Toxicology Reports 14 (2025) 101862

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Cytotoxicity evaluation of microbial sophorolipids and glucolipids using normal human dermal fibroblasts (NHDF) *in vitro*

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ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:
Fibroblasts
NHDF
Sophorolipids
Sophorosides
Cytotoxicity
Biosurfactants

ABSTRACT

Fibroblasts are considered a key player in the wound healing process. Although this cellular family is constituted by several distinct subtypes, dermal fibroblasts are crucial thanks to their ability to secrete pro-regenerative growth factors, extracellular matrix (ECM) proteins and their immune and anti-inflammatory role. Sophorolipids (SL), sophorosides (SS) and glucolipids (G), mono-unsaturated (C18:1) or saturated (C18:0), glycolipids derived from microbial fermentation of wild type or engineered yeast *Starmerella bombicola*, constitute a novel sustainable class of bio-based chemicals with interesting physicochemical characteristics, which allow them to form soft diverse structures from hydrogels to vesicles, micelles or complex coacervates with potential interest in skin regeneration applications. In this study, we first tested the cytocompatibility of a broad set of molecules from this family on normal human dermal fibroblasts (NHDF). Our results show that, up to an upper threshold (0.1 % w/v), the microbial glycolipids (SL-C18:1, G-C18:1, SS_{bola}-C18:1, SL-C18:0 and G-C18:0) under study were able to sustain cell growth. Furthermore, we selected the least cytotoxic glycolipids (SL-C18:1, SS_{bola}-C18:1, SL-C18:0) to study their potential to promote wound healing by measuring the gene expression of several key skin regeneration markers (i.e. collagen, elastin, transforming growth factor β, fibroblast growth factor …) using qPCR. Unfortunately, none of these glycolipids modulated the gene expression of molecules involved in tissue repair. However, this study aims to encourage the community to test this novel class of molecules for novel high-end biomedical applications.

Importance: Biosurfactants prepared by microbial fermentation are natural amphiphiles of growing importance, with the goal of replacing synthetic surfactants in commercial formulations. However, their cytotoxicity profile is still poorly known, especially for new molecules like single-glucose lipids or bolaform sophorolipids. This wants to contribute to all those applications, which could be developed with biosurfactants in contact with the skin (cosmetics, wound healing). We test the cytotoxicity of five structurally-related molecules (C18:1 and C18:0 sophorolipids, C18:1 and C18:0 single-glucose lipids, C18:1 di-sophoroside) against normal human dermal fibroblasts (NHDF) and evaluate the metabolic activity of the least toxic among them. To the best of our knowledge, cytotoxicity of these molecules, and of microbial biosurfactants in general, was never tested against NHDF.

1. Introduction

Skin wound healing is the most intensive commercial area where tissue engineering and regenerative medicine (TERM) solutions are being developed. This is due to the high socioeconomic impact imposed, especially due to aging populations [1]. For instance, the total spending on wound care alone has been estimated in 10 billion EUR per year just in France [2]. Most of the current cell therapies are focused on the use of stem cells and their secretome. However, most of the clinical trials have been performed with biomaterials such as soft hydrogels such as alginate, fibrin or collagen, encompassing bioactive molecules and possessing smart properties to maintain the wettability, combat bacterial infections, or increase the pro-regenerative environment [3,4].

Dermal fibroblasts are fundamental during skin wound healing thanks to their ability to synthesize *de novo* tissue matrix, to promote epidermalization and their immunoactivity role [5]. In this sense, they are responsible for the production of key pro-regenerative growth factors (i.e. transforming growth factor-β, platelet-derived growth factor,

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https://doi.org/10.1016/j.toxrep.2024.101862
Received 21 October 2024; Received in revised form 8 December 2024; Accepted 10 December 2024
Available online 10 December 2024

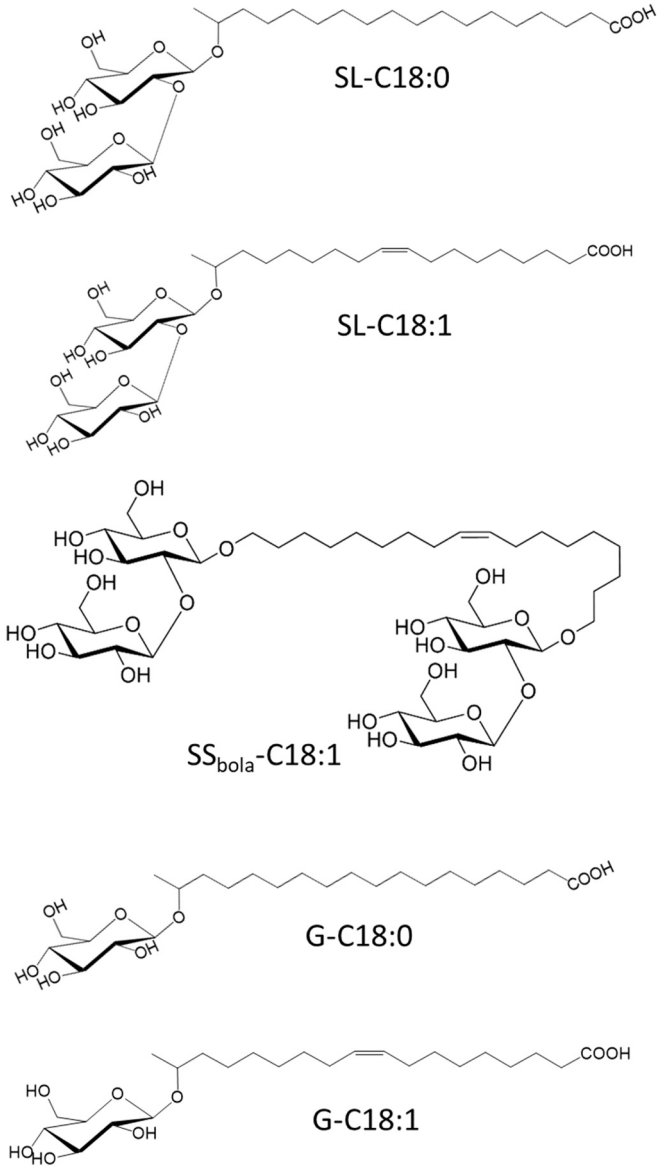


Fig. 1. Chemical structure of the microbial glycolipids used in this work.

fibroblast growth factor), inflammatory intermediates and immunomodulators (i.e. interleukins and tumour necrosis factor-α), extracellular matrix (ECM) molecules and enzymes involved in matrix remodelling (collagens, fibronectin, actin, elastin, matrix metalloproteinases). Furthermore, dermal fibroblasts play a central role in cell-cell communication processes with the other cell types involved in tissue repair (mast cells, macrophages, keratinocytes, endothelial cells or mesenchymal stem cells) [6–8]. Last, they increase tissue-resident cell migration and proliferation, modulate lymphocytic activity and the activation of macrophages, and secrete key chemotactic factors for development of neovasculature [9].

Biological amphiphiles, such as sophorolipids, sophorosides or glucolipids, are amphiphilic molecules, also known as biosurfactants, synthesised by microorganisms which have been previously used in the biomedical field due to their antimicrobial properties [10,11]. While these molecules have been extensively studied for industrial applications such as oil remediation, biosustainable surfactants or the production of personal healthcare products [12–14], little is known about their cell biocompatibility and their possible use in TERM applications. Their stunning self-assembly, in particular their hydrogel-forming ability [15,16], could be exploited to prepare soft scaffolds for TERM. This idea is gaining particular interest as it has been recently disclosed in the pioneering work conducted at the University of Ulster, U.K. [17,18].

The underlying hypothesis is the improved safety of biosurfactants towards mammalian cells as compared to synthetic surfactants. If this assumption is most likely reasonable, considering the biobased origin and chemical nature of many biosurfactants, the actual body of data is relatively scarce and scattered across many different molecules. Recently, Adu et al. [19] have reported that purified acidic sophorolipids, differently than sodium lauryl ether sulphate (SLES), have no deleterious effect on a 3D in vitro skin model colonized with *S. epidermidis*. However, they also state they do not test mammalian cells and they state that their conditions of work should be broadened for a better understanding. In a concomitant work [20], the same team shows the human keratinocyte (HaCaT) cell viability upon exposure to several biosurfactants and results are less straightforward. If acidic sophorolipids have no impact on cell viability up to 500 μg mL^{-1}, di-rhamnolipids decrease the cell viability to less than 30 % above 70 μg mL^{-1} while lactonic sophorolipids have comparable cytotoxicity to SLES, cell viability is none above about 70 μg mL^{-1}. These results are actually not surprising and demonstrate that this topic should be treated with rigor. As a matter of fact, if *in vitro* and *in vivo* studies are still rare, the interactions between biosurfactants and model lipid membranes are studied since decades [21–23] and recently reviewed [24]. The current knowledge shows that molecules like rhamnolipids or surfactin can modify the biophysical properties of phosphatidylcholine bilayer membranes, like the gel to liquid crystalline phase transition temperature, the interlamellar periodicity [22] and even their shape [23]. Further studies are then crucial to better apprehend the impact of biosurfactants on mammalian cells for health-related applications.

In an effort to understand their biological activity, we opted to treat human dermal fibroblasts with solutions of selected acidic sophorolipid standard as well as hydrogel-forming sophorolipids (SL) [25], sophorosides (SS) [26], and glucolipids (G) [27–29] to determine whether or not they could increase regenerative profiles for applications in skin regeneration and wound healing. For this reason, we studied the cytotoxicity of five structurally-related microbial amphiphiles, all non-acetylated in their open form (Fig. 1): a) sophorolipids C18:1 (SL-C18:1) and C18:0 (SL-C18:0) contain a di-glucose (sophorose) and a carboxylic acid at the other side of the aliphatic chain; b) glucolipids C18:1 (G-C18:1) and C18:0 (G-C18:0) contain a single glucose headgroup and also an opposite carboxylic acid; c) bolaform sophoroside (SS_{bola}-C18:1), structurally similar to SL-C18:1, replaces the COOH group by a second sophorose headgroup. Known for their antimicrobial properties, [30] the amphiphilic character of microbial amphiphiles also suggests toxicity towards human cells, today essentially known for acidic SL, lactonic SL and rhamnolipids on human keratinocytes [18,19]. The structural diversity in relationship to the complementary solution self-assembly properties [26,31] of the molecules chosen for this work will then help better understanding their cytotoxicity on normal human dermal fibroblasts (NHDF) using metabolic activity evaluation. We further studied the effect of the selected molecules, based on cytotoxicity, on the upregulation or downregulation of several key target genes in the wound healing process using quantitative gene expression techniques.

2. Materials and methods

2.1. Glycolipids

All glycolipids used in this work are provided by Amphistar, Belgium, and used as such: non-acetylated acidic C18:1 sophorolipids (SL-C18:1), non-acetylated acidic C18:1 glucolipids (G-C18:1), non-acetylated bolaform sophorolipids (SS_{bola}-C18:1). The saturated form of sophorolipids (SL-C18:0) and glucolipids (G-C18:0) was prepared from the mono-unsaturated compounds. All compounds were studied and used in previous works: SL-C18:1 [32], G-C18:1 [28], SS_{bola}-C18:1

Table 1
Selected reverse and forward sequences from selected gene targets.

TARGET	FORWARD (5'→3')	REVERSE (5'→3')
BAX	GATGATTGCCGCCGTGGACACAGA	GGAGGAAGTCCAATGTCCAGCCCA
COL1A1	TGGTGTGATGGGATTCCCTGGACC	CCTGAGCTCCAGCCTCTCCATCTT
COL3A1	GGTGCTCGAGGCAGTGATGGTCAA	GGCACCATTTGAACCAGGAGACCC
TGFB1	GGAGTTGTGCGGCAGTGGTT	GCCGGTAGTGAACCCGTTGATG
FGF-2	AGTGTGTGCTAACCGTTACCTGGC	GCCCAGTTCGTTTCAGTGCCACA
CXCL-12	CCATGTTGCCAGAGCCAACGTC	GAGTGGGTCTAGCGGAAAGTCC
FN1	TACCCACACGGTCCGGGACTCAAT	GCCTGTCAGAGTGGCACTGGTAGA
ACTA1	AATACTCGGTGTGGATCGGCGGCT	TGAGAAGTCGCGTGCTGGAGGT
18S	TTACAGGGCCTCGAAAGAGT	TGAGAAACGGCTACCACATC
ELN	CTTCCCCGCAGTTACCTTTC	TGTGGTGTAGGGAGTCCAT
MMP14	TCGCTGCCATGCAGAAGTTTTA	CTGGATGCAGAAAGTGATTTCATTA
KGF	AAGGCTCAAGTTGCACCAGGCAG	GTGTGTCGCTCAGGGCTGGAAC
TIMP1	GGCATCCTGTTGTTGCTGTGGC	CCCACGAACTTGGCCCTGATGA

[33]. SL-C18:0 and G-C18:0 were prepared by catalytic hydrogenation of SL-C18:1 and G-C18:1 according to a recently-published protocol [34]. The chemical structure of all compounds is given in Fig. 1.

2.2. Preparation of the different glycolipid solutions

Solutions with concentrations ranging from 3 % (w/v) to 0.0001 % (w/v) were prepared from the microbial glycolipids. For this, solutions were prepared in falcon tubes where warm complete cell culture medium and the appropriate mass of each molecule were vortexed. To ensure the complete dissolution, the pH was adjusted to 8.0 using small volumes of 1 M NaOH or 1 M HCl solutions. Then, solutions were vigorously vortexed again to ensure complete homogenisation and warmed up to 37 °C to be further sterilised by passing them through a 0.22 μm filter. Each solution was fabricated extemporaneously.

2.3. Normal human dermal fibroblast (NHDF) cell culture

NHDF were purchased from Merck® and stored in liquid nitrogen according to manufacturer's recommendations (Promocell). NHDF cells were cultured in complete cell culture medium (Glutamax™ Dulbecco's Modified Eagle's Medium (DMEM) at physiological pH supplemented with 10 % (v/v) foetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL Fungizone for expansion under normal culture conditions (37°C, 5 % CO_2). NHDF were exposed to Phosphate Buffered Saline (PBS) solution for 5 minutes, trypsinized to detach them and centrifuged at 1000 RPM for 5 minutes. Cells were resuspended in complete culture medium and seeded into wells of 6-well plates at a density of 30,000 cells/well. NHDF were cultivated for 3 days in normal culture conditions before being exposed to the different sophorolipids. All chemicals were purchased from Merck® unless otherwise specified.

2.4. Cytotoxicity evaluation

Cytotoxicity evaluation was performed at physiological pH 24 and 48 hours after treating NHDF cells (group size = 3, N-value = 3) with the different glycolipid solutions, using the Alamar Blue assay. Glycolipid solutions were removed and cells were carefully washed two times with warm phenol-free DMEM. Following, cells were incubated at 37 °C with 1 mL of a 10 μg/mL resazurin solution for 4 hours. For this, a stock solution of resazurin at 100 μg/mL was diluted 1:10 in phenol-free and serum-free DMEM culture medium. The supernatant was then collected from each well and distributed in 96-well plates in triplicates. Absorbance was measured at $\lambda = 560$ nm and $\lambda = 590$ nm using a Varioskan™ LUX multimode plate reader (ThermoFisher Scientific). Cells were carefully washed twice with phenol-free DMEM to remove excess resazurin solution and initial treatment solution were applied to further culture cells for the next time point. The percentage of resazurin reduction was calculated following manufacturer's instructions. Untreated NHDF cells at each timepoint were used as controls with an arbitrary value of 100 %. Results were expressed as a percentage compared to the control (untreated cells). Three samples per condition and timepoint were analysed.

2.5. RNA purification

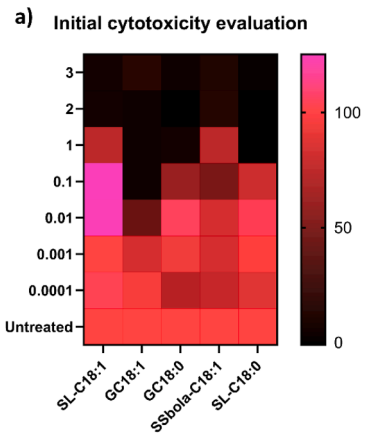
Total RNA was extracted by scratching adherent NHDF cells from treated and untreated control conditions and preserved using 1 mL TRIzol® reagent. Following, phase separation was performed by adding 0.2 mL of chloroform and a centrifugation step at 10,000*g* for 15 min. After collecting the aqueous phase, RNA purification was carried out using the RNeasy kit (Qiagen) according to manufacturer's instructions. Last, RNA concentration and purity was determined by UV spectrophotometry using the Varioskan™ LUX multiplate reader.

2.6. Reverse transcription into cDNA

1 μL of random primers (200 μM, Invitrogen) and 1 μL deoxyribonucleoside triphosphates (10 mM, Invitrogen) were added to 10 μL purified RNA aliquots (around 150–300 ng). Following denaturation of the secondary structure at 65 °C and random primer binding, 4 μL of 5x reaction buffer, 1 μL dithiothreitol (0.2 M) and 2 μL reverse transcriptase from Moloney Murine Leukaemia Virus (M-MLV) (Invitrogen) were added to the reaction mix. After 60 min at 37 °C, the reaction was stopped by heating at 70 °C for 10 min. The resulting cDNAs were stored at −20 °C until further use.

2.7. Real-time polymerase chain reaction (PCR)

Gene expression of BAX, COL1A1, COL3A1, FN-1, ACTA-1, ELN, TGFβ-1, FGF-2, CXCL-12, TIMP-1, MMP-14 and KGF was evaluated, after 24 hours in culture in presence of the different glycolipids, using reverse transcription quantitative PCR (qPCR). The quantification was performed using the Light Cycler FastStart DNA Mater plus SYBR Green I kit (Roche) in a Light Cycler 480 system (Roche). Appropriate primers (Thermofischer Scientific) are listed in Table 1. For cycling conditions, the initial Taq polymerase activation at 95 °C for 5 min was followed by 40 cycles. Each cycle consisted of 10 s denaturation at 95 °C, 15 s annealing at 60 °C and 15 s elongation at 72 °C. Then, a melting curve was generated by increasing the temperature from 60 °C to 95 °C at a rate of 0.1 °C/s to assess the reaction specificity. The results were analysed using a relative quantification following the Pfaffl method [35]. The efficiencies of the target and reference primer pairs were measured by producing a standard curve based on the amplification of a serial dilution of cDNAs. The mRNA transcript level of each target gene was normalised with the 18 s housekeeping gene. Fold changes in gene expression were calculated for each target gene relative to a calibration point, which is the normalised gene expression of this target gene for the untreated control NHDF cells after 24 hours in culture. The value 1 was arbitrarily given to this calibration point. Four samples per condition



b)

Cytotoxicity values

Molecule name	[molecule] (% w/v) 1	0.1	0.01	Untreated
SL-C18:1	69.18 ± 19.07	125.2 ± 44.98	124.45 ± 36.69	100
SS_{bola}-C18:1	68.14 ± 46.13	42.86 ± 20.43	78.52 ± 17.68	100
SL-C18:0	-1.03 ± 1.11	75.42 ± 9.8	104.79 ± 17.09	100

Fig. 2. Cytotoxicity evaluation of glycolipids on NHDF cells after 24 hours culture (group size = 3, N-value = 3). a) Heatmap representing cytotoxicity as the percentage difference in resazurin reduction between treated and untreated conditions. b) Cytotoxicity values for SL-C18:1, SS_{bola}-C18:1 and SL-C18:0, the molecules selected for further examination in the 1–0.01 % w/v concentration range. Values are shown as mean percentage ± SD.

were analysed and each PCR reaction was performed in triplicate. The minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines were followed.

2.8. Statistical analysis

All experiments were carried out at least twice and results were expressed as mean values ± standard deviation (SD). Cytotoxicity difference between treatments were analysed for each selected timepoint using the two-way ANOVA test followed by Turkey's test to ensure statistical significance. Differences in gene expression between treatments were analysed for each selected timepoint using the Kruskal-Wallis test followed by Dunn's test as a *post-hoc* test to ensure statistical significance. A p value < 0.05 was considered to be significant.

3. Results and discussion

3.1. Cytotoxicity evaluation

Cytotoxicity evaluation is one of the key biological characterisations to be done when using novel molecules for biomedical applications. The importance of healthy dermal fibroblasts in skin regeneration has been well discussed [36–39]. Also, the glycolipids chosen for this study have shown different interesting physicochemical characteristics, such as their ability to form hydrogels, vesicles or micelles [15,26,40]. Considering also novel results obtained at the University of Ulster [17, 18], these molecules are interesting candidates in the field of drug delivery and TERM [12,41]. As per our knowledge, there has been no cytotoxicity evaluation of these molecules on human fibroblasts. Initial cytotoxicity studies were performed. As shown in the heatmap in Fig. 2a, significant cytotoxicity was appreciated, regardless the treatment, for concentrations higher than 1 % (w/v) if compared with untreated conditions after just 24 hours culture (****, $p < 0.0001$). On the contrary, cytotoxicity values from concentrations lower than 0.01 % (w/v) were similar to those obtained for untreated conditions (ns, $p > 0.1$). In the middle concentration range (1–0.01 % w/v), significant cytotoxicity was observed for G-C18:1, G-C18:0 and SL-C18:0. In contrast, in this same interval, SL-C18:1 increased NHDF cell metabolic activity when compared to untreated conditions (i.e., SL-C18:1 vs. Untreated 0.1 % w/v, ***, $p < 0.001$). In this context, SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 were selected for further experimentation, and Fig. 2b shows the cytotoxicity values of each of the selected molecules in the 1–0.01 % w/v concentration range.

Further cytotoxicity evaluation was performed on NHDF cells treated with SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 for up to 48 hours. In general, metabolic activity levels were constant over the 48 hours culture. As seen in Fig. 3, metabolic activity levels were lower after 24 hours culture. In this first period, cells are usually under stress due to exposure to chemicals in solution and activity levels tend to be lower. However, there was a sharp decline when 1 % w/v treatments were used (****, $p < 0.0001$), indicating that these molecules are cytotoxic for NHDF cells at high concentration levels. Metabolic activity levels were comparable to those for untreated cells after 48 hours for SL-C18:1 and SL-C18:0 treatments, particularly for low concentrations (0.001 % w/v). However, interestingly, metabolic activity was significantly reduced to approximately 50 % when using SS_{bola}-C18:1 treatment for both timepoints at 0.1 % w/v concentration (****, $p < 0.0001$).

Understanding the structure-cytoxicity relationship between the

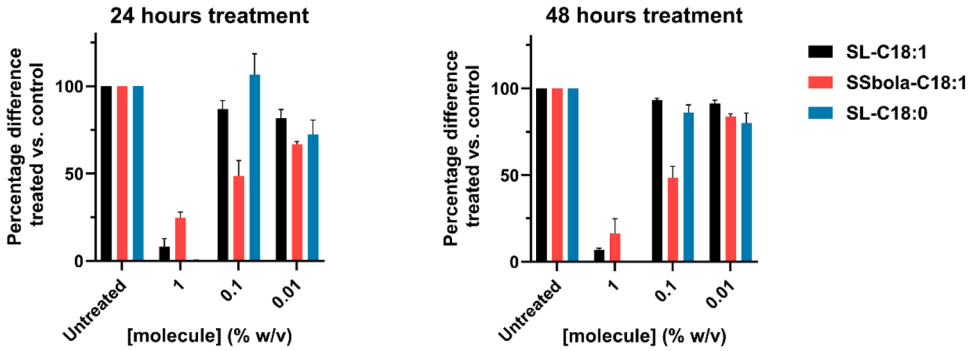


Fig. 3. Cytotoxicity evaluation of SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 on NHDF cells (group size = 3, N-value = 3) showing metabolic activity data over a 48-hour treatment. Statistical significance evaluated using two-way ANOVA test followed by Turkey's test (not shown in the figure).

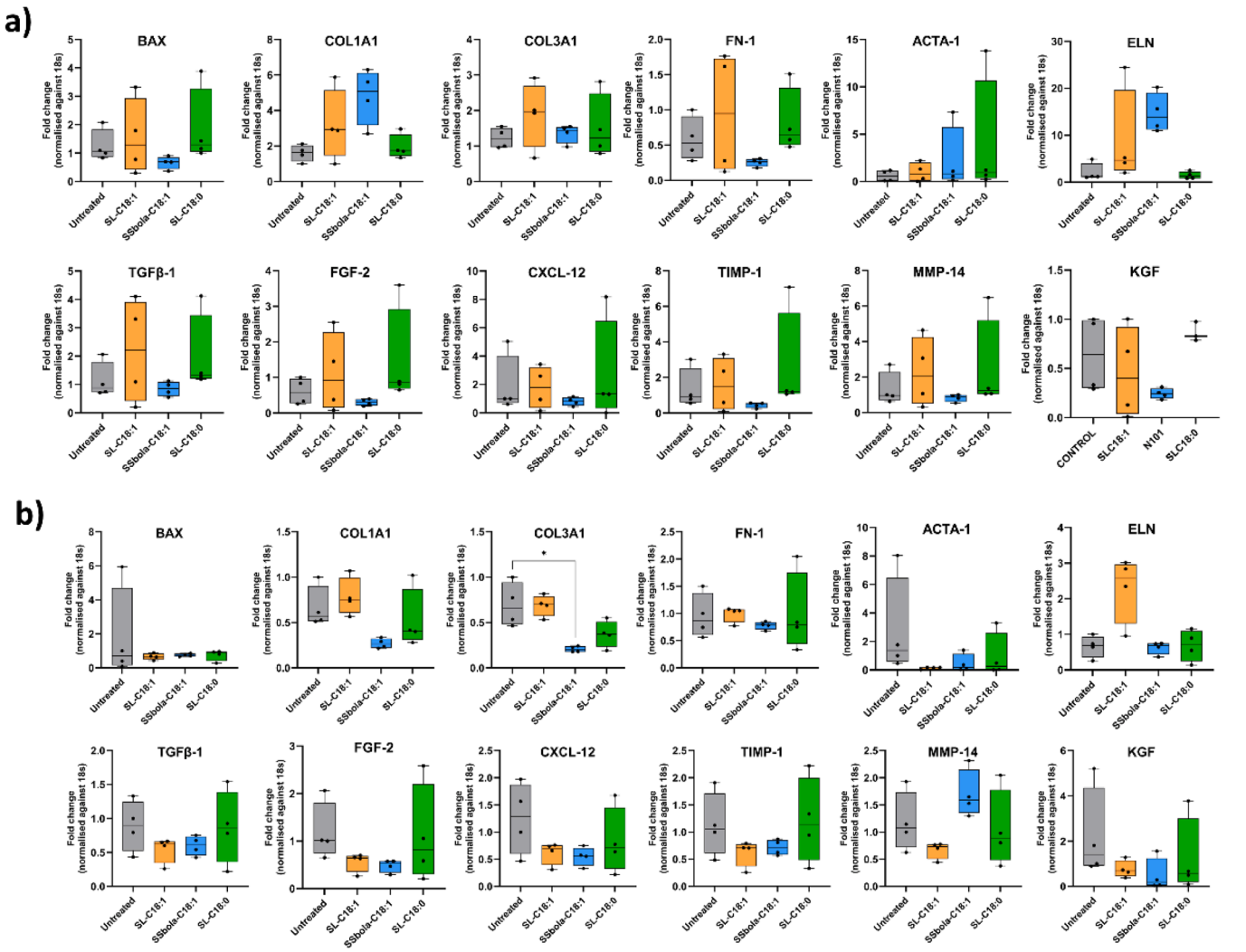


Fig. 4. Gene expression analysis after 24 h SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 on NHDF cells showing fold changes for a) treatments with concentration at 0.1 % w/v and b) treatments with concentrations at 0.01 % w/v. Statistical significance evaluated using Kruskal-Wallis test followed by Dunn's test.

selected molecule and NHDF is not straightforward, simply due to the lack of data in the literature concerning all of these molecules. Broadly speaking, a number of microbial glycolipids (sophorolipids, rhamnolipids, trehalolipids) were shown to interact with bilayer models membranes [42,43] but also with human keratinocytes [18,20]. On a qualitative level, the higher toxicity of G-C18:1 and G-C18:0 after 24 h could be explained by their ability to self-assemble into liquid crystalline membranes themselves [31], probably explaining their affinity towards phospholipid bilayers. Unpublished small X-ray scattering data from our group shows the permeability of model phospholipid (DOPC) bilayer membranes towards G-C18:1, while recent work demonstrates that G-C18:1 and G-C18:0 have a higher inhibitory action towards specific bacteria when compared to acidic sophorolipids (C18:1, C18:0) and sophorosides. Pala et al. [43] shows that the minimum inhibitory concentration (MIC) for both G-C18:1 (5a in [43] and G-C18:0 (9a in [43] is often lower than for SL-C18:1, SL-C18:0 and SS_{bola}-C18:1 (2, 6 and 5a in [43], respectively) for various pathogens. Cui et al. [44] have found similar results by comparing G-C18:1 (higher inhibition for most pathogens) and SL-C18:1, but they propose that the actual MIC may depend on pH, where lower MICs, promoting higher inhibition, are found at more acidic pH. However, it was not clear whether or not the effect on the MIC could actually be attributed to the pH.

As for possible differences between SL-C18:1 and SL-C18:0, the effect of unsaturation on the cytotoxicity is less clear and more prone to speculation. This being said, the results in this work show a comparable cytotoxicity and these agree with Pala et al. [43], who did not experience major inhibitory differences between the two compounds. This could be probably explained by the fact that both molecules have a similar anionic surfactant behaviour assembling into micelles at pH above 7.4 [31], which is the physiological pH at which toxicity experiments are generally conducted. It is then not unreasonable that both compounds have comparable cytotoxicity. Concerning SS_{bola}-C18:1, it seems that this compound has a comparable cytotoxicity to acidic sophorolipids, however, it is unclear the reason why its cytotoxicity after 48 h is more pronounced. Given the practically inexistent data existing for this molecule, we prefer not to disclose any speculative interpretation.

The cytotoxicity data constitute a precious database pour further applications, however translating them from isolated cells to an *in vivo* context is a complex challenge. In the field of skin tissue engineering, hydrogels are commonly used as a formulation. Their primary advantage lies in maintaining hydration of the wound bed, which promotes wound healing. Typically, such hydrogels are prepared from biopolymers at concentrations ranging from 0.05 wt% to 4 wt% [45–47]. Hydrogels prepared from microbial amphiphiles, like SL-C18:0, G-C18:1 or SSbola-C18:1, require concentrations between at least 1 wt% and 4 wt% [16,26,28] a range which is lethal for NHDF cells and which may pose risks to tissues, depending on the hydrogel's stability and the

release of soluble molecules. As an alternative, microbial glycolipids could be combined with other biopolymers, such as collagen, alginate, chitosan, or silk fibroin, [48–51] to create composite hydrogels with potentially enhanced properties.

3.2. Gene expression analysis on key markers for dermal regeneration

Fibroblasts are a key cellular component for skin wound healing [52] due to their capacity to secrete pro-regenerative growth factors, ECM proteins, and immunomodulators [9,53–57]. Cytotoxicity evaluation results were able to exclude treatments at high concentrations; therefore, we focused this analysis on SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 treatments with 0.1–0.01 % w/v concentrations after culturing NHDF cells for 24 hours. Regardless of the conditions, the total RNA extracted was similar with a mean value of 237.6 ± 45 ng/µL. We measured the gene expression of NHDF subjected to the aforementioned treatments to investigate differences in cell apoptosis (BAX), ECM proteins (COL1A1, COL3A1, FN-1, ACTA-1, ELN), pro-regenerative growth factors (TGFβ-1, FGF-2, CXCL-12, KGF) and matrix remodellers (TIMP-1 and MMP-14). As seen in Fig. 4a for treatments at 0.1 % w/v concentrations and Fig. 4b for treatments at 0.01 % w/v concentrations, results showed no significant variations in gene fold changes compared to control (ns, $p > 0.01$).

4. Conclusions

Dermal fibroblasts are key players governing the skin wound healing process due to their capacity to secrete ECM proteins and pro-regenerative growth factors. Microbial glycolipids, here sophorolipids, sophorosides and glycolipids with different unsaturation, are novel sustainable molecules obtained by microbial fermentation from several yeasts and bacteria. Due to their physicochemical characteristics, it has been shown that by modulating their pH and/or temperature, for example, it is possible to form soft hydrogels, with possible output in the field of tissue regeneration. In this study we have shown that, up to a certain concentration, several of these molecules were not toxic for NHDF: out of all tested molecules, we were able to show that SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 sustained NHDF cell growth *in vitro* for concentrations up to approximately 0.1 % w/v for 48 hours continuous exposure. G-C18:1 and G-C18:0 were excluded due to their higher cytotoxicity, probably explained by their higher tendency to behave as membrane-forming lipids, thus showing a pronounced tendency to integrate and disrupt cell membranes. For this reason, we studied changes in gene expression for the key elements of the regenerative milieu (mainly growth factors and ECM proteins and remodellers) for SL-C18:1, SS_{bola}-C18:1 and SL-C18:0 only. However, we found no significant gene expression changes. This work might encourage researchers in the field to advance towards finding different biomedical applications for this class of molecules.

CRediT authorship contribution statement

Niki Baccile: Supervision, Conceptualization. **Christophe Hélary:** Writing – review & editing, Supervision, Conceptualization. **Sergio Oliveira Formoso:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Vincent Chaleix:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was possible due to funding from the Agence Nationale de la Recherche (ANR), France, project SELFAMPHI ANR-19-CE43-0012-01. Dr. Sophie L. K. W. Roelants (Amphistar, Belgium) is warmly acknowledged for her assistance in the biosurfactants management.

Conflict of interest

Authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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