



**HAL**  
open science

## Direct Synthesis of Peptide-Containing Silicones: A New Way to Bioactive Materials

Julie Martin, Mohammad Wehbi, Cécile Echalié, Sylvie Hunger, Audrey Bethry, Xavier Garric, Coline Pinese, Jean Martínez, Lubomir Vezekov, Gilles Subra, et al.

### ► To cite this version:

Julie Martin, Mohammad Wehbi, Cécile Echalié, Sylvie Hunger, Audrey Bethry, et al.. Direct Synthesis of Peptide-Containing Silicones: A New Way to Bioactive Materials. *Chemistry - A European Journal*, 2020, 26, pp.12839 - 12845. 10.1002/chem.202001571 . hal-04816986

**HAL Id: hal-04816986**

**<https://hal.science/hal-04816986v1>**

Submitted on 3 Dec 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright



# Chemistry A European Journal

 **Chemistry  
Europe**  
European Chemical  
Societies Publishing

## Accepted Article

**Title:** Direct synthesis of peptide-containing silicone. A new way for bioactive materials

**Authors:** Ahmad Mehdi, Martin Julie, Mohammad Wehbi, Cecile Echalié, Sylvie hunger, Audrey Bethry, Xavier garric, coline Pinese, jean Martinez, Lubomir vezenkov, and gilles subra

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

**To be cited as:** *Chem. Eur. J.* 10.1002/chem.202001571

**Link to VoR:** <https://doi.org/10.1002/chem.202001571>

WILEY-VCH

# Direct synthesis of peptide-containing silicone. A new way for bioactive materials.

Julie Martin,<sup>[a,b]</sup> Mohammad Wehbi,<sup>[a]</sup> Cécile Echalié,<sup>[a,b]</sup> Sylvie Hunger,<sup>[b]</sup> Audrey Bethry,<sup>[b]</sup> Xavier Garric,<sup>[b]</sup> Coline Pinese,<sup>[b]</sup> Jean Martinez,<sup>[b]</sup> Lubomir Vezenkov,<sup>[b]</sup> Gilles Subra\*<sup>[b]</sup> and Ahmad Mehdi\*<sup>[a]</sup>

This work is dedicated to COVID-19 victims

---

[a] Dr. Julie Martin, Dr. Mohammad Wehbi, Dr. Cécile Echalié, Pr. Ahmad Mehdi  
ICGM Univ. Montpellier, CNRS, ENSCM, Montpellier, France.  
E-mail: ahmad.mehdi@umontpellier.fr

[b] Dr. Julie Martin, Dr. Cécile Echalié, Dr. Sylvie Hunger, Dr. Audrey Bethry, Pr. Xavier Garric, Dr. Coline Pinese, Pr. Jean Martinez, Dr. Lubomir Vezenkov, Pr. Gilles Subra  
IBMM Univ. Montpellier, CNRS, ENSCM, Montpellier, France.  
E-mail: gilles.subra@umontpellier.fr

Supporting information for this article is given via a link at the end of the document.

## ABSTRACT

A simple and efficient way to synthesize peptide-containing silicone materials is described. Silicone oils containing a chosen ratio of bioactive peptide sequences were prepared by acid-catalyzed copolymerization of dichlorodimethylsilane, hybrid dichloromethyl peptidosilane and either Si-vinyl or Si-H functionalized monomers. Functionalized silicone oils were first obtained and then after hydrosilylation cross-linking, bioactive PDMS based materials were straightforwardly obtained. The introduction of an antibacterial peptide yields PDMS materials showing an interesting activity against *Staphylococcus Aureus*. In the same way, RGD ligands-containing PDMS demonstrated improved cell adhesion properties. This generic method was fully compatible with the stability of peptides and thus opened the way to the synthesis of a wide range of biologically active silicones.

## INTRODUCTION

Polydimethylsiloxane (PDMS), also known as silicone, is a mixed organic/inorganic hydrophobic polymer displaying very interesting properties<sup>1</sup> such as high degree of oxygen permeability,<sup>2</sup> thermal and electrical resistance,<sup>3</sup> as well as being considered biocompatible.<sup>4</sup> Thanks to these features, it was used in many industrial applications such as antifoams, coatings, sealants and insulation. In the past decades, PDMS has been extensively adopted for the elaboration of microfluidic chips<sup>5</sup> that found useful applications in the

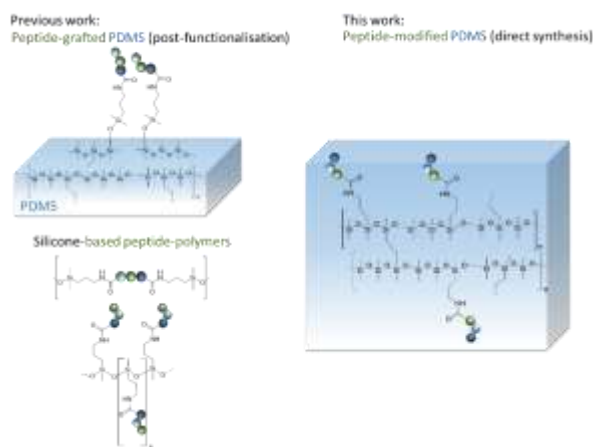
fields of diagnostics, screening or biosensing.<sup>67</sup> Thanks to its biocompatibility, PDMS occupies an important position in the field of soft implantable biomedical devices.<sup>8,9</sup> As most synthetic polymers used in healthcare, PDMS is bio-inert i.e. it does not present any biological activity. For more complex therapeutics applications, these polymers have to be functionalized in order to obtain the chosen propertie(s) (e.g. targeting, cellular penetration, cellular adhesion, antimicrobial activity, better detection for imaging etc.). Moreover, the surface of PDMS is highly hydrophobic leading to unselective adsorption and subsequent denaturation of proteins.<sup>10</sup> Very early, simple chemical modifications (e.g. alkyl chains, cationic and anionic groups) of the hydrophobic native PDMS surface have been used to improve its biocompatibility,<sup>11</sup> while more complex molecules such as sugar,<sup>12</sup> poly(L-lactic acid),<sup>13</sup> peptides and proteins<sup>14,15</sup> have been immobilized on PDMS to afford chosen bioactivities to the material. These modifications were performed by multi-step grafting chemistry, first introducing a functional group on the PDMS surface and then reacting with the molecule to be immobilized. Advantageously, our group showed recently that it was possible to graft peptides on silicone of medical devices in a single step by using hybrid bioorganic-inorganic molecules bearing an alkoxysilane group. This strategy was first applied to the modification of silicone catheter surfaces by antibacterial peptides to get an antibacterial material with high immediate efficiency by comparison to similar silver-embedded materials.<sup>16</sup> In the same way, bioactive peptides promoting wound-healing were successfully immobilized on silicone dressings.<sup>17</sup> These strategies concerned surface modification of bulk silicone by post-functionalization.

In contrast, direct strategies can be envisioned to offer peptide-containing silicone. We already reported on the polymerization of peptides using silane chemistry to get linear<sup>18</sup> and comb-like peptide-polymers assembled through Si-O-Si bonds and without any DCDMS copolymerization (**Figure 1**).<sup>19</sup> However, these hybrid peptide polymers are limited to their modest polymerization degree (DP < 30) and, despite the similarities in their backbone; they did not display comparable physicochemical properties than PDMS.

Herein, we describe the direct synthesis of PDMS polymers incorporating bioactive peptides in a controlled ratio to get peptide-containing cross-linked PDMS. We hypothesized that a low amount of bioactive peptide compared to silicone should be enough to induce a desired effect. To demonstrate that, we prepared two types of bioactive-PDMS with various molar % (0.01 to 10 mol%) of peptide. Taking into account the dramatic number of infections appearing subsequently to a medical device implantation<sup>20</sup>, we

chose to incorporate an antibacterial peptide. Alternatively, we prepared a silicone containing a derivative of fibronectin peptide sequence to improve the cell-adhesion properties of the material. In order to prove the biological properties of the two type peptide-containing-PDMS adapted assays were performed: antibacterial assays for the materials containing hybrid peptide **2** and cell adhesion and proliferation assays for the materials containing hybrid peptide **3**.

By contrast to post-grafted silicone, the modified PDMS materials present peptides homogeneously distributed in the bulk. This will ensure the durability of the functionalization which cannot be altered by surface erosion or mechanical damage. This could afford durable and dedicated properties to implantable medical devices and general public healthcare devices such as nipples, lens, catheters etc. As an additional level of modularity, we propose a generic method based on the cross-linking of hybrid bioorganic-silicone oils which could be chosen and mixed in an appropriate ratio to get diverse peptide containing PDMS materials on-demand. Thus, the first step of our study was to optimize the synthesis of a hybrid PDMS polymeric oil.



**Figure 1.** Different strategies using hybrid silane-peptides for the synthesis of peptide-containing silicone and polymers.

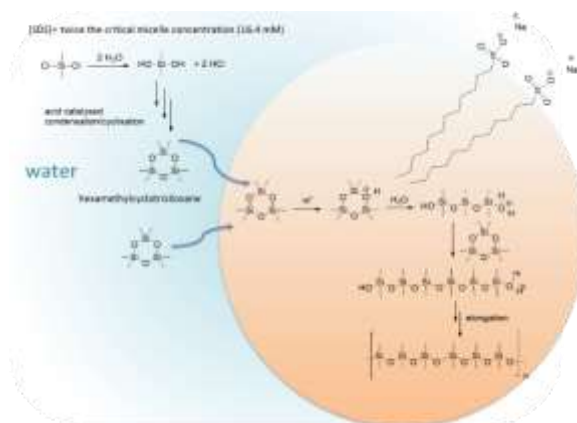
## RESULTS AND DISCUSSION

Different silicone oils were prepared. They are composed of three types of building-blocks. (i) Dichlorodimethylsilane (DCDMS), which will yield the main polymeric repeat of PDMS; (ii) dichloromethylsilane (DCMS) or dichloromethylvinylsilane (DCMVS), which are the siege of cross-linking between linear PDMS chains by hydrosilylation; (iii) hybrid building blocks displaying one

dichloromethylsilane moiety and either a fluorescent dye (hybrid compound **1**) or a bioactive peptide sequence (hybrid peptide **2** and **3**). Fluorescent compound **1** was used to easily visualize the covalent incorporation of a hybrid building block with the PDMS chains. The hybrid peptide **2** is a cationic peptide, displaying two arginine residues and the hybrid peptide **3** is a linear peptide derived from fibronectin sequence (i.e. GRGDSP). The first one was selected for its antibacterial properties and the second one for its ability to enhance the cell adhesion.<sup>21</sup> Peptides **2** and **3** were first synthesized on solid support, using Rink amide or 2-Chloro chlorotriyl PS resin respectively. Peptide **3** was silylated on solid support while peptide **2** was cleaved, then silylated in solution, according to a well-established procedure.<sup>19</sup>

The polymerization of dichlorosilyl blocks was performed using sodium dodecyl sulfate ([SDS]= 110 mM) as a surfactant in water at 60 °C. These experimental conditions were compatible with the stability of peptides. DSC hydrolyses in water producing dimethyldisilanol and hydrochloric acid, which catalyzes both hydrolysis and condensation processes. In these experimental conditions, three molecules of disilanol condensed into hexamethylcyclotrisiloxane,<sup>22</sup> entering the core of SDS micelles. Acid-catalyzed hydrolysis induced both opening on the cycle and growing (**Scheme 1**).



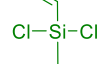
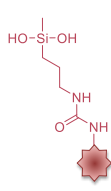
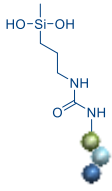
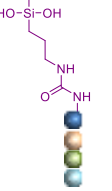
Non-functionalized PDMS (**P0**), PDMS functionalized with 1, 2.5, 5 and 10 mol% of DCMS (**P1**, **P3**, **P5** and **P7** respectively) and PDMS functionalized with 1, 2.5, 5 and 10 mol% of DCMVS (**P2**, **P4**, **P6** and **P8** respectively) were prepared using the procedure mentioned above. The experimental percentage of incorporation of these functional monomers were calculated by integration and comparison in <sup>1</sup>H NMR of the monomer peak to the Si-CH<sub>3</sub> peak. The PDMS oils were recovered by 75 to 91% yields. Polymers were then characterized by size-exclusion chromatography (SEC) at a concentration of 10 mg/ml in chloroform (CHCl<sub>3</sub>) (see Figure 7 in Supporting Information). The molecular weight of the polymers ranged from 12600 (**P5**) to 28200 (**P8**). The introduction of the Si-H monomer yields shorter polymer lengths probably slowing down the polymerization whereas the introduction of Si-Vinyl has no effect on the size, except for the 10 mol% (**P8**) where the length is a little longer than the pristine PDMS. <sup>29</sup>Si NMR analyses of unfunctionalized PDMS showed a single peak at -22 ppm, corresponding to the siloxane -(OSi(Me)<sub>2</sub>)<sub>n</sub>- whereas, several additional peaks were obtained for -OSi(RMe)- with R = H, Vinyl or peptide. Noteworthy, the <sup>29</sup>Si NMR confirmed the absence of any uncondensed monomer byproduct.



**Scheme 1.** SDS promoted polymerization of dichlorodimethylsilane (DCDMS)

As a first examples of functionalization, we incorporated the hybrid fluorescein **1** at 0.01 or 0.1 mol% and hybrid peptide **2** and **3** at 0.01 to 10 mol% to get bioorganic PDMS-modified oils **P9** to **P20** (**Table 1**). These modified PDMS oils containing hybrid compound were prepared with the same protocol than **P2**, **P4**, **P6** and **P8** with DMCVS. At the low concentrations (0.01-0.1 mol%), it was not possible to detect the presence of the peptide in the copolymers by NMR while the incorporation of hybrid fluorescein at the same concentrations was clearly perceived by the color changes of **P9** and **P10** modified-PDMS oils, pale yellow (0.01% mol of compound **1**) to dark yellow (0.1% mol of compound **1**) respectively (**Figure 2**).

**Table 1.** Composition of different functional PDMS.

Polymer	mol% of Silylated building blocks				Yield (%)	MW <sup>a</sup>			
	DCDMS	DCMS	DCMVS	Experimental % <sup>b</sup>					
									
<b>P0</b>	100	-	-	-	-	-	-	84	23100
<b>P1</b>	99	1	-	1	-	-	-	88	14800
<b>P2</b>	99	-	1	1.4	-	-	-	91	22200
<b>P3</b>	97.5	2.5	-	2.3	-	-	-	85	22500
<b>P4</b>	97.5	-	2.5	3.8	-	-	-	89	20200
<b>P5</b>	95	5	-	3.1	-	-	-	83	12600
<b>P6</b>	95	-	5	6.7	-	-	-	79	22100
<b>P7</b>	90	10	-	8.5	-	-	-	75	18600
<b>P8</b>	90	-	10	13	-	-	-	76	28200
<b>P9</b>	97.5	-	2.5	3.6	0.01	-	-	92	33500
<b>P10</b>	97.5	-	2.5	3.0	0.1	-	-	86	72900
<b>P11</b>	97.5	-	2.5	4.4	-	0.01	-	98	57400
<b>P12</b>	97.5	-	2.5	4.5	-	0.1	-	88	36000
<b>P13</b>	97.5	-	2.5	2.8	-	1	-	n.d.	60034
<b>P14</b>	97.5	-	2.5	2.3	-	5	-	n.d.	79301
<b>P15</b>	97.5	-	2.5	3.4	-	10	-	n.d.	61623
<b>P16</b>	97.5	-	2.5	2.5	-	-	0.01	n.d.	28990
<b>P17</b>	97.5	-	2.5	2.8	-	-	0.1	n.d.	28315
<b>P18</b>	97.5	-	2.5	3.0	-	-	1	n.d.	38284
<b>P19</b>	97.5	-	2.5	3.0	-	-	5	n.d.	70780
<b>P20</b>	97.5	-	2.5	2.3	-	-	10	n.d.	81601

<sup>a</sup> determined by SEC, <sup>b</sup> experimental percentage of DCMS and DCMVS determined by <sup>1</sup>H RMN integration from PDMS oils before any cross-linking, n.d. non determined due to the small quantity synthetize

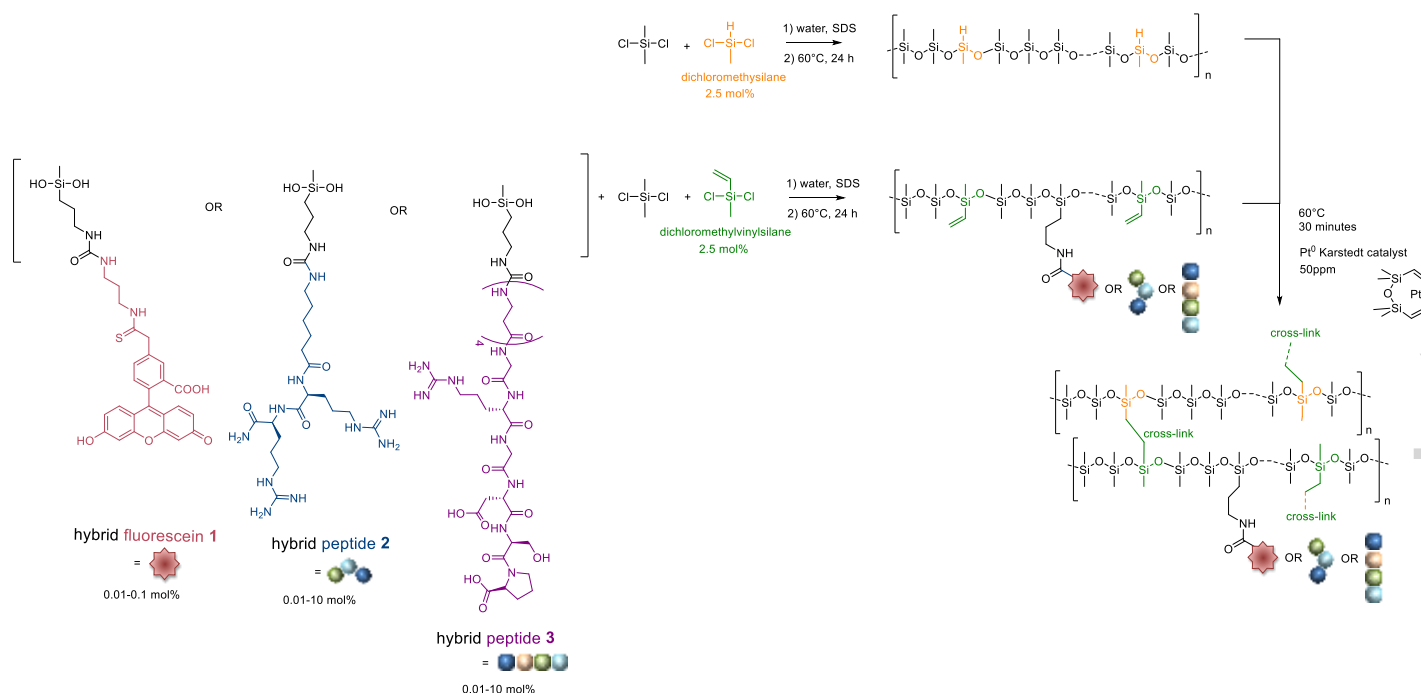




**Figure 2.** 2.5 mol% vinyl-PDMS oil **P4** (left), Fluorescein containing-PDMS oil **P9** (middle) and **P10** (right).

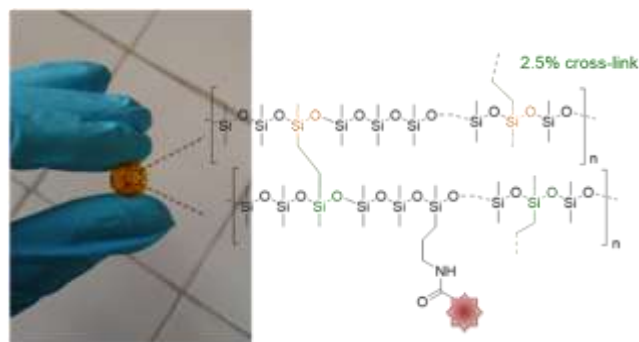
Therefore, PDMS containing higher concentration of peptide were synthesized in order to detect both hybrid peptides **2** and **3** by  $^1\text{H}$  NMR, and so prove their integration inside the silicone backbone (see Figure 16 in Supporting Information).

Having functionalized PDMS oils in hand, we set up the synthesis of silicone materials by cross-linking two modified PDMS displaying the same percentage of vinyl groups and Si-H functions (**Scheme 2**). These functional PDMS materials, noted **M1** to **M16**, were obtained by hydrosilylation using the Karstedt's catalyst. The same amount of each complementary oils was mixed up with 50 ppm Karstedt's catalyst then spread into a glass slide and put in the oven at 60 °C for 30 min.



**Scheme 2.** Direct synthesis of peptide-containing silicone (or fluorescent-silicone) by hydrosilylation-mediated cross-linking of functionalized silicone oils.

We have investigated the effect of crosslink's ratio (from 1% to 10%) on the mechanical properties of PDMS by nano-indentation. We observed a linear progression of the indentation modulus of the materials obtained when the crosslinking amount was between 2.5 and 10%. Above 10% cross-linking, the material loses its elasticity and became more susceptible to break under pressure. On the contrary, below 2.5% cross-linking, no solid material was obtained and, characterization by indentation was not performed. Taking into account these results, we chose arbitrary the 2.5% for the following syntheses of hybrid silicone material containing peptide or fluorescein (**Figure 3**).



**Figure 3.** PDMS material **M6** (2.5% cross-linking, containing 0.5 mol% of hybrid fluorescein **1**) removed from a circular mold.

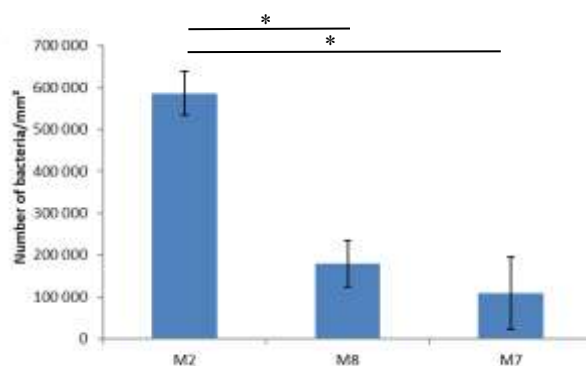
We then prepared peptide-containing-PDMS materials **M7** and **M8** containing respectively 0.005 mol% and 0.05 mol% of hybrid peptide **2**, from hybrid silicone oils. For more clarity, **M7** and **M8** would be also noted as  $M_{0.005}^2$  and  $M_{0.05}^2$ , **2** for the peptide and the index number for the theoretical percentage of incorporation. Si-H and peptide containing Si-Vinyl silicone oils were mixed and drop-casted on microscope glass slides ( $\sim 2 \text{ cm}^2$ ) in the presence of Karstedt's catalyst. After 30 min at  $60^\circ \text{C}$ , the cross-linking is over and thin layers ( $\sim 100 \mu\text{m}$ ) of PDMS-based antibacterial were obtained. 3 samples were prepared for each material as well as a non-functionalized PDMS materials **M2** obtained from **P3** and **P4** PDMS oils (**Table 2**).

**M7** ( $M_{0.005}^2$ ) and **M8** ( $M_{0.05}^2$ ) were evaluated for antibacterial activity against the *Staphylococcus aureus* gram positive bacteria. This bacteria strain is responsible for many nosocomial infection and is commonly found on contaminated medical devices.<sup>23,24</sup> The antibacterial activity was investigated by using a method described in normalized guidelines with minor modifications (ISO 22196).  $10 \mu\text{l}$  of bacterial solution ( $10^5 \text{ CFU}\cdot\text{mL}^{-1}$ ) was dropped on each sample and recovered with  $1 \text{ cm}^2$  sterile glass coverslip. After 24 h at  $37^\circ \text{C}$ , bacteria were detached from supports by washing and sonication. The viable bacteria in solution were spread on agar plates for CFU determination. The assay was performed in triplicate. The percentage of inhibition was calculated on the basis of the number of bacteria found on non-functionalized PDMS material (**M2**).

**Table 2.** Composition of different functional PDMS materials obtained from functionalized PMDS oils.<sup>a</sup>Average of 10 indentation modulus ( $E_{IT}$ ) obtained by indents at 60 $\mu$ N and 100 $\mu$ N.

Material	Obtained from 50/50 mol%					
	V-PDMS	H-PDMS	Xlink%	$E_{IT}$ (MPa) <sup>a</sup>	mol% peptide	
<b>M1</b>	<b>P1</b>	<b>P2</b>	1	n.d.	0	
<b>M2</b>	<b>P3</b>	<b>P4</b>	2.5	1.61	0	
<b>M3</b>	<b>P5</b>	<b>P6</b>	5	2.66	0	
<b>M4</b>	<b>P7</b>	<b>P8</b>	10	5.68	0	
<b>M5</b>	$M_{0.005}^1$	<b>P3</b>	<b>P9</b>	2.5	3.09	0
<b>M6</b>	$M_{0.05}^1$	<b>P3</b>	<b>P10</b>	2.5	2.70	0
<b>M7</b>	$M_{0.005}^2$	<b>P3</b>	<b>P11</b>	2.5	1.49	0.005
<b>M8</b>	$M_{0.05}^2$	<b>P3</b>	<b>P12</b>	2.5	1.12	0.05
<b>M9</b>	$M_{0.5}^2$	<b>P3</b>	<b>P13</b>	2.5	0.26	0.5
<b>M10</b>	$M_{2.5}^2$	<b>P3</b>	<b>P14</b>	2.5	0.28	2.5
<b>M11</b>	$M_5^2$	<b>P3</b>	<b>P15</b>	2.5	0.18	5
<b>M12</b>	$M_{0.005}^3$	<b>P3</b>	<b>P16</b>	2.5	1.23	0.005
<b>M13</b>	$M_{0.05}^3$	<b>P3</b>	<b>P17</b>	2.5	0.35	0.05
<b>M14</b>	$M_{0.5}^3$	<b>P3</b>	<b>P18</b>	2.5	0.29	0.5
<b>M15</b>	$M_{2.5}^3$	<b>P3</b>	<b>P19</b>	2.5	1.12	2.5
<b>M16</b>	$M_5^3$	<b>P3</b>	<b>P20</b>	2.5	0.77	5

Results are reported in **Figure 4**. The number of bacteria found on functionalized **M2** (over 580 000 bacteria/mm<sup>2</sup>) was much higher than the number found on peptide-containing materials (110 000 - 180 000) which corresponded to a decrease of 70% and 81.5 % for **M7** ( $M_{0.005}^2$ ) and **M8** ( $M_{0.05}^2$ ) respectively. This result clearly demonstrated that the peptide kept its antibacterial properties when covalently linked on silicone, avoiding the bacterial adhesion on surfaces, and that only small amount of peptide is necessary to get an antibacterial activity.



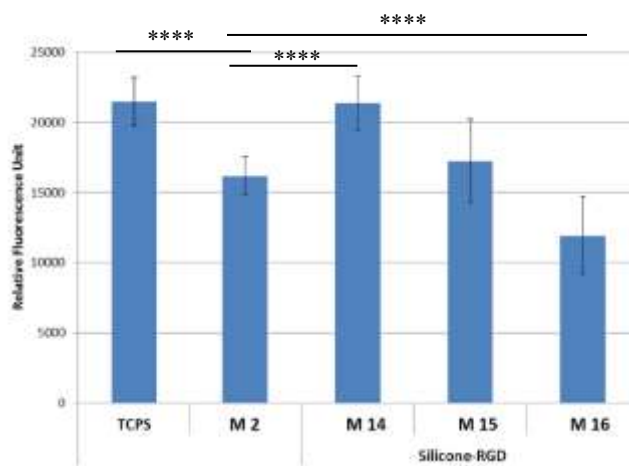
**Figure 4.** Antibacterial activity of silicone materials **M2**, **M7** ( $M_{0.005}^2$ ) and **M8** ( $M_{0.05}^2$ ) against *S. aureus* after 24 hours incubation. Results are presented as a mean of 3 experiments and error bars represent SD, \*p < 0.05 (Non-parametric Mann-Whitney test).

Moreover, as the peptide is covalently immobilized on the polymer chains of the materials, one could expect a prolonged activity compared to a simple antibacterial incorporation. Such material with sustained antibacterial activity is particularly attractive for medical device conception such as catheter, laryngeal masks and septum.

We also prepared peptide **3**-containing-PDMS materials (**M12** ( $M_{0.005}^3$ ), to **M16** ( $M_5^3$ ), containing from 0.005 mol% to 5 mol% hybrid peptide **3**), from hybrid silicone oils. Si-H (**P3**) and peptide containing Si-Vinyl silicone oils (**P16-P20**) were mixed and drop-casted on glass coverslip of approximately 1 cm<sup>2</sup>, in the presence of Karstedt's catalyst at the equivalence of 50 ppm. After a 30 min treatment at 60 °C in an oven, the cross-linking was complete and thin layers (~100 μm) of hybrid PDMS were finally obtained.

RGD-containing PDMS **M14** ( $M_{0.5}^3$ ), **M15** ( $M_{2.5}^3$ ) and **M16** ( $M_5^3$ ), assayed for the adhesion of L929 fibroblasts and compared to non-functionalized PDMS materials **M2** (**Figure 5**). Highly favorable 'Treated for Cell Culture Polystyrene' (TCPS) was used as a positive control. L929 fibroblasts cells were deposited onto the different materials. After 4h, an assay was performed to quantify cell viability (i.e., PrestoBlue, Invitrogen). Once reagent added, viable cells convert it into intensely redfluorescent dye. Higher is the fluorescent signal better is the cell adhesion onto the surface. Cell adhesion was significantly higher on RGD-PDMS **M14** ( $M_{0.5}^3$ ), compared to control **M2** (control) (\*\*\*\*p<0.0001). More interestingly, cell adhesion on **M14** ( $M_{0.5}^3$ ) and **M15** ( $M_{2.5}^3$ ) were similar to this obtained with the positive TCPS control witnessing the

importance of the RGD peptide, which turned the PDMS into a surface suitable for proliferation. Surprisingly, the cell adhesion was lower with **M16** ( $M_5^3$ ) which incorporated more RGD peptides, being even less efficient than the silicone control, probably because of a too high density of peptides on the surface. Thus, the incorporation of 0.5 mol% of **3** was enough to promote fibroblast adhesion. The bioactive effect of the peptide was confirmed by the cells morphology on materials. L929 cells on non-modified PDMS material (**M2**) revealed a rounded shape after 96h of culture compared with cells grown on 0.5% RGD peptide (**M14** ( $M_{0.5}^3$ )). The morphology of L929 fibroblasts on **M14** ( $M_{0.5}^3$ ) is similar to cells on TCPS (see Figure 19 in supporting information). These observations correlated with results obtained by measuring metabolic activity. These observations show the importance of the RGD peptide incorporation in the material to enhance the cells adhesion and thus provide bioactivity to unmodified material.



**Figure 5.** Fibroblast cell adhesion on silicone materials **M2**, **M14** ( $M_{0.5}^3$ ), **M15** ( $M_{2.5}^3$ ) and **M16** ( $M_5^3$ ) after 4h incubation. Results are presented as mean and error bars represent SD (n=5). P-values < 0.05 (\*), P < 0.01 (\*\*), P < 0.001 (\*\*\*), or P < 0.0001 (\*\*\*\*) (One-way ANOVA test followed by Tukey's multiple comparison test)

Several studies describing the modification of silicone with antibacterial peptides or cell-adhesion peptides have been reported in literature.<sup>16,25–27</sup> However and in strong contrast, the approach we reported here is completely different. Indeed, the bioactive peptides were covalently incorporated in the silicone oils before the preparation of PDMS materials. This strategy is highly generic as different oils can be mixed together and cross-linked, the peptide moiety being either carried by the vinyl-functionalized PDMS or by the Si-H functionalized PDMS. Moreover, different types of peptides can be introduced together for a

synergistic effect. Combinations of bioactive moieties can be introduced on the same linear PDMS oil or on differently modified PDMS oils. In these first examples, we used a short cationic peptide and a 9-mer linear peptide to demonstrate how generic was the strategy. Hydrosilylation proceeded chemoselectively between Si-H and Si-Vinyl function versus carboxylic acids, alcohols, amides, phenols and amines, enabling the reticulation of PDMS in the presence of unprotected peptides. This strategy opens the way to the design of any tailored bioactive silicone material by combination of the suitable bioactive silicone oils prepared with hybrid dichloromethylsilyl-biomolecules.

## CONCLUSION

Several studies describing the modification of silicone with antibacterial peptides or cell-adhesion peptides have been reported in literature.<sup>16,25–27</sup> However and in strong contrast, the approach we reported here is completely different. Indeed, the bioactive peptides were covalently incorporated in the silicone oils before the preparation of PDMS materials and without any activation or treatment of the PDMS material as needed with post-functionalization strategies. This strategy is highly generic as different oils can be mixed together and cross-linked, the peptide moiety being either carried by the vinyl-functionalized PDMS or by the Si-H functionalized PDMS and does not need any protection on its side chain. Moreover, different types of peptides can be introduced together for a synergistic effect and incorporated with a controlled ratio. Combinations of bioactive moieties can be introduced on the same linear PDMS oil or on differently modified PDMS oils. In these first examples, we used a short cationic peptide and a 9-mer linear peptide to demonstrate how generic the strategy was. Hydrosilylation proceeded chemoselectively versus carboxylic acids, alcohols, amides, phenols and amines, enabling the reticulation of PDMS in the presence of unprotected peptides. This strategy opens the way to the design of any tailored bioactive silicone material by combination of the suitable bioactive silicone oils prepared with hybrid dichloromethylsilyl-biomolecules.

## EXPERIMENTAL SECTION

**Synthetic procedures of compound 1.** Fluorescein-5-isothiocyanate (FITC) (120 mg, 0.3 mmol) was solubilized into anhydrous DMF. DIEA (100  $\mu$ L, 1.8 mmol) was added and then N-Boc-1,3-propanediamine (62  $\mu$ L, 0.36 mmol). The mixture was flushed under argon, stirred for 1h at r.t. and finally concentrated *in vacuo*. The Boc protecting group was removed by TFA treatment for 2h at r.t., and the TFA was removed *in vacuo*. The hybrid fluorescein was precipitated in Et<sub>2</sub>O and recovered by centrifugation. The product was then solubilized in anhydrous DMF. DIEA (500  $\mu$ L, 2.4 mmol) and isocyanatopropyl dichloromethylsilane (80  $\mu$ L, 0.36 mmol) were added. The mixture was flushed by argon and stirred 1h at r.t. After concentration of the solvent *in vacuo*, the product was purified by a PLRP-S column and lyophilized.

**Synthetic procedures of P9.** SDS (0.925 g, 3mmol) was dissolved in 30 mL of H<sub>2</sub>O. The mixture was heated up 60°C on a reflux. The three different monomers were added at the same time in the mixture: DCDM (4.8 mL, 39 mmol), DCMVS (0.11 mL, 0.9 mmol) and compound **1** (2.5 mg, 0.004 mmo). The polymerization was let for 16h. The polymer was then extracted by chloroform, washed three times by water, dried by MgSO<sub>4</sub> and concentrated *in vacuo*.

**Synthetic procedures of M5. P9 and P3** (20mg each) were both solubilized in THF (100 $\mu$ L) in a haemolysis tube. Karstedt catalyst was added to the solution (10 $\mu$ L of a 0.1 g/L solution in THF, corresponding to 50ppm). The solution was disposed on a glass slide. The THF was evaporated under the hood at room temperature and then the slide was placed in a 60°C oven for 30 min.

## Acknowledgments

Peptide syntheses were performed using the facilities of SynBio3 IBISA platform supported by ITMO cancer.

**Keywords:** Bioactive material • Silylated peptide • Silicone • Hydrosilylation • Antimicrobial

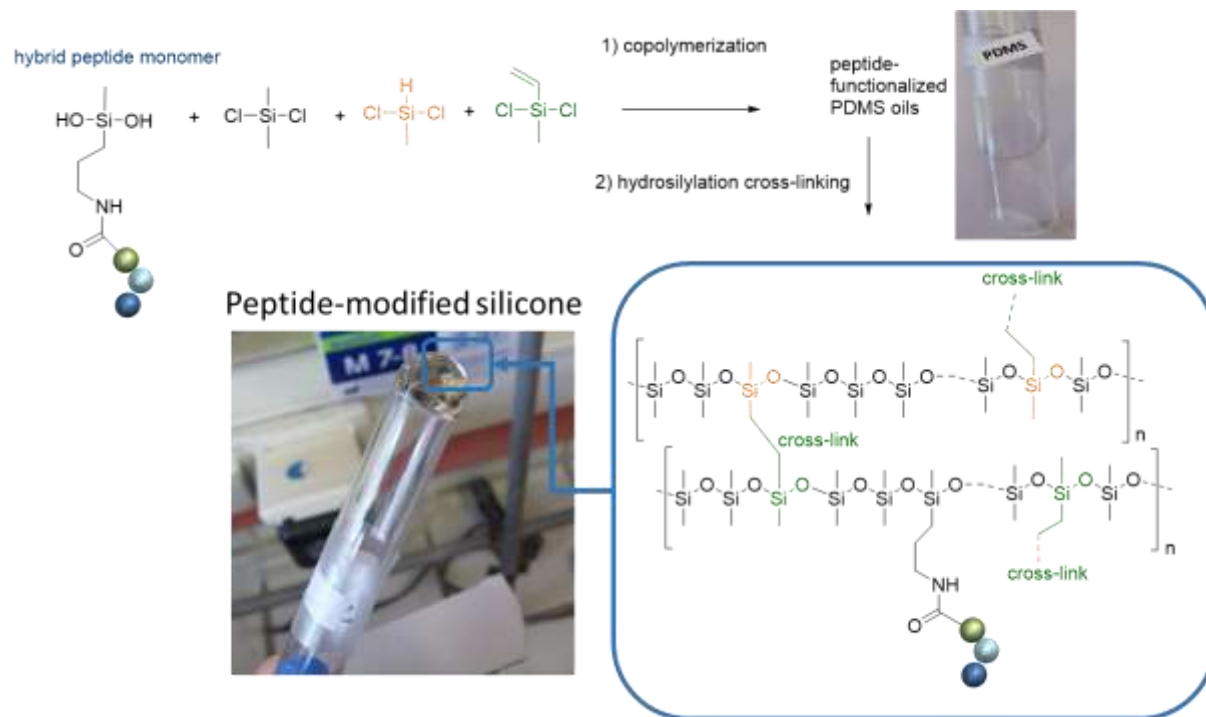


## REFERENCES

- (1) Owen, M. J. Properties and Applications of Silicones. In *Advances in Silicones and Silicone-Modified Materials*; ACS Symposium Series; American Chemical Society, 2010; Vol. 1051, pp 13–18.
- (2) Chekina, N. A.; Pavlyuchenko, V. N.; Danilichev, V. F.; Ushakov, N. A.; Novikov, S. A.; Ivanchev, S. S. A New Polymeric Silicone Hydrogel for Medical Applications: Synthesis and Properties. *Polym. Adv. Technol.* **2006**, *17* (11–12), 872–877. <https://doi.org/10.1002/pat.820>.
- (3) Madsen, F. B.; Yu, L.; Daugaard, A. E.; Hvilsted, S.; Skov, A. L. A New Soft Dielectric Silicone Elastomer Matrix with High Mechanical Integrity and Low Losses. *RSC Adv.* **2015**, *5* (14), 10254–10259. <https://doi.org/10.1039/C4RA13511C>.
- (4) Lee, J. N.; Jiang, X.; Ryan, D.; Whitesides, G. M. Compatibility of Mammalian Cells on Surfaces of Poly(Dimethylsiloxane). *Langmuir* **2004**, *20* (26), 11684–11691. <https://doi.org/10.1021/la048562+>.
- (5) Nge, P. N.; Rogers, C. I.; Woolley, A. T. Advances in Microfluidic Materials, Functions, Integration, and Applications. *Chem. Rev.* **2013**, *113* (4), 2550–2583. <https://doi.org/10.1021/cr300337x>.
- (6) Zhang, R.; Gong, H.-Q.; Zeng, X. D.; Sze, C. C. A High-Throughput Microfluidic Biochip to Quantify Bacterial Adhesion to Single Host Cells by Real-Time PCR Assay. *Anal Bioanal Chem* **2013**, *405* (12), 4277–4282. <https://doi.org/10.1007/s00216-013-6826-0>.
- (7) Carroll, M. J.; Stopfer, L. E.; Kreeger, P. K. A Simplified Culture System to Examine Soluble Factor Interactions between Mammalian Cells. *Chem. Commun.* **2014**, *50* (40), 5279–5281. <https://doi.org/10.1039/C3CC47507G>.
- (8) Salamone, J. C.; Salamone, A. B.; Swindle-Reilly, K.; Leung, K. X.-C.; McMahon, R. E. Grand Challenge in Biomaterials-Wound Healing. *Regen Biomater* **2016**, *3* (2), 127–128. <https://doi.org/10.1093/rb/rbw015>.
- (9) Bhatt, S.; Pulpytel, J.; Arefi-Khonsari, F. Low and Atmospheric Plasma Polymerisation of Nanocoatings for Bio-Applications. *Surface Innovations* **2015**, *3* (2), 63–83. <https://doi.org/10.1680/sufi.14.00008>.
- (10) Bartzoka, V.; McDermott, M. R.; Brook, M. A. Protein-Silicone Interactions. *Adv. Mater.* **1999**, *11* (3), 257–259. [https://doi.org/10.1002/\(SICI\)1521-4095\(199903\)11:3<257::AID-ADMA257>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1521-4095(199903)11:3<257::AID-ADMA257>3.0.CO;2-S).
- (11) Silver, J. H.; Lin, J. C.; Lim, F.; Tegoulia, V. A.; Chaudhury, M. K.; Cooper, S. L. Surface Properties and Hemocompatibility of Alkyl-Siloxane Monolayers Supported on Silicone Rubber: Effect of Alkyl Chain Length and Ionic Functionality. *Biomaterials* **1999**, *20* (17), 1533–1543.
- (12) Beppu, K.; Kaneko, Y.; Kadokawa, J.; Mori, H.; Nishikawa, T. Synthesis of Sugar-Polysiloxane Hybrids Having Rigid Main-Chains and Formation of Their Nano Aggregates. *Polym. J* **2007**, *39* (10), 1065–1070. <https://doi.org/10.1295/polymj.PJ2006268>.
- (13) Maeda, H.; Kasuga, T. Control of Silicon Species Released from Poly(Lactic Acid)-Polysiloxane Hybrid Membranes. *J Biomed Mater Res A* **2008**, *85* (3), 742–746. <https://doi.org/10.1002/jbm.a.31506>.
- (14) Chen, H.; Brook, M. A.; Sheardown, H. D.; Chen, Y.; Klenkler, B. Generic Bioaffinity Silicone Surfaces. *Bioconjugate Chem.* **2006**, *17* (1), 21–28. <https://doi.org/10.1021/bc050174b>.
- (15) Calabrese, D. R.; Wenning, B.; Finlay, J. A.; Callow, M. E.; Callow, J. A.; Fischer, D.; Ober, C. K. Amphiphilic Oligopeptides Grafted to PDMS-Based Diblock Copolymers for Use in Antifouling and Fouling Release Coatings. *Polymers for Advanced Technologies* **26** (7), 829–836. <https://doi.org/10.1002/pat.3515>.
- (16) Pinese, C.; Jebors, S.; Echalié, 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. *Advanced Healthcare Materials* **2016**, *5* (23), 3067–3073. <https://doi.org/10.1002/adhm.201600757>.
- (17) Pinese, C.; Jebors, S.; Emmanuel Stoeber, P.; 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. *Materials Today Chemistry* **2017**, *4*, 73–83. <https://doi.org/10.1016/j.mtchem.2017.02.007>.
- (18) 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* (12), 3778–3782. <https://doi.org/10.1002/anie.201411065>.
- (19) 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* (3), 243–247. <https://doi.org/10.1002/psc.2757>.

- (20) Darouiche, R. O. Treatment of Infections Associated with Surgical Implants. *N. Engl. J. Med.* **2004**, *350* (14), 1422–1429. <https://doi.org/10.1056/NEJMra035415>.
- (21) Masurier, N.; Tissot, J.-B.; Boukhriss, D.; Jebors, S.; Pinese, C.; Verdie, P.; Amblard, M.; Mehdi, A.; Martinez, J.; Humblot, V.; et al. Site-Specific Grafting on Titanium Surfaces with Hybrid Temporin Antibacterial Peptides. *J. Mater. Chem. B* **2018**. <https://doi.org/10.1039/C8TB00051D>.
- (22) Palaprat, G.; Ganachaud, F. Synthesis of Polydimethylsiloxane Microemulsions by Self-Catalyzed Hydrolysis/Condensation of Dichlorodimethylsilane. *Comptes Rendus Chimie* **2003**, *6* (11), 1385–1392. <https://doi.org/10.1016/j.crci.2003.09.002>.
- (23) Darouiche, R. O. Device-Associated Infections: A Macroproblem That Starts with Microadherence. *Clin Infect Dis* **2001**, *33* (9), 1567–1572. <https://doi.org/10.1086/323130>.
- (24) García-Aparicio, L.; Blázquez-Gómez, E.; Martín, O.; Krauel, L.; de Haro, I.; Rodó, J. Características Bacterianas y Significado Clínico de Catéteres Ureterales Doble J En Niños. *Actas Urológicas Españolas* **2015**, *39* (1), 53–56. <https://doi.org/10.1016/j.acuro.2014.04.008>.
- (25) Prijck, K. D.; Smet, N. D.; Rymarczyk-Machal, M.; Driessche, G. V.; Devreese, B.; Coenye, T.; Schacht, E.; Nelis, H. J. *Candida Albicans* Biofilm Formation on Peptide Functionalized Polydimethylsiloxane. *Biofouling* **2010**, *26* (3), 269–275. <https://doi.org/10.1080/08927010903501908>.
- (26) Lim, K.; Chua, R. R. Y.; Saravanan, R.; Basu, A.; Mishra, B.; Tambyah, P. A.; Ho, B.; Leong, S. S. J. Immobilization Studies of an Engineered Arginine–Tryptophan-Rich Peptide on a Silicone Surface with Antimicrobial and Antibiofilm Activity. *ACS Applied Materials & Interfaces* **2013**, *5* (13), 6412–6422. <https://doi.org/10.1021/am401629p>.
- (27) Mishra, B.; Basu, A.; Chua, R. R. Y.; Saravanan, R.; Tambyah, P. A.; Ho, B.; Chang, M. W.; Leong, S. S. J. Site Specific Immobilization of a Potent Antimicrobial Peptide onto Silicone Catheters: Evaluation against Urinary Tract Infection Pathogens. *J. Mater. Chem. B* **2014**, *2* (12), 1706–1716. <https://doi.org/10.1039/C3TB21300E>.

## Table of Contents



A simple and efficient way to synthesize peptide-containing silicone material was described. Antibacterial peptide containing silicone oils were prepared. Resulting peptide-functionalized silicone oils were then cross-linked by hydrosilylation, yielding active PDMS materials in a single step. This generic method can be applied to any peptide sequence and thus opens the way to the synthesis of a wide range of biologically active silicones.