

19. Mishra G, Bhattacharyya S, Bhatia V, Ateeq B, Sharma A, Sivakumar S: **Direct intranuclear anticancer drug delivery via polydimethylsiloxane nanoparticles: in vitro and in vivo xenograft studies**. *ACS Appl Mater Interfaces* 2017, **9**: 34625-34633.

20. Wu D, Zhao B, Dai Z, Qin J, Lin B: **Grafting epoxy-modified hydrophilic polymers onto poly(dimethylsiloxane) microfluidic chip to resist nonspecific protein adsorption**. *Lab Chip* 2006, **6**:942-947.

21. Wu D, Qin J, Lin B: **Self-assembled epoxy-modified polymer coating on a poly(dimethylsiloxane) microchip for EOF inhibition and biopolymers separation**. *Lab Chip* 2007, **7**:1490-1496.

22. Yeh S-B, Chen C-S, Chen W-Y, Huang C-J: **Modification of silicone elastomer with zwitterionic silane for durable antifouling properties**. *Langmuir* 2014, **30**:11386-11393.

23. •• Pinese C, Jebors S, Echalier C, Licznar-Fajardo P, Garric X, Humblot V, Calers C, Martinez J, Mehdi A, Subra G: **Simple and specific grafting of antibacterial peptides on silicone catheters**. *Adv Healthc Mater* 2016, **5**:3067-3073.

A direct grafting of an antimicrobial peptide is described, using a simple activation of the PDMS by oxygen plasma. The main point is the use of silylated peptides, without any linker or further activation.

24. Pinese C, Jebors S, Stoebner PE, Humblot V, Verdié P, Causse L, Garric X, Taillades H, Martinez J, Mehdi A *et al.*: **Bioactive peptides grafted silicone dressings: a simple and specific method**. *Mater Today Chem* 2017, **4**:73-83.

25. Boateng S, Lateef SS, Crot C, Motlagh D, Desai T, Samarel AM, Russell B, Hanley L: **Peptides bound to silicone membranes and 3d microfabrication for cardiac cell culture**. *Adv Mater* 2002, **14**:461-463.

26. Lateef SS, Boateng S, Hartman TJ, Crot CA, Russell B, Hanley L: **GRGDSP peptide-bound silicone membranes withstand mechanical flexing in vitro and display enhanced fibroblast adhesion**. *Biomaterials* 2002, **23**:3159-3168.

27. Sui G, Wang J, Lee C-C, Lu W, Lee SP, Leyton JV, Wu AM, Tseng H-R: **Solution-phase surface modification in intact poly (dimethylsiloxane) microfluidic channels**. *Anal Chem* 2006, **78**:5543-5551.

28. Makamba H, Kim JH, Lim K, Park N, Hahn JH: **Surface modification of poly(dimethylsiloxane) microchannels**. *Electrophoresis* 2003, **24**:3607-3619.

29. Bowden N, Huck WTS, Paul KE, Whitesides GM: **The controlled formation of ordered, sinusoidal structures by plasma oxidation of an elastomeric polymer**. *Appl Phys Lett* 1999, **75**:2557-2559.

30. Chen H, Brook MA, Sheardown HD, Chen Y, Klenkler B: **Generic bioaffinity silicone surfaces**. *Bioconjug Chem* 2006, **17**:21-28.

31. Mikhail AS, Jones KS, Sheardown H: **Dendrimer-grafted cell adhesion peptide–modified PDMS**. *Biotechnol Prog* 2008, **24**:938-944.

32. Mikhail AS, Ranger JJ, Liu L, Longenecker R, Thompson DB, Sheardown HD, Brook MA: **Rapid and efficient assembly of functional silicone surfaces protected by PEG: cell adhesion to peptide-modified PDMS**. *J Biomater Sci Polym Ed* 2010, **21**:821-842.

33. Ferreira P, Carvalho Á, Correia TR, Antunes BP, Correia IJ, Alves P: **Functionalization of polydimethylsiloxane membranes to be used in the production of voice prostheses**. *Sci Technol Adv Mater* 2013, **14**:055006.

34. • Prijck KD, Smet ND, Rymarczyk-Machal M, Driessche GV, Devreese B, Coenye T, Schacht E, Nelis HJ: ***Candida albicans* biofilm formation on peptide functionalized polydimethylsiloxane**. *Biofouling* 2010, **26**:269-275.

Two types of UV light activation of PDMS are described. Authors present the direct activation of PDMS surface and the activation through a photo-initiator (i.e. benzophenone).

35. Smet ND, Rymarczyk-Machal M, Schacht E: **Modification of polydimethylsiloxane surfaces using benzophenone**. *J Biomater Sci Polym Ed* 2009, **20**:2039-2053.

36. •• Li B, Chen J, Wang JH-C: **RGD peptide-conjugated poly (dimethylsiloxane) promotes adhesion, proliferation, and collagen secretion of human fibroblasts**. *J Biomed Mater Res A* 2006, **79**:989-998.

UV light activation of azido groups is presented here. Upon irradiation one side of the hetero-bifunctional spacer is converted into nitrene functions, which are able to react with PDMS methyl groups.

37. Vladkova TG, Keranov IL, Dineff PD, Youroukov SY, Avramova IA, Krasteva N, Altankov GP: **Plasma based Ar^+ beam assisted poly (dimethylsiloxane) surface modification**. *Nucl Instrum Methods Phys Res B Beam Interact Mater At* 2005, **236**:552-562.

38. Lim K, Chua RRY, Saravanan R, Basu A, Mishra B, Tambyah PA, Ho B, Leong SSJ: **Immobilization studies of an engineered arginine–tryptophan-rich peptide on a silicone surface with antimicrobial and antibiofilm activity**. *ACS Appl Mater Interfaces* 2013, **5**:6412-6422.

39. Mishra B, Basu A, Chua RRY, Saravanan R, Tambyah PA, Ho B, Chang MW, Leong SSJ: **Site specific immobilization of a potent**

antimicrobial peptide onto silicone catheters: evaluation against urinary tract infection pathogens. *J Mater Chem B* 2014, **2**:1706-1716.

40. Rambarran T, Gonzaga F, Brook MA: **Generic, metal-free cross-linking and modification of silicone elastomers using click ligation**. *Macromolecules* 2012, **45**:2276-2285.

41. •• Calabrese DR, Wenning B, Finlay JA, Callow ME, Callow JA, Fischer D, Ober CK: **Amphiphilic oligopeptides grafted to PDMS-based diblock copolymers for use in antifouling and fouling release coatings**. *Polym Adv Technol* 2015, **26**:829-836.
The synthesis of vinyl functionalized PDMSs by copolymerization with a vinyl silane is presented. The resulting vinyl PDMSs are reacted with N-ter cysteinyl-peptides.

42. Akers PW, Dingley AJ, Swift S, Nelson ARJ, Martin J, McGillivray DJ: **Using neutron reflectometry to characterize antimicrobial protein surface coatings**. *J Phys Chem B* 2017, **121**:5908-5916.

43. Rytkönen J, Arukuusk P, Xu W, Kurrikoff K, Langel Ü, Lehto V-P, Närvänen A: **Porous silicon–cell penetrating peptide hybrid nanocarrier for intracellular delivery of oligonucleotides**. *Mol Pharm* 2014, **11**:382-390.

44. Maurice V, Slostowski C, Herlin-Boime N, Carrot G: **Polymer-grafted silicon nanoparticles obtained either via peptide bonding or click chemistry**. *Macromol Chem Phys* 2012, **213**:2498-2503.

45. • Aucoin L, Griffith CM, Pleizier G, Deslandes Y, Sheardown H: **Interactions of corneal epithelial cells and surfaces modified with cell adhesion peptide combinations**. *J Biomater Sci Polym Ed* 2002, **13**:447-462.
This is the only example of hydroxyl-modified PDMSs obtained by microwave plasma polymerization of allyl alcohols. After activation with tresyl chloride, modified PDMS are reacted with the free N-ter of peptides.

46. Lim K, Chua RRY, Bow H, Tambyah PA, Hadinoto K, Leong SSJ: **Development of a catheter functionalized by a polydopamine peptide coating with antimicrobial and antibiofilm properties**. *Acta Biomater* 2015, **15**:127-138.

47. Jebors S, Cecillon S, Faye C, Enjalbal C, Amblard M, Mehdi A, Subra G, Martinez J: **From protected trialkoxysilyl-peptide building blocks to bioorganic–silica hybrid materials**. *J Mater Chem B* 2013, **1**:6510-6515.

48. Echalier C, Kalistratova A, Ciccione J, Lebrun A, Legrand B, Naydenova E, Gagne D, Fehrentz J-A, Marie J, Amblard M *et al.*: **Selective homodimerization of unprotected peptides using hybrid hydroxydimethylsilane derivatives**. *RSC Adv* 2016, **6**:32905-32914.

49. Jebors S, Ciccione J, Al-Halifa S, Nottelet B, Enjalbal C, M'Kadmi C, Amblard M, Mehdi A, Martinez J, Subra G: **A new way to silicone-based peptide polymers**. *Angew Chem Int Ed* 2015, **54**:3778-3782.

50. Jebors S, Pinese C, Nottelet B, Parra K, Amblard M, Mehdi A, Martinez J, Subra G: **Turning peptides in comb silicone polymers**. *J Pept Sci* 2015, **21**:243-247.

51. Martin J, Wehbi M, Echalier C, Pinese C, Martinez J, Subra G, Mehdi A: Submitted for publication 2019.