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Using sophorolipids as an antiadhesive or release strategy to fight *S. aureus* catheter-related infections

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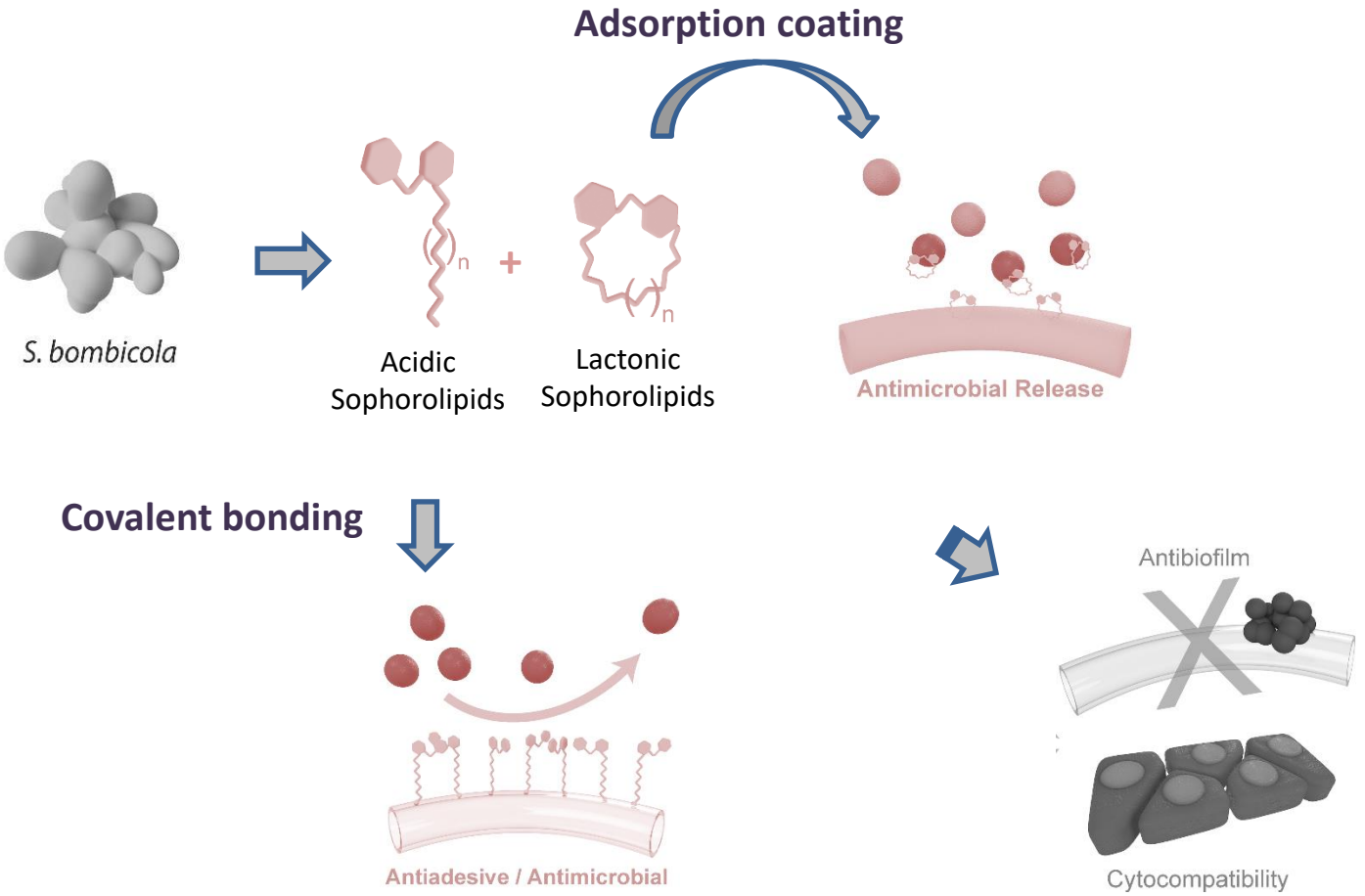
Using sophorolipids as an antiadhesive or release strategy to fight *S. aureus* catheter-related

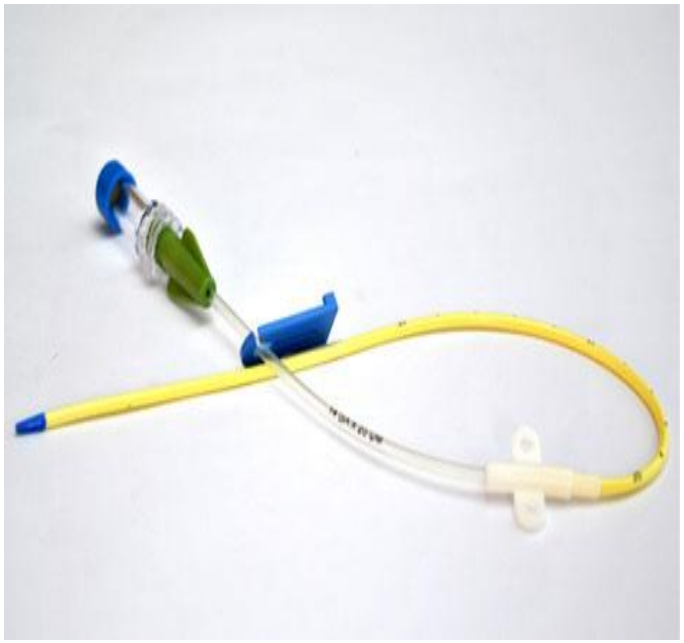


Abstract: Among medical devices, blood stream catheters are certainly included in the most used. Nevertheless, *S. aureus* catheter-related infection are of great concern and developing strategies to prevent bacteria colonization remain a big challenge. This work aimed to modify medical grade silicon surfaces with sophorolipids, glycolipid biosurfactant, endowed with antimicrobial and antiadhesive properties. Two approaches were carried out: i) an antiadhesive strategy that uses the covalent bonding of sophorolipids to silicone surface and ii) a release-based strategy with the isolated most active sophorolipids adsorbed to the surface. *S*ophorolipids were produced by *Starmerella bombicola,* purified and isolated by automatic flash chromatography and identified using UHPLC-MS and RMN. Highest antimicrobial activity was observed with the isolated C18:0 and C18:1 diacetylated lactonic sophorolipids, that presented a minimum inhibitory concentration of 50 µg mL^{-1}. After functionalization, surfaces were evaluated by contact angle measurement, FTIR-ATR and AFM analysis. The antiadhesive strategy, using a mixture of acidic sophorolipids covalently bonded to the silicone surface, exhibited a biofilm reduction of 90% with no interference observed with HaCaT human cells. Concerning the release approach using the isolated C18:1 diacateylated lactonic sophorolipid, 5 log units reduction was observed on the biofilm formation and no reduction in HaCaT cells viability. Referring to the results above, sophorolipids seem promising biomolecules to prevent the occurrence of *S. aureus* catheter-related infections.

Keywords: Anti-adhesive; Antibacterial surface; Biofilm; Infection; Release; *S. aureus.*

Introduction, Objective

- ✓ Actually biomedical stents are in an increasing use to rescue multiple lives;
- ✓ The main drawback of using biomedical stents is the Healthcare associated infections (HAIs);
- ✓ 43.3 % of HAIs are belong to blood stream catheters and originated by *S. aureus* bacterial biofilm
- ✓ Antimicrobial molecules (antibiotics) prone to develop bacteria resistance.



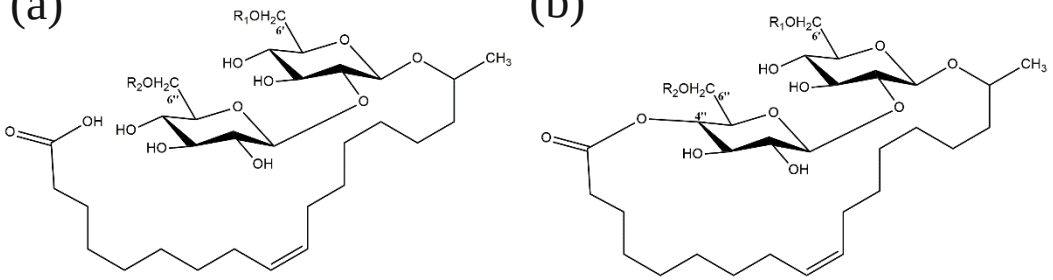
Strategies not prone to develop bacteria resistance and to overcome *S. aureus* colonization are needed

Introduction, Methods

1. Sophorolipids biosurfactants production

- ✓ Sophorolipids (SLs) produced as a mixture of acidic and Lactonic structure by *S. bombicola*
- ✓ SLs' properties: amphiphilic structure, antimicrobial and antiadhesive properties.

(a) (b)



R_1=R_2=H;
R_1=H and R_2=$COCH_3$;
R_1=$COCH_3$ and R_2=H;
R_1=R_2 = $COCH_3$

(a) SLs in acidic and (b) SLs in lactonic form,

2. SLs' down processing stream methodologies

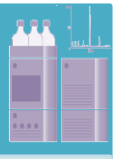
Isolation

- Liquid-Liquid extraction (ethyl acetate)

Purification

- Combi flash

Identification

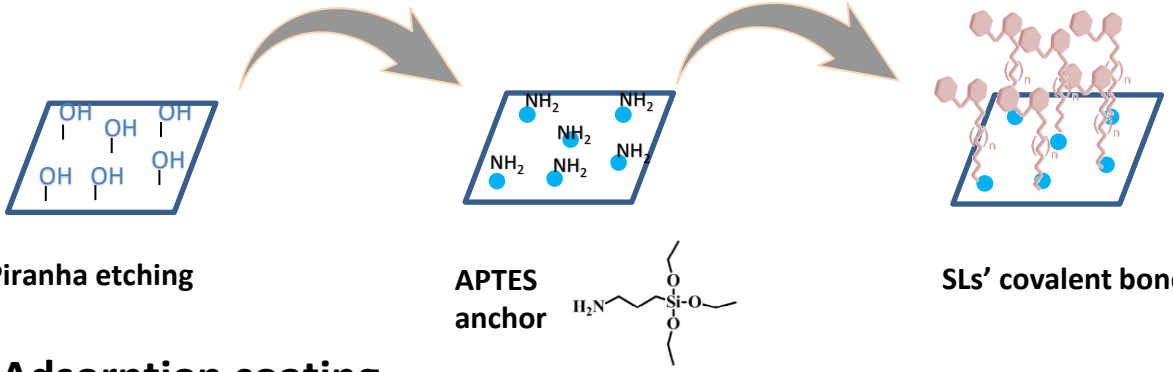


- UHPLC-MS/MS
- NMR

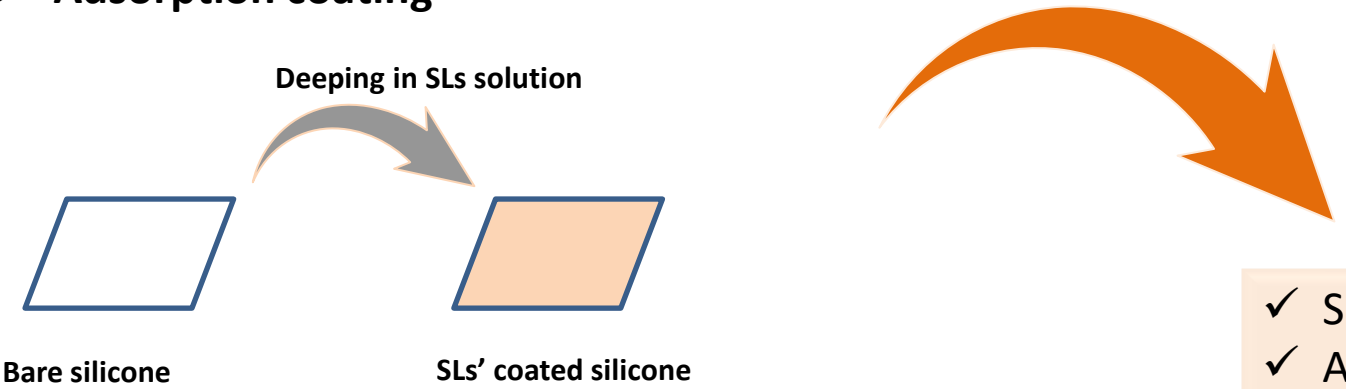
Introduction, Methods

3. Silicone surface functionalization using SLs biosurfactants

- **Covalent bonding**



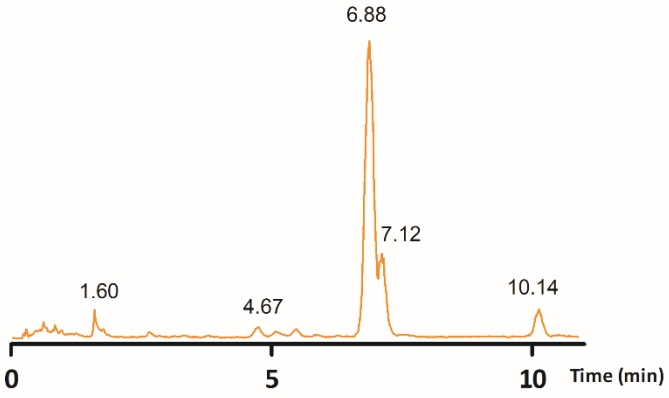
- **Adsorption coating**



- ✓ Surfaces characterization
- ✓ Anti-microbial assay
- ✓ Anti-Biofilm assay
- ✓ Cytocompatibility assay
- ✓ Release assay

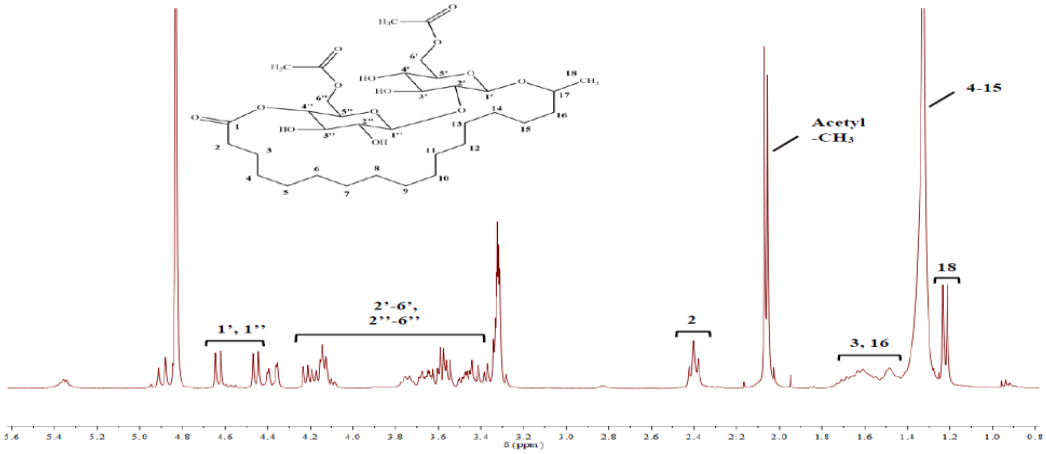
Results and discussion

1. UHPLC-MS/MS identification



RT (min)	$[M+Na]^+$	$[M+H]^+$	MW	Sophorolipid
0.84	729	707	706	A–C18:1 deacetylated
0.96	687	665	664	A–C18:1 monoacetylated
1.6	729	707	706	A-C18:1 diacetylated
3.79	669	647	646	L–C18:1monoacetylated
4.76	709	687	686	L-C18:2 diacetylated
5.09; 5.46	685	663	662	L-C16:0 diacetylated
6.9; 7.12	711	689	688	L-C18:1 diacetylated
10.14	713	691	690	L-C18:0 diacetylated

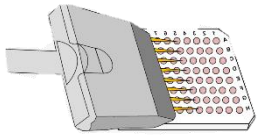
2. NMR identification



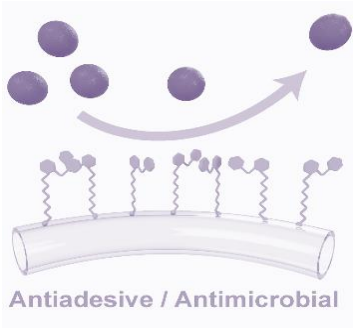
^{1}H NMR spectrum of the isolated L-C18:1 diacetylated SL in MeOD.

Results and discussion

3. SLs ' purification and Minimum Inhibitory Concentration (MIC)



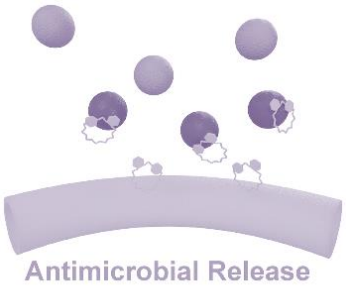
	Sophorolipid	Purity	MIC (μg mL^{-1})
Purified	**L-C18:0 diacetylated SL**	73.6	50
	L-C18:1 diacetylated SL	94.6	50
	L-C18:2 diacetylated SL	76.4	200
	L-C18:3 diacetylated SL	88.8	200
	A-C18:1 deacetylated SL	87.2	> 800
Mixtures	**SLs_{OA}**	-	50
	L-SLs_{OA}	-	100
Levofloxacin (control)		-	0.25



A-C18:1 deacetylated SL
SLs_{OA}

Covalent bonding

Release coating

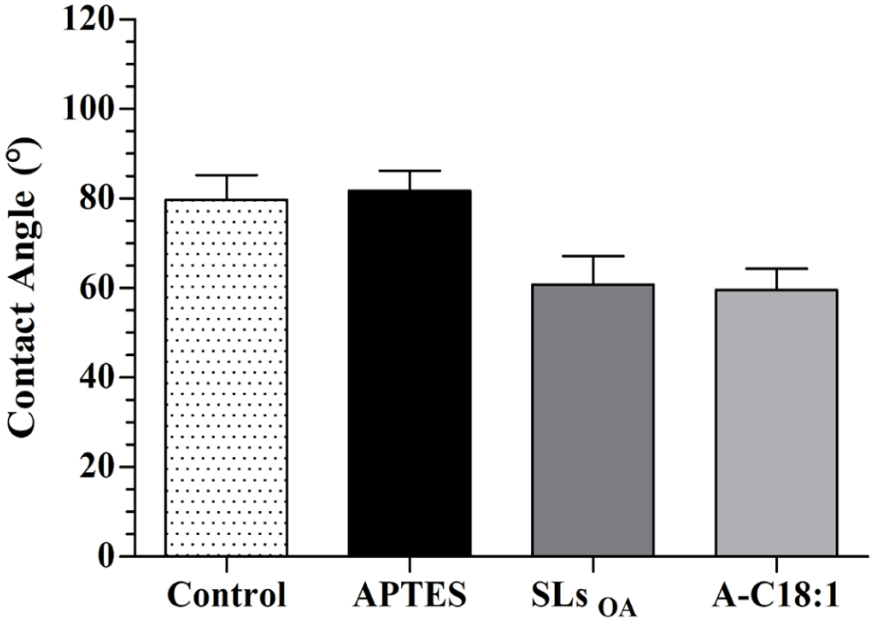


L-C18:0 diacetylated SL
L-C18:1 diacetylated SL
L-SLs_{OA}

Results and discussion

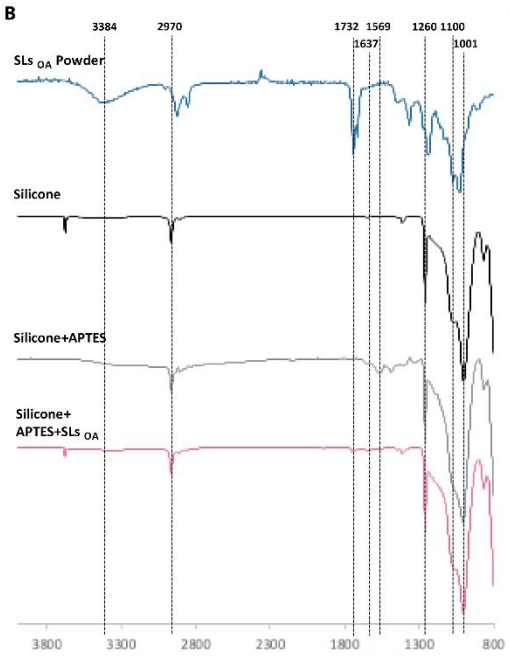
4. Evaluation of silicone surface functionalization by chemical modification (covalent bonding)

Contact angle

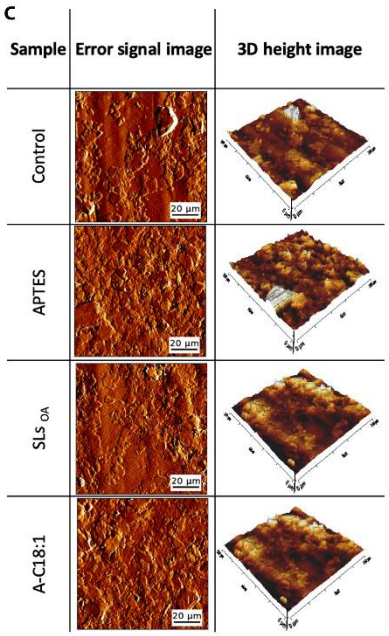


Decrease of 20º in the Contact angle of the functionalized substrate comparing to the control

FTIR



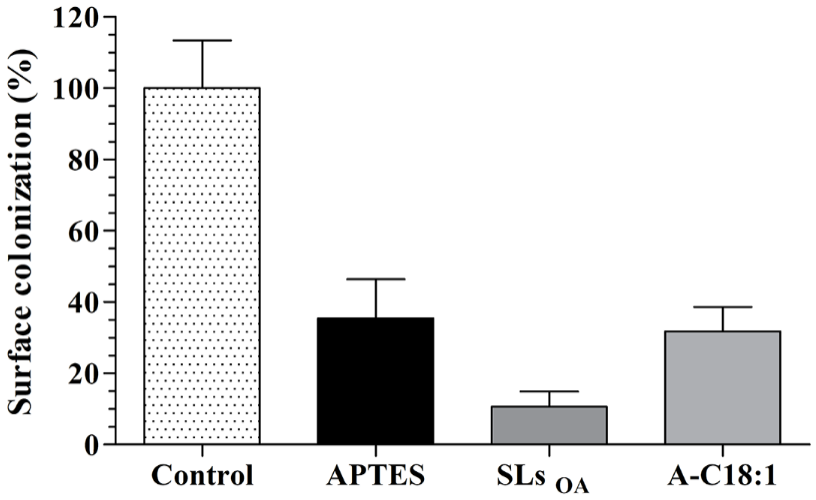
AFM



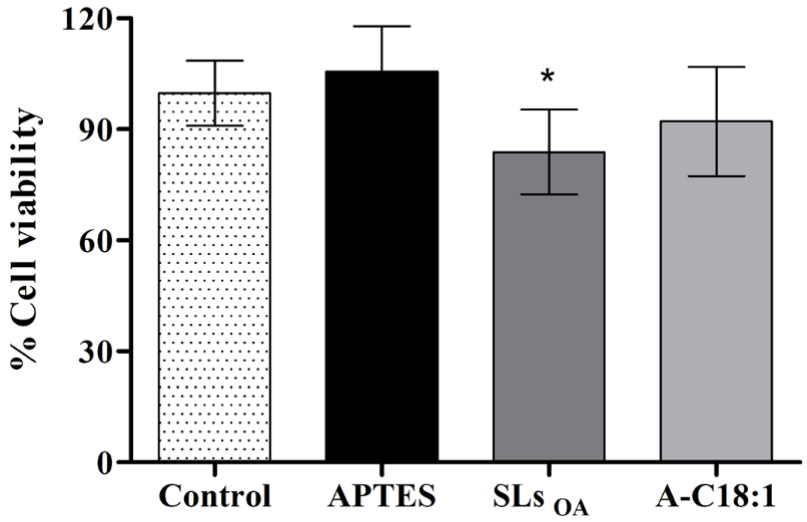
The appearance of the amide group on the functionalized samples confirms the covalent linkage of SLs on the silicone surface also an increase in the surface roughness have been observed

Results and discussion

4. Evaluation of silicone surface functionalization by chemical modification



90% and 70% of biofilm reduction was observed with the functionalized samples comparing to the control



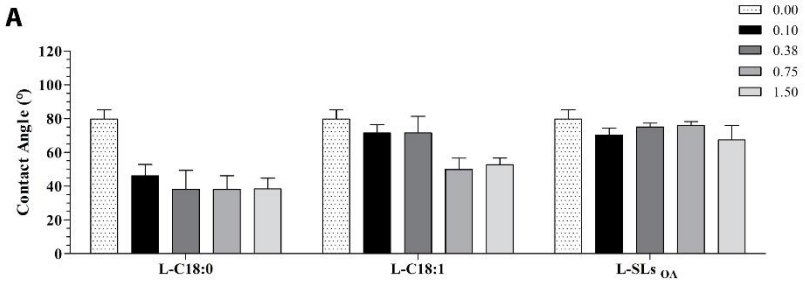
70% of the metabolic activity was maintained comparing to the control

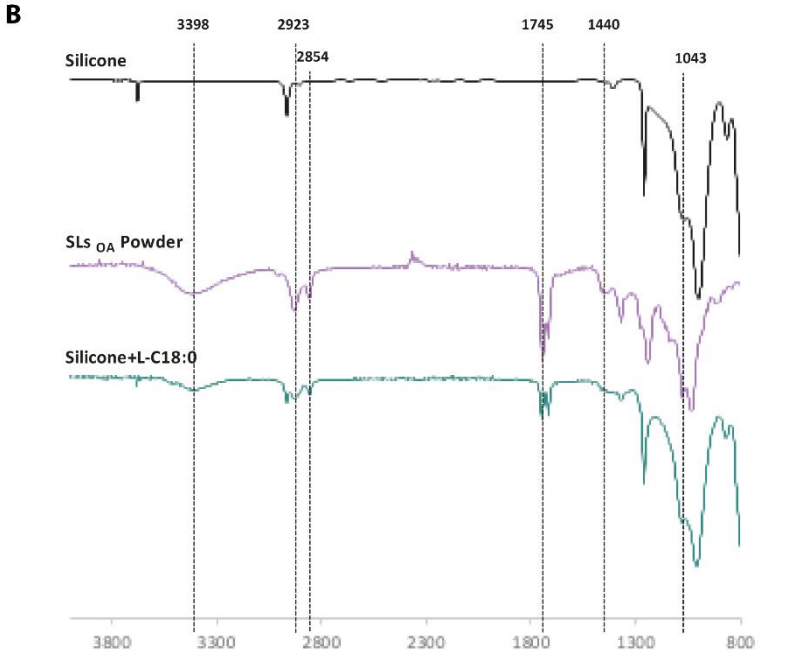
Covalent bonding shows an antiadhesive effect and cytocompatibility

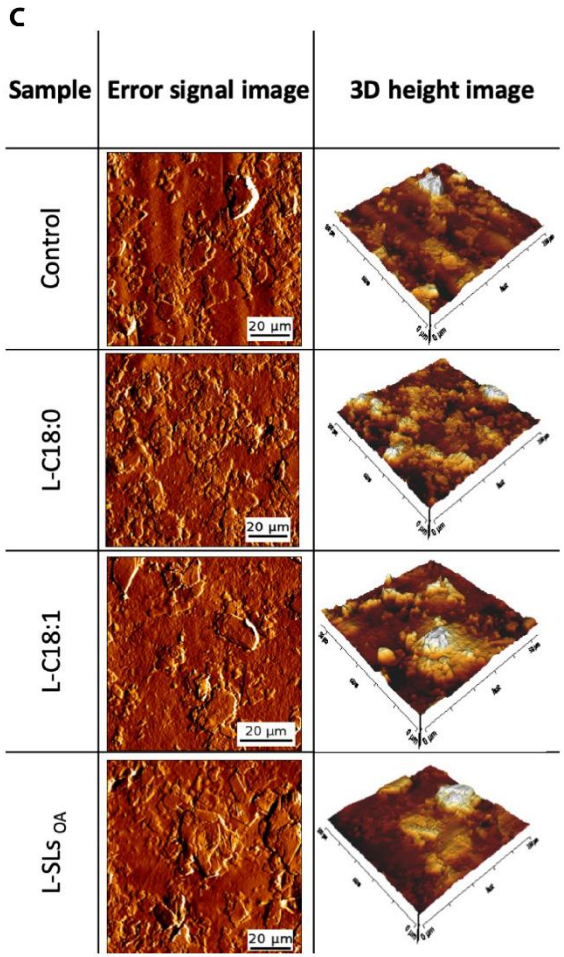
Results and discussion

4. Evaluation of silicone surface functionalization by L-SLs' adsorption

➢ Surface characterization





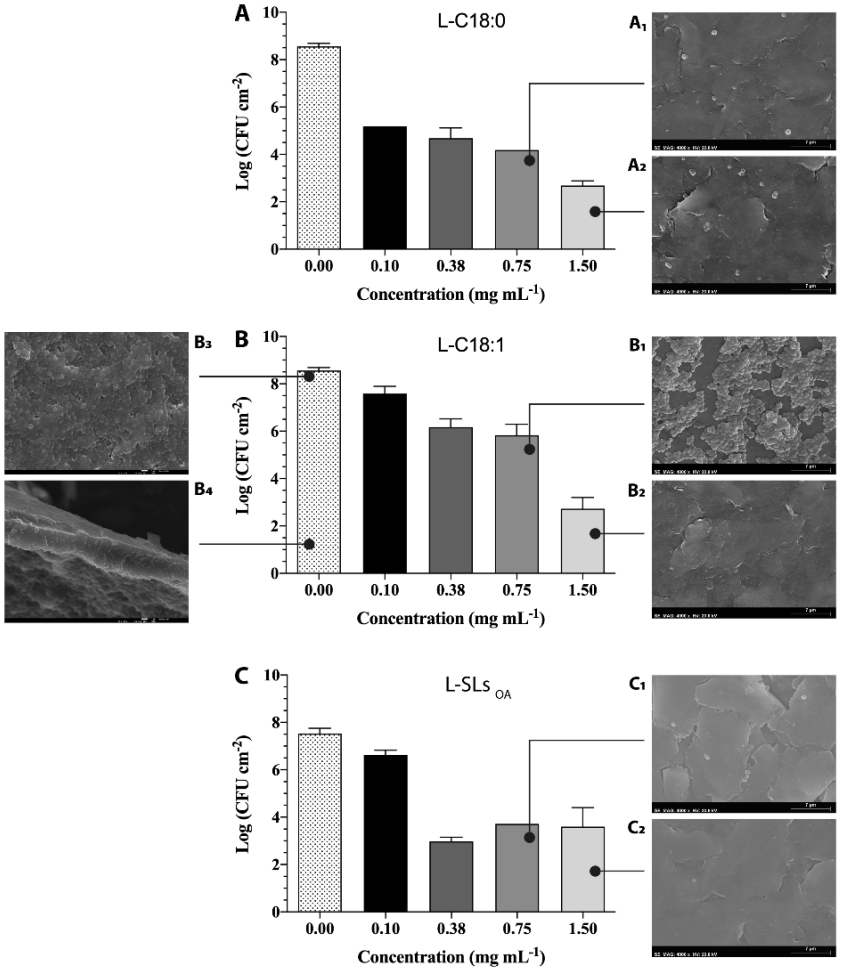


- ✓ Decrease in the contact angle measurement especially when coated with the isolated L-C18:0 with all the concentrations and comparing to the control;
- ✓ FTIR spectra confirm the coating: specific SLs' bands appear on the coated substrates

Results and discussion

4. Evaluation of silicone surface functionalization by L-SLs' adsorption

➢ Anti-biofilm assay



Sessile bacteria CFU counts:

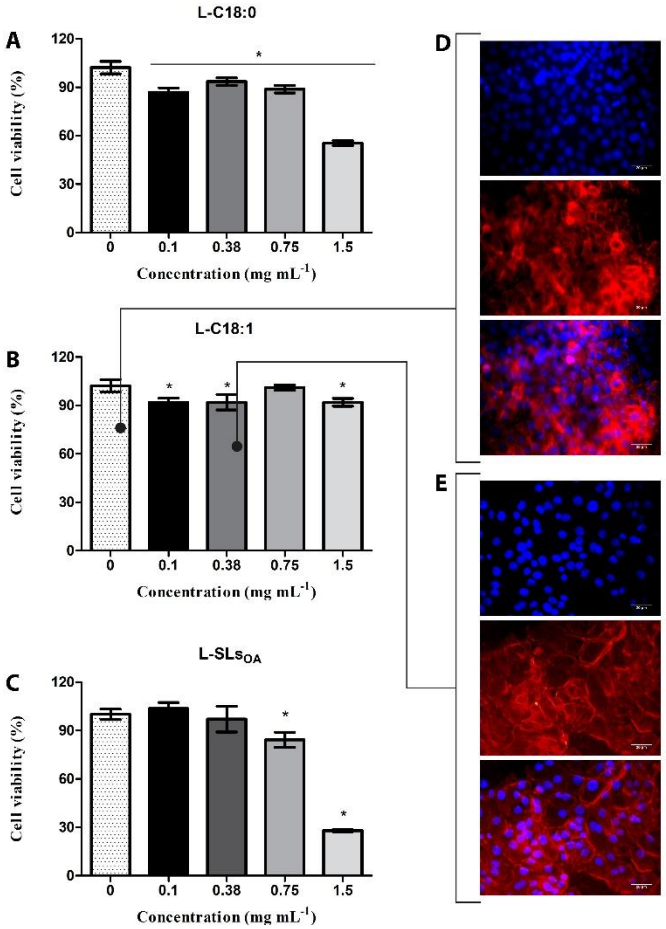
a decrease of 6 log units on coated silicone with L-C18:0 diacetylated SL (A) and a decrease of 5 log units on coated silicone L-C18:1 diacetylated SL (B) when a concentration of 1.5 mg mL^{-1} was used.

SEM images of *S. aureus* biofilm on representative silicone segments. A_1: L-C18:0 diacetylated SL 0.75 mg mL^{-1}; A_2: L-C18:0 diacetylated SL 1.50 mg mL^{-1}; B_1: L-C18:1 diacetylated SL 0.75 mg mL^{-1}; B_2: L-C18:1 diacetylated SL 1.50 mg mL^{-1} B_3: Control (top observation); B_4: Control (side observation). C_1: L-Mixture SLs 0.75 mg mL^{-1}; C_2: L-Mixture SLs 1.50 mg mL^{-1}.

Results and discussion

4. Evaluation of silicone surface functionalization by L-SLs' adsorption

➢ Cytocompatibility assay



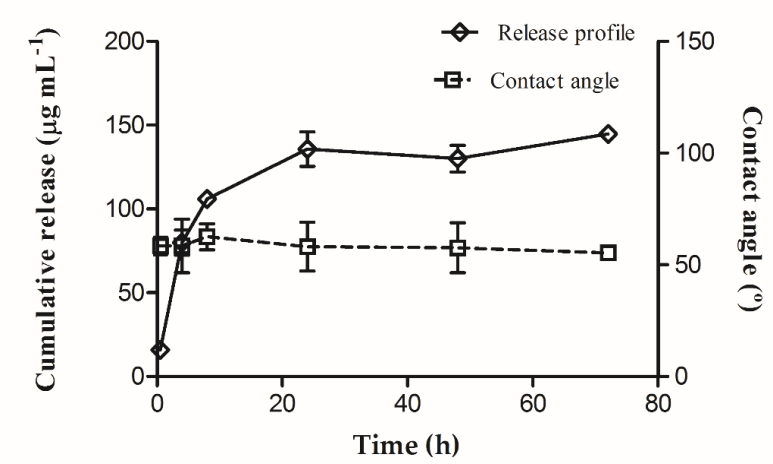
A–**C: HaCaT cell viability** in direct contact assay with different silicone specimens adsorbed with SLs after 48 h of proliferation L-C18:0 diacetylated SL (A), L-C18:1 diacetylated SL (B) and L-Mixture SLs (C). *P < 0.05 (mean ± SD, n = 8). D and E: Immunochemistry analysis of 48 h HaCaT cell line growth on surface of plain silicone (D) and L-C18:1 diacetylated SL (E). Nuclei were stained with DAPI (blue); actin was stained with rhodamine phalloidin (red) (scale bar: 20 µm); Merged images are in pink and blue.

L-C18:1 diacetylated SL: a promising candidate for silicon coating

Results and discussion

4. Evaluation of silicone surface functionalization by L-SLs' adsorption

- **Release assay**



Cumulative release of L-C18:1 diacetylated SL from silicone surface (202 ± 14 µg. cm^{-2}) and water contact angle measurements through time.

The coating approach seems to be promising in *S. aureus* biofilm fighting

Conclusions

- ✓ SLs biosynthezised by *S. bombicola were extracted, purified, isolated and identified;*
- ✓ Two strategies on silicone surface modification by SLs were evaluated: the antiadhesive and the release strategies using the acidic and the lactonic forms respectively;
- ✓ A reduction of the *S. aureus* biofilm was observed with the two approaches, however the coating by adsorption of the L- C18:1 diacetylated SL seems to be the more effective when considering the anti-biofilm and the cytocompatibilty assays.

Acknowledgments

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