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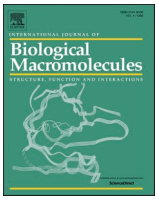

Exploring the potential of *Halomonas* levan and its derivatives as active ingredients in cosmeceutical and skin regenerating formulations

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ABSTRACT

Demand on natural products that contain biological ingredients mimicking growth factors and cytokines made natural polysaccharides popular in pharmaceutical and cosmetic industries. Levan is the β-(2-6) linked, nontoxic, biocompatible, water-soluble, film former fructan polymer that has diverse applications in pharmacy and cosmeceutical industries with its moisturizing, whitening, anti-irritant, anti-aging and slimming activities. Driven by the limited reports on few structurally similar levan polymers, this study presents the first systematic investigation on the effects of structurally different extremophilic *Halomonas* levan polysaccharides on human skin epidermis cells. *In-vitro* experiments with microbially produced linear *Halomonas* levan (HL), its hydrolyzed, (hHL) and sulfonated (ShHL) derivatives as well as enzymatically produced branched levan (EL) revealed increased keratinocyte and fibroblast proliferation (113–118 %), improved skin barrier function through induced expressions of involucrin (2.0 and 6.43 fold changes for HL and EL) and filaggrin (1.74 and 3.89 fold changes for hHL and ShHL) genes and increased type I collagen (2.63 for ShHL) and hyaluronan synthase 3 (1.41 for HL) gene expressions together with fast wound healing ability within 24 h (100 %, HL) on 2D wound models clearly showed that HL and its derivatives have high potential to be used as natural active ingredients in cosmeceutical and skin regenerating formulations.

1. Introduction

Cosmeceuticals are products that combine the benefits of cosmetics and pharmaceuticals and are applied topically to the skin. They contain physiologically or biologically active ingredients and are distinct from cosmetics in that they can improve skin appearance while also treating skin ailments with medicine-like features [1–6]. With increasing demand for natural cosmeceuticals by conscious consumers who pay particular attention to product ingredients, the cosmetic industry has sought alternative natural ingredients that mimic naturally occurring growth factors and cytokines. Desired functionalities for natural polysaccharides used in skin care cosmetics' development include antioxidant, anti-wrinkle, anti-inflammatory, anti-acne, anti-tyrosine activity, biocompatibility, and non-toxicity [6–11]. Functional polysaccharides derived from various sources such as plants, marine organisms, animals, fungi, or microbes, such as hyaluronic acid (HA), xanthan gum, and cellulose, are preferred in cosmetic formulation technologies due to their film-forming, thickening, emulsifying, conditioning, gelling, and suspending properties, as well as their bioactivity, biocompatibility, and biodegradability features [7,12–14]. Besides these, bacterial exopolysaccharides (EPS) like levan from *Bacillus subtilis* and *Zymomonas mobilis* [15], poly-gamma-glutamic acid from *B. subtilis* and *Chromohalobacter canadensis* 28 [16,17] are also proposed as potential ingredients to be used in cosmeceutical industry due to their sustainable and economical large-scale production and downstream processing features. In fact, halophilic extremophile species like *Chromohalobacter* or *Halomonas* are considered as an interesting and promising source of bacterial EPSs for the cosmetic sector due to their added advantages like high yields and non-sterile production under saline conditions [13,15].

Levan is a β-(2-6) linked, nontoxic, biocompatible, water-soluble, film former and strongly adhesive fructose homopolymer, a fructan, that has diverse applications in pharmacy and cosmeceutical industries with its skin moisturizing, whitening, anti-irritant, anti-aging and slimming activities [15,18]. In an earlier study, cytotoxicity, cell proliferative, anti-inflammatory, and moisturizing activity of *Zymomonas mobilis* levan were tested by Kim et al. [19] and high cell viability,

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reduced IL-1α in SLS-induced 3D artificial skin and similar moisturizing and proliferative effects comparable to HA were observed. Then, the effect of *Zymomonas* levan on Matrix metalloproteinase (MMP) activity was investigated by Sturzoiu et al. [20] on skin burned or wounded rats and activation of MMP-2 and MMP-9, which were responsible for new tissue formation, was observed. More recently, *Zymomonas* levan based injectable dermal filler hydrogels showed enhanced cell proliferation of human adult dermal fibroblasts (hADF), increased collagen type I expression compared to HA and anti-wrinkle formation in mice model as reported by Choi et al. [21].

Bacillus levans were also studied for cosmeceutical applications. Abdel-Fattah et al. [22] reported strong free radical scavenging activity for both native and sulfated levan from *Bacillus subtilis* where the sulfated derivative showed increased cell cycle arrest (S and G2-M) in human hepatocarcinoma cell line (HepG2). Domżał-Kędzia et al. [23] investigated the potential application of levan produced by *B. subtilis* natto in cosmetics. The effect of low molecular weight levan on cellular viability on human dermal fibroblasts (NHDF) was investigated and no cytotoxicity was observed. Then the same group reported that *Bacillus subtilis* levan nanoparticle containing and surfactin stabilized nano-emulsive cream, tonic and gel formulations improved skin hydration, elasticity, reduced wrinkle depth and also skin discoloration [24]. da Silva et al. [25] investigated moisturizing, antioxidant activity and stability of *Bacillus subtilis natto* levan and almond oil containing bio-cosmetic for 90 days and high hydration capacity, antioxidant activity and stability were observed. Also *Bacillus siamensis* levan was tested for cosmetics and high anti-oxidant activity, non-toxicity to keratinocyte (HaCaT) and fibroblast (NHDF) skin cells, high anticancer activity on Hela, HCT116, HepG2 and A549 cells as well as high water and oil holding capacities were reported [26].

Enzymatically produced *Erwinia amylovora* levan was also proposed as a strong candidate for cosmeceutical applications due to its rheological properties and gel-forming features [27]. More recently, hydroxyapatite (Hap) embedded *Erwinia herbicola* levan based injectable dermal filler was reported to improve fibroblast proliferation, *in-vivo* collagen production with easy injectability and improved anti-wrinkle efficacy [28].

In addition to these mesophilic levans, *Halomonas smyrnensis* produces a bioactive polymer called *Halomonas* levan (HL), which has been studied extensively for its potential applications. These include its use in adhesive multilayer and freestanding films [29,30], bioactive laser deposited surfaces [31–34], biocompatible peptide and protein drug microcarrier systems [35,36], hydrogels [37–39], and PLGA-HL based drug delivery systems for curcumin [40]. HL has also shown promise in the development of nanoparticles with resveratrol release [41], as well as in exhibiting antioxidant, anticancer [42], and anticoagulant activities [18,43]. In addition to these, our group has previously reported its use in paper conservation [44] and as an adsorptive bisphenol A remover [45]. Considering previous reports on the uses of levan from various non-extremophilic microorganisms in cosmetic formulations, we were curious about the added value of the microbial source and structural features of levan polysaccharide in cosmeceutical and skin regeneration applications. Hence in this study, human skin epidermis keratinocyte (HaCaT) and dermis fibroblast (PCs-201-012) cells were incubated with structurally different *Halomonas* levans and then their proliferation, type I collagen, hyaluronic acid (HA), skin barrier functioning involucrin (*INV*) and filaggrin (*FLG*) gene expressions in addition to wound healing abilities were investigated for the first time. For this, not only microbially produced linear *Halomonas* levan (HL) and its chemically modified forms (hydrolyzed, hHL and sulfonated, ShHL) but also branched levan (with approximately 10 % degree of branching) produced by recombinant levansucrase enzyme (EL), were used. *In-vitro* experiments revealed improved cell proliferation, skin barrier function and fast wound healing ability which in turn clearly showed that HL and its derivatives have high potential to be used as natural active ingredients in cosmeceutical and skin regenerating formulations.

2. Materials and methods

2.1. Levan production and purification

Microbial *Halomonas* levan (HL) production by *Halomonas smyrnensis AAD6T* strain was performed in semi-chemical medium (consisting of; 137.2 g/L NaCl; 7 g/L K_2HPO_4; 50 g/L sucrose; 0.1 g/L $MgSO_4{\cdot}_7H_2O$; 2 g/L KH_2PO_4; 1 g/L $(NH_4)_2SO_4$; 0.5 g/L peptone from casein; 0.8 mg/L thiamine; 50 mM boric acid (H_3BO_3); and trace elements (0.36 mg/L $MnCl_2{\cdot}_4H_2O$; 0.44 mg/L $ZnSO_4{\cdot}_7H_2O$; 2.3 mg/L $FeSO_4{\cdot}_7H_2O$; 0.05 mg/L $CuSO_4{\cdot}_5H_2O$) in a controlled bioreactor system (Biostat B, Sartorius, Germany) at working volume of 5 L at constant temperature (37 °C) and pH (7) with agitation rate of 200 rpm and aeration rate of 0.1 vvm as previously described [46]. At the end of fermentation, cells were harvested from culture broth by centrifugation at 10,000 ×*g* for 20 min and recovery of levan in the supernatant was performed by ethanol precipitation (1:1 *v*/v) overnight at −20 °C. After removal of HL pellet by centrifugation at 10,000 ×*g* for 20 min, it was resuspended in dH_2O and dialyzed against several runs of dH_2O for at least 4 days (cut-off: 14 kDa) to remove salt and other impurities. Further purification of HL to remove residual proteins was performed by passing it through a DEAE-Sepharose CL-6B weak anion-exchange column. Finally, HL was freeze-dried (Leybold Lyovac GT 2,) and stored for further experiments.

Enzymatic levan production was performed *via* recombinant *Halomonas smyrnensis* levansucrase enzyme as previously described by Kırtel et al. [47]. Briefly, purified levansucrase was mixed with 2 L substrate solution (5.36 g/L K_2HPO_4, 2.62 g/L KH_2PO_4, 100 g/L sucrose, 204.5 g/L NaCl and 15 mM EDTA, pH 5.9) and incubated overnight at 15 °C. Recovery of EL and its further purification was performed as described above.

2.2. Synthesis of levan derivatives

2.2.1. Microwave assisted hydrolysis of Halomonas levan

Microwave-assisted acid hydrolysis of HL was performed as described by Selvi et al. [39] through dissolving 5 % (*w/v*) HL in 2 % (*v/v*) acetic acid solution and microwave irradiation for 60 s at 600 W. Three volumes of ethanol were added to the solution and the precipitated hydrolyzed *Halomonas* Levan (hHL) samples were recovered by centrifugation, vacuum dried, and then powdered for further analysis.

2.2.2. Sulfonation of Halomonas levan

Powdered hHL was used for sulfonation as previously described by Erginer et al. [18]. Briefly, chlorosulfonic acid (CSA, $ClSO_3H$) was added dropwise to hHL/pyridine suspension in an ice bath and then after 24 h reaction period, saturated sodium carbonate was added to neutralize and stop the reaction. The organic pyridine phase was removed and the precipitated ShHL was dissolved in distilled water and dialyzed against distilled water for several days for purifying the polymer from salt and pyridine residues. Purified ShHL freeze-dried, and the degree of sulfonation was calculated according to Erginer et al. as 2.91 [18].

2.3. Chemical characterization

The structural integrity of the polymers was investigated by Fourier Transform – Infrared Spectroscopy (FT-IR; Jasco FT/IR 470). The scanning was conducted in the attenuated total reflection mode (ATR) the spectra were obtained at the scanning range of 400–4000 cm^{-1} with an average of 32 scans and a resolution of 4 cm^{-1}. Molecular weights of the polymers were analyzed by multi-angle laser light scattering - gel permeation chromatography (MALLS-GPC).

2.4. In vitro cell viability test

In-vitro cell viability tests of HL and its chemically modified derivatives (hHL, ShHL) and EL were performed *via* 2D cell proliferation

analysis. Human keratinocyte (HaCaT) and primary human dermal fibroblast (PCS-201-012) cells are used to test effect samples on different sections of skin. HA is a very common polysaccharide used in the cosmetic industry to help skin hydration and it is known to support cellular growth in ECM. Thus, HA was also used as a positive control in cell viability experiments. HaCaT and PCS-201-012 cells were seeded in 96 well plates (5×10^3/well) in 100 μL/well DMEM complete media and incubated overnight for cellular attachment 5 at.% CO_2 incubator at 37 °C. After cell attachment, media was removed and samples were added onto cells in DMEM media (0 μg/mL as control, 10, 100, 500 and 1000 μg/mL) and incubated for 24, 48 and 72 h. At the end of the incubation period, WST-1 ((4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio] 1,3benzenedisulfonate) solution (Roche, Switzerland) was added onto cells and incubated for 2 h and absorbances were measured *via* GloMax®-Multidetection system (Promega) at 450 nm.

2.5. In-vitro collagen production

Collagen production and expression were analyzed *via* Type I collagen production by ELISA and CoL1A1 gene expression by Q-RTPCR. Collagen secreted outside the cell and precursor collagen (inside the cell) were estimated by Abbkine Human Collagen ELISA1 (China) kit according to the manufacturer's protocol. For this, dermal fibroblast cells were seeded into 24 well plates at the density of 8×10^4 cells/well and incubated for 24 h for cellular attachment. After 24 h, media was replaced with 500 μg/mL HL, hHL, ShHL and EL containing treatment media and incubated for 48 h. Ascorbic acid was used as a positive control (50 μg/mL) [48].

2.6. Cell cycle analysis

The nuclear DNA content of the cells labeled with propidium iodide was measured quantitatively *via* flow cytometry to analyze the cell cycle distribution (G1/S/G2/M) of the cellular population. Briefly, dermal fibroblasts (PCS-201-012) at the density of 3×10^5 cells/well were seeded to 6 well plates and incubated for 72 h with HL, hHL, ShHL and EL (500 μg/mL). After incubation media was removed, cells were trypsinized and centrifuged. Pellet was washed with PBS and cells were precipitated. 100 μL PBS was added onto cells and pipetted and vortexed gently. Cells were fixed by the addition of 70 % ice cold ethanol and incubation on ice for 1 h. Then the cell pellet was collected and washed with 1 mL PBS. 1 mL of PI (NeoFroxx, Germany) working dye (0.1 mg/mL) (0.1 % Triton X-100, RNase, PBS) was added onto the pellet and incubated for 1 h at 37° C in dark. After incubation, cells were analyzed in BD Accuri™ C6 (USA) flow cytometer and G1/S and G2 phases were recorded. Cells incubated with DMEM media only were accepted as control.

2.7. RNA isolation and quantitative RT-PCR

To investigate the expression of epithelial barrier functioning proteins of filaggrin (*FLG*), involucrin (*IVL*) in HaCaT cells, type I collagen (CoL1A1) and HA synthesizing enzyme hyaluronan synthase 3 (*HAS3*) in PCS-201-012 cells, total RNA was extracted using Tripure reagent (Roche, Switzerland), after being cultivated with levan samples (500 μg/mL). cDNAs were synthesized using Fire script RT cDNA kit (Solis Bio dyne, Germany). Quantitative RT-PCR was performed on Light cycler 96 (Roche, Switzerland) using SYBR Green FastStart Essential DNA Green Master Mix (Roche, Switzerland) according to the manufacturer's instructions with the primers listed in Table 1. Gene expression levels were normalized to the expression of *GAPDH* and Pfaffl efficiency method was used for fold change calculations [49].

2.8. In-vitro scratch assay

Human keratinocyte cell line (HaCaT) was used to determine the effects of HL, hHL, ShHL and EL (500 μg/mL) on wound closure created 2D scratch model *in-vitro*. Cells were seeded on 24 well cell culture plates at the density of 4×10^5 and incubated until confluency. Then, linear scratches were generated by 100 μL pipette tips across the center of each well and wound healing was observed for 48 h during cultivation with samples. Images were taken by Gen5 image 2.09 Cytation 3 (Biotek, USA) from same locations after 0, 24 and 48 and hours and Image J was used for the calculation of wound closure and wound width as previously described by Justus et al. [50] and Pinto et al. [51]. Each sample was tested in duplicates and two random wound zones were collected at the beginning of the experiments and the same zones were used for further calculations. Ten wound width measurements for each zone were performed with Image J and graphs were generated using GraphPad Prism 5. Calculation of wound closure was applied as described by Pinto et al. [51] according to the following Eq. (1).

$$\%Wound\ Closure = \frac{(Initial\ Scratch\ Width - Final\ Scratch\ Width)}{(Initial\ Scratch\ Width)} \times 100 \quad (1)$$

Table 1
List of gene-specific primers used for expression analysis.

Gene symbol	Primer sequence [5′-3′]
GAPDH F:	5′-AAG-GTG-AAG-GTC-GGA-GTC-AA-3′
GAPDH R:	5′-AAT-GAA-GGG-GTC-ATT-GAT-GG-3′
Filaggrin (*FLG*) F:	5′-TTT-CTG-GTT-TGT-CTG-CTT-GC-3′
Filaggrin (*FLG*) R:	5′-CTG-GAC-ACT-CAG-GTT-CCC-AT-3′
Involucrin (*IVL*) F:	5′-ACT-GAG-GGC-AGG-GGA-GAG-3′
Involucrin (*IVL*) R:	5′-TCT-GCC-TCA-GCC-TTA-CTG-TG-3′
Collagen I (*CoL1A1*) F:	5′-CAG-CGC-TGG-TTT-CGA-CTT-3′
Collagen I (*CoL1A1*) R:	5′-CCA-CCA-TCG-TGA-GCC-TTC-3′
Hyaluronan Synthase 3 (*HAS3*) F:	5′-TCT-CCC-AGC-CCA-TCT-GAA-3′
Hyaluronan Synthase 3 (*HAS3*) R:	5′-CCC-AGC-TCG-CTC-AGT-AGA-AA-3′

2.9. Statistical analysis

Statistical analysis was performed with Graph Pad Prism 5 and One Way Anova and Two Way Anova with 95 % confidential interval (CI) was used together with Tukey test and Dunnett's tests. A p-value <0.05 was considered statistically significant ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.).

3. Results and discussion

3.1. Chemical modifications and characterization

Levan polysaccharide was produced both microbially and enzymatically and the purified polymers as well as their chemical derivatives were subjected to chemical characterization to confirm their structural integrity. FTIR analysis (Fig. 1) shows characteristic peaks of levan polysaccharides. Bands around 3200 cm^{-1}, 2900, and 2950 cm^{-1} were the vibration of —OH, —CH_2OH group and C—H stretching of fructofuranose residues, respectively. Bands around 1430–1200 cm^{-1} were the result of C—H vibrations together with aromatic skeletal vibrations. Glycosidic linkage of C—O—C vibrations of fructofuranose rings and glycosidic linkages at 1120–1020 and 950 cm^{-1} were as reported by our previous studies. Levan form *Bacillus subtilis* (BL) was used as reference [18,46,52]. The success of sulfation and hydrolysis modification of HL polymer was also analyzed by FT-IR. New bands around 1215 and 810 cm^{-1}were determined to be S═O and C—O—SO_3 groups stretching and not found in native levan [18,53]. hHL and EL gave similar peaks to native HL thus it was concluded that sulfonation, hydrolysis and enzymatic production of levan were successful. The chemical structure EL and microbially produced levans (HL) are known to differ such that EL has a 10 % degree of branching according to NMR analysis performed [54] while HL is linear with negligible branching. The molecular weight

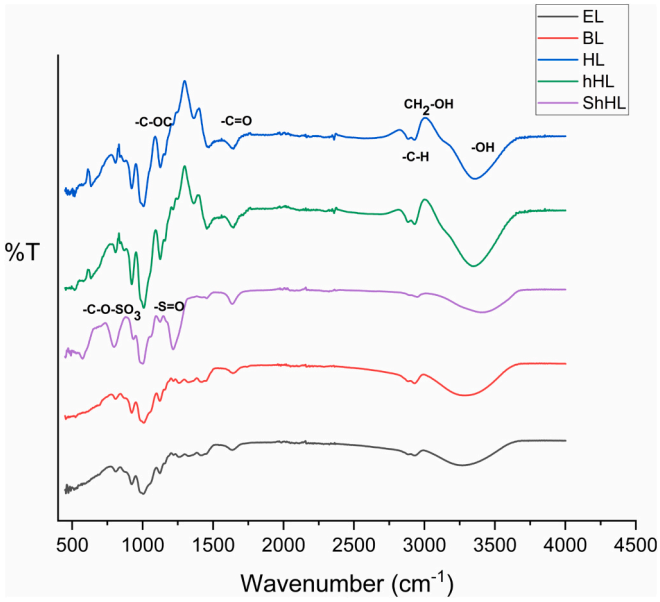

Fig. 1. FT-IR spectra of EL (a), HL (b), BL (c), hHL (d) and ShHL (e).

of HL efficiency of hydrolysis (hHL) were analyzed by MALLS-GPC and molecular weights were found to be 468 ± 3.4 kDa with the polydispersity of 1.221 ± 0.04 for hHL where native HL was known to be 2000 kDa [43,55]. The molecular weight of EL was recorded as 5130 ± 0.0263 kDa with polydispersity of 1.023 ± 0.372 *via* HELEOS-II MALS and WYATT DynaPro DLS GPC detector systems.

3.2. In-vitro skin cell compatibility

Human keratinocyte cells (HaCaT) from epidermis section of skin and Fibroblasts (PCS-201-012) from dermis section of skin are commonly used for construction of 3D skin models, cosmetic testing studies to enlighten specific pathways and responses to testing materials and give valuable results about candidate active ingredients in formulations. Thus, in this study, keratinocytes and fibroblasts were used to investigate the effect of structurally different levans in different sections of skin. Different doses (0,100, 250, 500, and 1000 μg/mL) of HL, hHL, ShHL, EL, and HA were incubated with PCS-201-012 and HaCaT cells for 24, 48, and 72 h to observe their effects on cell viability (Figs. 2 and 3). Cells incubated with complete media only (0 μg/mL) were used as control. HA is known to be concentrated in the extracellular matrix of connective tissues, especially in the dermis section of skin harboring collagen and elastin fibers. It is one of the most common active ingredients that are used in cosmeceuticals (gels, emulsions, or serums) as a skin smoother and moisturizer [56]. Thus, it has been used as a standard for comparative evaluation of the cellular viability of HL and its derivatives.

At the end of the incubation period, almost all doses of HL showed higher viability than the control group for 24, 48, and 72 h for the dose of 500 μg/mL (114.9 %, 118.5 % for the first two days) Thus, the dose of 500 μg/mL was concluded to be the best dose for HL with highest viability results. Almost all samples showed good biocompatibilities for all days. The highest dose of viability was estimated as 250 μg/mL for the first two days (98.78 % and 112. 5 %) while 500 μg/mL(91.90 %) was recorded as the best for 72 h for the sample of hHL. Almost all doses of ShHL showed good viability, but the dose of 100 and 250 μg/mL showed the highest viability at the end of the first day. Doses of 500 and 1000 μg/mL of EL showed greater viability than the control for each day. Viability results of HaCaT cells after cultivation with HA (Fig. 2) showed that for the first 2 days increased viability was observed in dose-dependent manner while the maximum viability was observed as 108.7 % (500 μg/mL) for 24 h, 119.1 % at the end of 48 h (250 μg/mL) and 118 % (100 μg/mL) at the end of 72 h.

Human dermal fibroblast cell line PCS-201-012 was preferred to test the effects of *Halomonas* levan samples and HA for 24, 48 and 72 h. Results were shared in Fig. 3. It can be concluded from the graph that the viability of cells for the first two days showed higher results for all doses of HL (100, 250, 500 and 1000 μg/mL) for the 72 h results, the highest viability was observed for the dose of 250 μg/mL.

Highest viability for hHL recorded as 110.2 % (250 μg/mL) for 24 h, 114.5 % (1000 μg/mL) for 48 h and 106. 7 % (100 μg/mL) for 72 h. For the first 24 and 48 h, almost all doses of ShHL showed control similar or higher viability but at the end of 72 h, highest viability was recorded as 113.5 % (100 μg/mL). Nearly all doses of EL showed high viability and similar results with the control group for each day. All doses of HA showed high viability for each day. The highest viability for HA was recorded as 106.8 % (100 μg/mL) after the first 24 h while the day 3, the dose of 100 μg/mL showed control equal highest viability. When all samples were compared for the viability of HaCaT cells, it can be concluded that HL and EL standout with their high viability when compared with control and HA. HL, hHL, and ShHL stand out with their high fibroblast proliferative ability for each day for all doses even higher than HA. Branched levan, EL, showed similar viability results almost close or even a little higher than the control group and higher than HA when day 2 and 3 were evaluated. Linearity and lower molecular weight of HL and hHL, when compared with partially branched one EL (10 %) [54], might affect structural conformation, solubility, free hydroxyl groups and cell membrane interaction positively, and result in increased cellular viability.

3.3. Cell cycle analysis

The distribution of cell cycle phases of PCS-201-012 fibroblast cells after 72 h of treatment with levan samples were determined by flow cytometry *via* measuring PI-stained DNA content and results are shared below (Table 2). When the percentage of cells in each phase was compared with the control group, HL showed a higher percentage at phase G2/M.

Cell cycle histogram ratios showed that samples of HL, hHL and EL, increased cellular proliferation of fibroblasts according to high S and G2/M ratios which was generally seen in proliferating populations. The fibroblast and keratinocyte WST-1 cell proliferation results (Figs. 2 and 3) are consistent with the increased S and G2/M ratios observed in flow cytometry analysis. Therefore, the results do not indicate cell cycle arrest, as the fibroblast cells continued to proliferate.

3.4. Type I collagen synthesis

Three sublayers of the skin, called epidermis, dermis, and hypodermis from anterior to interior, have different subsections. The epidermis, which is the outermost layer, is a stratified epithelium layer that protects the skin from infection, chemical or mechanical damage, and maintains skin hydration with the help of corneocytes and natural moisturizing factors (such as Pyrrolidone carboxylic acid (PCA), lactic acid, and urea) that occur by the breakdown of the FLG protein, which is vital for skin barrier function. The dermis is separated from the epidermis by the basement membrane, and fibroblast cells, together with extracellular matrix ingredients like HA, collagen (Type I and III, which account for 90 % of total collagen), and elastin, bind cells together and support skin elasticity, giving it a healthy and young-looking appearance [13,57–62]. Thus, proliferation of cells at epidermis and dermis, improvement in skin barrier function, increase in production of Type I collagen, HA, and reduction the activity of enzymes that lead to the breakdown of those components in ECM are in focus for healthy, young looking, hydrated skin. Type I collagen is mostly common in skin and bone tissues that are synthesized as procollagen and secreted into dermal-extracellular space and have anti-wrinkle effects on UV-irradiated skin. Synthesis and loss

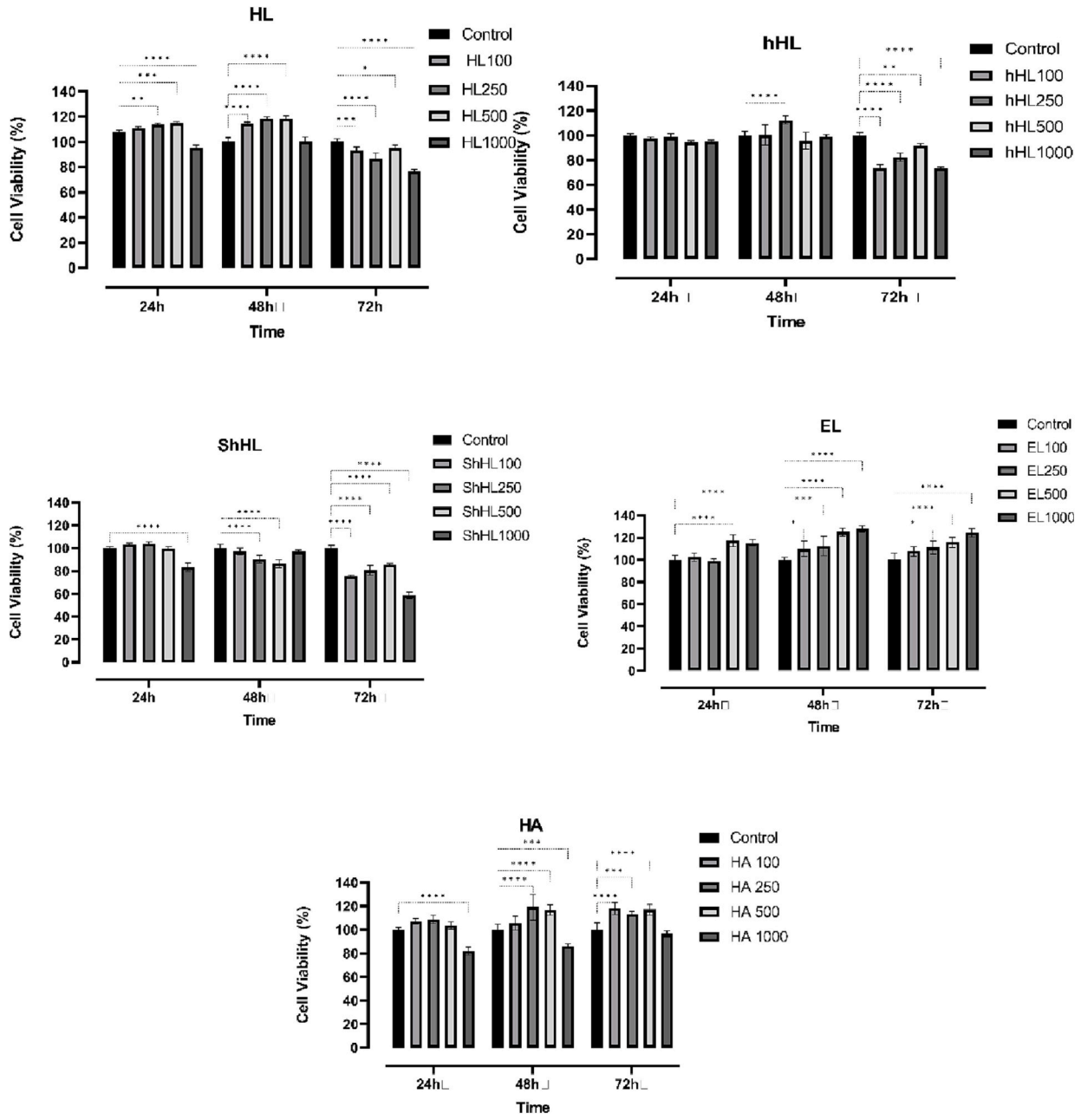

Fig. 2. Viability results of HaCaT (Human Keratinocyte Cell line) cells after being cultivated with HL, hHL, ShHL, EL and HA for 24, 48 and 72 h. (A *P* value below 0.05 was considered as statistically significant).

of this collagen type are known to be related to longevity and aging [59,63]. Loss of type 1 collagen (a structural protein) in dermis is known to be the major cause of wrinkle formation in dermis layer of skin [64].

Synthesis of type I collagen by human dermal fibroblasts after being cultivated with HL, hHL, ShHL, EL (500 μg/mL) as well as ascorbic acid (Asc) as positive control [48] were analyzed by ELISA (Fig. 4).

At the end of the 48-hour incubation period, the levels of type I collagen in cells incubated with the HL sample were very close to the control and higher than those incubated with Ascorbic acid. When the supernatants collected from the cells were investigated, the EL sample showed the highest levels of type I collagen among the samples, followed by the HL sample. Based on these results, it can be concluded that the amounts of synthesized and secreted collagen type I were higher than the procollagen levels inside the cells at the end of the 48 h for the Asc, HL, and EL samples, while the other samples showed higher amounts of type I procollagen inside the cells. ShHL showed lower type I collagen production compared to the other levan types; however, the procollagen levels were high, which could be explained by the comparatively slower release rates of collagen from the cells.

3.5. Skin barrier function

FLG and *IVL* genes are known to play vital roles in keratinocyte differentiation and skin barrier function in epidermis. FLG is known to be an essential protein involved in epidermal corneocyte development and intracellular activity of this protein maintains stratum corneum hydration and physiological pH balance [65]. Another cell envelope protein, IVL is known to secrete early stages of keratinocyte differentiation and cell envelope formation is formed in late stages of differentiation at stratified epithelia. Thus, the expression of *IVL* is accepted as an important precursor of epithelial barrier formation. Down-regulation of this gene is known to lead to structural abnormalities in skin and hair

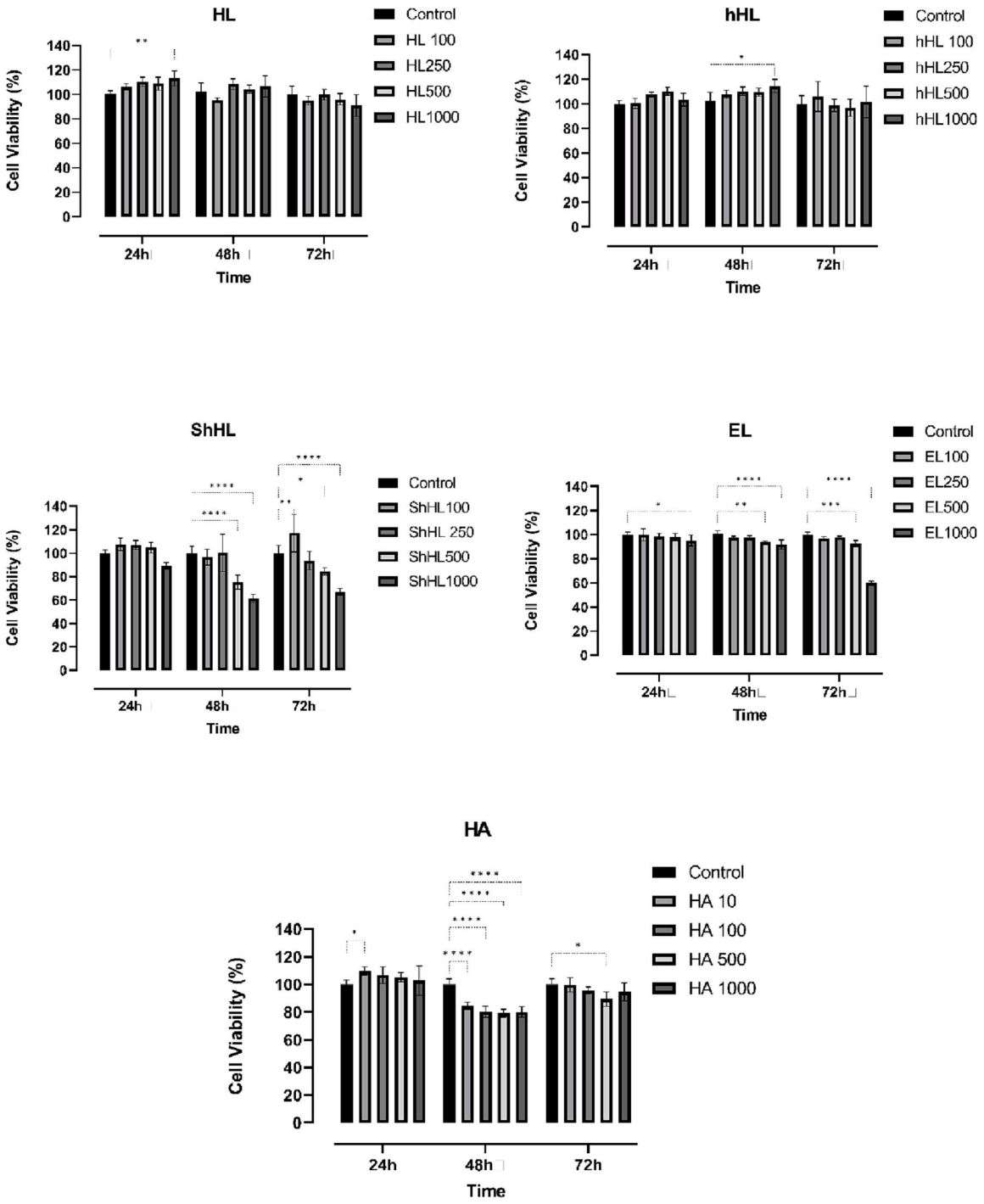

Fig. 3. Viability results of PCS-201-012 (Human Fibroblast Cell line) cells after being cultivated with HL, hHL, ShHL, EL and HA for 24, 48 and 72 h. (A *P* value below 0.05 was considered as statistically significant).

Table 2
Cell cycle percentage of fibroblast cells after incubation with samples.

Sample	G1 (%)	S (%)	G2/M (%)
Control	66.0	10.6	17.1
HL	41.1	17.9	27.5
hHL	61.8	13.9	19.1
ShHL	74.7	10.6	11.8
EL	63.8	13.6	18.6

[66,67]. Expression profiles of *FLG* and *IVL* in the HaCaT cell line were recorded (Fig. 5A and B) as upregulation of *FLG* in HL, hHL, ShHL and EL with the fold changes of 1.10, 1.74, 3.89, 1.49, respectively and upregulation for *IVL* for HL and EL with fold changes of 2.0 and 6.43, respectively. Expression profiles of target genes human *CoL1A1* and *HAS3* in PCS-201-012 cell line were (Fig. 5C and D) recorded for *CoL1A1* in ShHL as upregulation with the fold change of 2.63 while expression of *HAS3* was upregulated in the cells treated with HL with the fold change value of 1.41.

Under normal circumstances, the synthesis of collagen is known to be slow. However, when the results of type I collagen production were

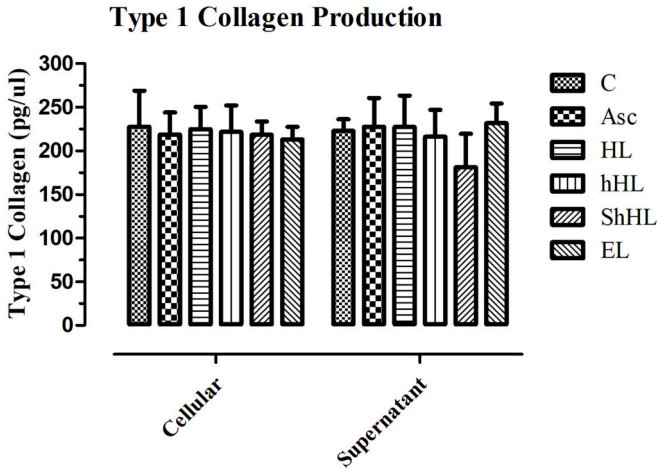

Fig. 4. Type I collagen production of the samples after being cultivated with PCS-201-012 cells for 48 h.

evaluated by ELISA and q-RT PCR together, it was found that the ShHL sample could increase collagen production if incubated for a longer period. ShHL exhibited the highest collagen expression among all *Halomonas* levan samples. The ELISA results also showed a higher amount of procollagen type I inside cells that were incubated with ShHL, while the amount of secreted type I collagen was lower than the amount of procollagen. Nevertheless, the expression of COL1A1 was higher than that of the control group.

Presence of sulfonate groups (—SO_3Na) is known to increase wettability, electronegativity and decreased adsorption of proteins and glycosaminoglycan-protein interactions are affected with the distribution and the number of sulfate groups. Sulfonate rich, heparin mimetic surfaces are also known to bind and stabilize cell growth factors like fibroblast growth factor (bFGF) through ionic bonding and increase proliferation of the cells. Heparin mimetic polymers are also known to increase expression of bFGF or decrease expression of transforming growth factor (TGF-β1) play important roles in cell adhesion, proliferation, wound healing, and ECM synthesis and decrease degradation of it through matrix and collagen degrading proteases. These proteases have huge impact on hypertrophic scar formation. Thus, heparin mimetic groups are known to regulate fibrotic scar formation through regulation of fibroblast proliferation and ECM degradation through MMPs activation. Dermal fibroblast proliferation and synthesis of collagen and other ECM molecules and their synergistic action with growth factors (GFs) are vital for wound healing. Keloid formation or scleroderma (fibroblast and collagenous rich ECM) may result when interaction of those molecules is affected and heparin mimetic polymers are known to regulate this mechanism through interaction with positively charged proteins of

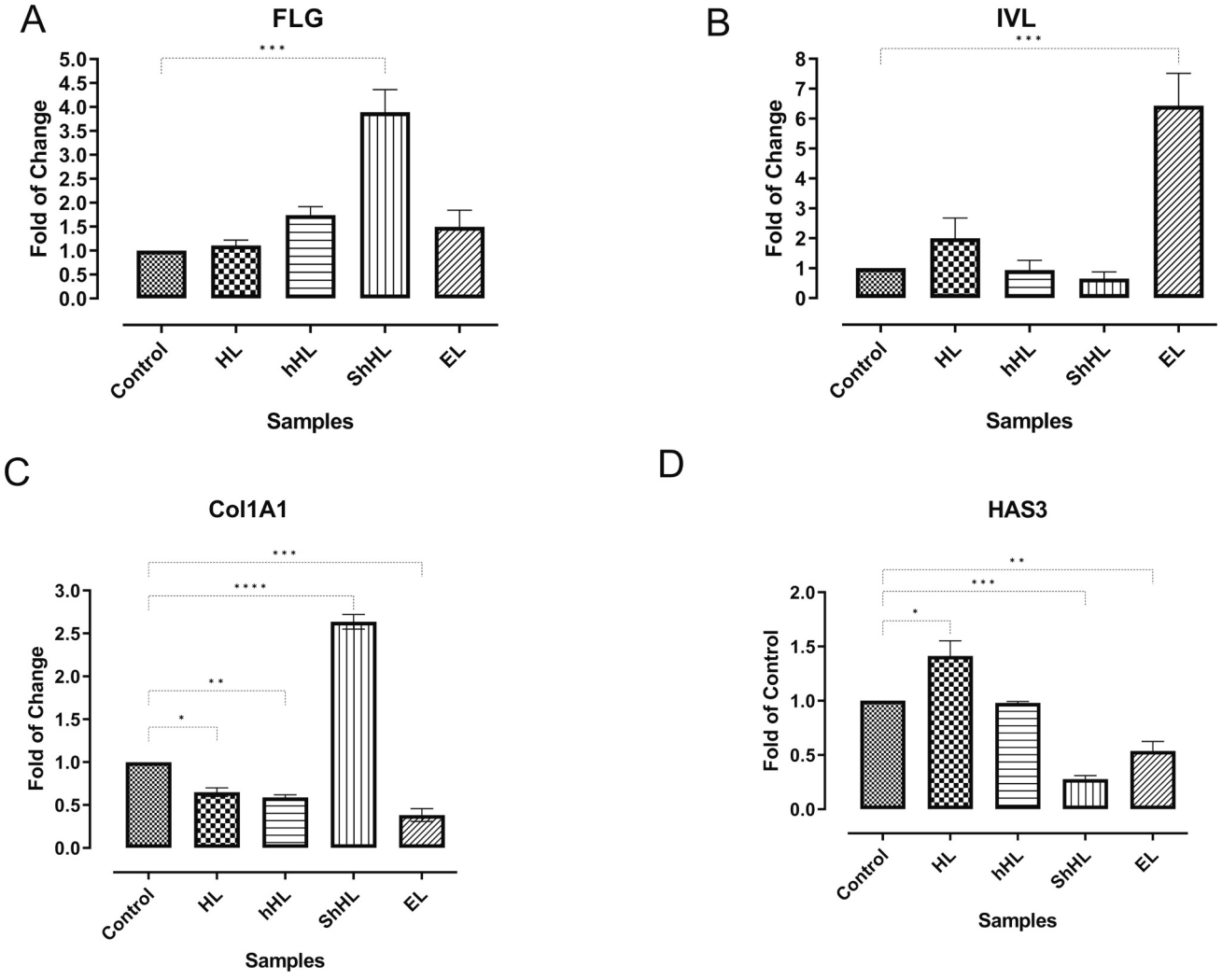

Fig. 5. Fold change of A) *FLG* B) *IVL* gene expression in HaCaT cell line C) *Col1A1* and D) *HAS3* gene expression in PCS-201-012 cells after being cultivated with samples for 24 h. (A *P* value below 0.05 was considered as statistically significant).

GFs with their negatively charged sulfate groups [68–70]. Sulfated polysaccharides provide moist environment with their natural viscous structures in nature and inhibit scab's development which facilitates the proliferation and migration of the cells leading to new tissue formation in wound zones. However, high concentration of sulfated polysaccharides is known to reduce activity of fibroblast keratinocyte growth factor 7 which is related with cell proliferation and migration [71]. Fucoidan, fucan, highly sulfated galactan are other sulfated exopolysaccharides that have anti-inflammatory anti-oxidant and anti-aging activities and highly preferred in cosmetic formulations [72]. Red microalgae (porphyridium) derived sulfated polysaccharide is reported to be an excellent candidate to substitute HA with its lubricant and anti-oxidant activity [73]. Improvement in amount of cornified envelope maturation in stratum corneum and reinforcement of dermal-epidermal junction is observed in another study with sulfated polysaccharide of red algae (*Porphyridium cruentum*). Study proposed sulfated polysaccharide as a promising candidate to be used in topically applied moisturizers to prolong their effect and improve characteristics of dry and aged facial skin [74].

Atopic dermatitis (AD) is a skin disorder where barrier disruption, chronic pruritis, eczematous skin occurs with reduced expression of *FLG*, *IVL* and loricin (*LOR*) and impaired barrier function. Topical usage of steroids is reported to improve inflammation, normalize trans epidermal water loss and upregulate *FLG* and or *LOR* expressions [75]. Sulfated polysaccharides like chondroitin sulfate, heparan sulfate and fucoidans were also used as a humectant AD moisturizer [77–79]. Sulfated polysaccharide from *Aphanothece sacrum*, sacran, was proposed as a topical treatment that improves corneocyte derived parameter [79]. In this study, highly sulfated (2.91 DS) hydrolyzed levan (ShHL) showed an increase in *FLG* and *IVL* expression when compared to native one. Sulfate groups are thought to be effective in this expression difference. Results obtained from gene expression analysis can be concluded as usage of levan and its derivatives increase barrier function and hydration of skin through regulation of *FLG* and *IVL* expression.

3.6. In-vitro wound healing

Body response to gain the normal structure of tissue and its function after traumas, diabetes related conditions or surgery is called wound healing which contains four main stages, namely hemostasis, inflammation, proliferation, and remodeling. Keratinocytes respond to epidermal violation starts the first stage, hemostasis, followed by clotting and vasoconstriction through secretion of growth factors and pro-inflammatory cytokines. Inflammation stage involves neutrophil reaction to wound area and secreted TGF-β induce monocyte differentiation into macrophages which produce fibroblastic growth factors and start angiogenesis. Proliferative stage starts with the vascular endothelial growth factor (VEGF) and FGF induced granulation tissue formation where ECM components like collagen and glycosaminoglycan production and angiogenesis occurs. Final stage of wound healing, re-modelling phase is the slowest phase which can last from months to years. Secreted type III collagen is replaced by type I collagen in second week and peaks around third week and is balanced by degradation. Abnormal ECM production, diminishing level of growth factors, increasing of inflammatory interleukins and tumor necrosis factor (TNF) prevent proliferation stage and remodeling phase [80]. Wound related secondary unwanted situations like infection and aesthetic reasons require treatments with not only rapid wound closure but also with minimal scar tissue formation and reduced pain. Non-sticky, microenvironment provider with non-toxicity, anti-microbial and anti-allergic activities are essentials of skin wound dressings [13,81–83]. Collagen is known to support fibroblasts' cell-cell and cell-ECM interactions and allows new tissue formation in third and fourth stages of wound healing together with growth factors, cytokines, integrin's and MMPs [84]. Maintaining moisture and hydration of wounded area is known to enhance the quality of the formed tissue [85]. Thus, development of cosmeceuticals that contain biologically active wound healing and cell proliferation supporting polysaccharides are in need. Fructans are strong candidates for components of wound healing creams with their hydrophilicity, high moisturizing activity and cellular proliferation supporter abilities. *Halomonas* levan polymer with different branching and active groups with previously reported bioactivities are the strong candidates for wound healer formulation and scaffold design. Results obtained from *in-vitro* scratch assay and decreases in wound width and wound closure (%) for the first 48 h were given in Fig. 6 for the levan samples. When compared to the control group, all samples showed considerable wound closure while HL showed the best effect with the total closure of the wound at the end of 48 h and this result was followed by hHL and EL. For the 24-hour period, percentage closure of the wound was recorded as; 30.70, 89.66, 65.17, 52.56, and 49.98 % (Table 3) for C, HL, hHL, ShHL and EL, respectively (Figs. 6 and 7). The fastest closure was observed for HL flowed by hHL for the first 24 h interval. After 48 h, almost complete closures were observed for HL and EL samples (Fig. 6B).

Scratch assay images showed that samples of HL, hHL and EL closed and connected zones after 24 h or closed completely (Fig. 7) With the branched levan EL, an interesting effect on cells was observed where after 24 h, cells started to lose their connection with neighboring cells and started to migrate through the wounded area and closed it almost completely after 48 h.

It is well known that bioactivity of polymers is affected by their chain length, charge, structure (chain length or branching), flexibility, structural conformation in aqueous environment and these properties also play vital role in polysaccharide and membrane interactions. The branching degree of the polysaccharide is a structural feature associated with the presence of linked monosaccharide residues or linked chains to their backbone and the surface charge of cells is known to be neutralized by branched molecules [86–88]. The presence of hydroxyl groups is known to increase hydrophilicity and cells are known to prefer surfaces to attach and proliferate that have hydroxyl (CH_2OH) or carboxylic acid (—COOH) groups [89]. Moreover, larger molecules are known to adopt more preferred conformations for membrane binding than smaller ones [87,88]. Fructans are known to stabilize cell membrane more efficiently than glucans with their more flexible chains and the smaller size of the furanose rings compared to bulky pyranoses of glucans thus effect cellular physiology in positive way through increased membrane interactions [90]. Chain length, branching, type, charges, structural conformation and flexibility in aqueous environment of fructans and even application dose of these molecules is known to regulate their bioactivity and cellular responses like barrier function protection on gut epithelial barrier through different signaling pathways. Neutral charge of fructans allows them to interact with lipid bilayer of cell membranes through nonpolar interactions through hydroxyls. Interactions between different DP inulins and mono – and bilayer lipidic systems and chain length dependent strong interaction were reported to be linked to linearity and flexibility of furanose rings in long chains [87,91]. Branched structure of fructans are thought to provoke disordered arrangements of their chains, spatially, which could result in decreased physical contact with the cell membrane. On the other hand, larger molecules are known to adopt more preferred conformations for membrane binding than smaller ones. The flexibility of furanose rings in levan backbone and helix conformation in aqueous environments make it easier to interact with membranes when compared with the structural rigidity of other polymers. Phosphate groups of liposomes are known to interact with hydroxyls of fructans through hydrogen bonding and levan is thought to anchor phosphatidylcholine of the cellular membrane through hydrogen bonding and scaffold-like branched structure of fructans affect this interaction [92]. Fructan structure dependent immunomodulation through inhibition and activation of different pathways (MYD88) and by leucine rich repeats of TLRs (2, 3, 4, 7 and 9) are known and β (2 → 6) bonds in agave fructans are reported to modulate immunity beneficially. Fernández-Lainez et al. [88] showed improved intestinal barrier function of epithelial cells through the protein kinase phosphorylation of

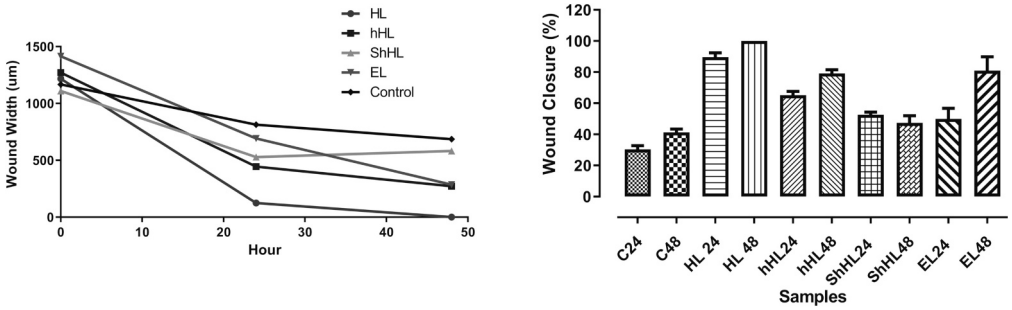

Fig. 6. Decrease at wound width (A) and wound closure % (B) of HaCaT cells after being cultivated with samples from hour 0 to 48.

Table 3
Measurement of wound closure rates expressed as the difference between wound width at different intervals (24 and 48 h).

Samples	Percentage wound closure (%)[a]	
	24 h	48 h
Control	30.37 ± 4.83	41.28 ± 4.41
HL	89.65 ± 5.41***	100.00 ± 0.00***
hHL	65.17 ± 4.95***	79.15 ± 4.46***
ShHL	52.56 ± 3.45***	47.37 ± 9.28^{ns}
EL	49.98 ± 13.16***	80.92 ± 17.46***

[a] The data were expressed as mean ± S.D. of triplicate values. One-way ANOVA, followed by Dunnett's test was used for statistical analysis (significant at ***$p < 0.05$ *versus* ns for no significance).

occludin proteins in tight junctions when incubated with long chain linear inulins and cells treated with long chain linear fructans did not increase secretion of pro-inflammatory cytokine, IL-8, in the presence of inflammatory compounds [88]. Immune modulatory and anti-irritant activities of cosmetics are important because of controlling skin allergies *via* maintaining skins pathogen defense mechanism, toxin, and inflammatory responses through immune system cells and cytokines. An association of helical conformation with lymphocyte stimulatory activity was shown for fructans. This conformation was evidenced at increasing mixed-type fructan concentration by atomic force microscopy, showing a concentration-dependent formation of helical conformation which is associated with a higher lymphocyte proliferative activity *in vitro* [93]. Azizov et al. [94] have reported that inulin derived from *Arctium lappa* L. (Compositae) (burdock) has been found to be beneficial in the treatment of various skin conditions, such as eczema, ulcers, and in promoting fast wound healing [94]. Immune modulatory activity of onion (*Allium cepa*) inulin-type fructans (DP 3-6 FOSs) were tested *in vitro* with primary murine peritoneal macrophage, peritoneal exudate cells (PECs) culture. The reported results indicate that there was an increase in cellular proliferation, nitric oxide production, intracellular free radical production, and phagocytic activity [95]. Zhao et al. [96] investigated immunoregulatory activity of natural fructan polysaccharides POP-1 and PCP-1 ((2 → 1)-linked β-D-fructofuranose (Fruf) backbone and (2 → 6)-linked β-D-Fruf side chains with an internal α-D-glucopyranose ((Glcp) in neokestose) isolated from *Polygonatum odoratum* and *P. cyrtonema* [96]. Cell viability, phagocytic activity and IL-6 production were analyzed on RAW 264.7 macrophage cells and improved cell viability and IL-6 production were observed on macrophages in the presence of PCP-1. A 1-kestotriose type fructan was extracted from garlic (*Allium sativum* L.), and it was found to have a protective effect on UVB-induced human keratinocyte cells (HaCaT) by increasing cell viability [97]. In another study, when inulin extracted from Jerusalem artichoke and chicory were tested with human keratinocytes, they showed increased cellular proliferation (5 mg/mL) and when added into cosmetic formulations, absence of skin irritation was reported by Nizioł-Łukaszewska et al. [98]. ROS decrease and improved proliferation was observed on levan containing anti-oxidant cosmetic PROTEOLEA® incubated blue light irradiated keratinocyte (HaCaT) [99]. APOLLUSKIN® (Silab) containing fructan extracted from dandelion, *Taraxacum officinale*, was reported to have skin radiant and anti-irritant activities in a patent application [100]. Oxidized inulin and ZrO_2 nanoparticle stabilized hybrid collagen scaffolds were reported to have good compatibility with mouse mesenchymal stem cells (mMSCs; C3H10T1/2) and human osteoblast cells (MG-63) and 60 % wound closure in the first 24 h on stem cell wound model were observed [101]. Catechol conjugated levan hydrogels were investigated by Osman et al. [102]. Strong adherence to hydrated porcine skin was three times higher than fibrin glue adhesive and not only rapid blood clotting but also no immunogenic response was observed together with fast wound healing in rat incision model compared to control. Carboxymethyl levan based nanoparticle system is used for delivery of human epidermal growth factor (hEGF) and improved skin moisturization and regeneration were observed through increased aquaporin 3 and collagen expression and 100 % cell proliferation for 6 weeks [103]. Effect of silver nanoparticle loaded levan gel on wound healing was investigated by Kubavat et al. [104] in rat excision wound model [104]. Reduction in wound area, bactericidal effect against *E. coli* and *B. subtilis* with anti-inflammatory effect were observed. Levan from *Bacillus subtilis* MZ292983 was reported to have accelerated burn healing activity on rats and antimicrobial activity against *E. coli* and *S. aureus* [105].

Fungi derived compounds like pullulan, schizophylan, glucans, chitin, scleroglucan, are commonly used for cosmetic products with shear thining, IL-8 producing, emulsifying, stabilizing, binding, antioxidant, wound healing, cell migrating, proliferating UV protecting, moisturizing abilities [106–111]. Seo et al. [112] showed wound closure on male ICR mice with its re-epithelialization and dermal contraction inducer abilities of β-glucan isolated from *Schizophyllum commune* fungi [112]. Muthuramalingam et al. [113] showed skin regenerating and reduced cutenous scarring ability of β-glucan-based hydrogel on mice full-thickness wound model through increased expression of TGF-β3, cytokeratin 10 and 14 on keratinocytes [113]. Effect of *β*-glucan containing Imunoglukan P4H® cream is tested on 105 patients with AD and improvement in symptoms of itching, dryness and flare are observed [114]. Improved barrier function, moisturization and reduced inflammation observed through reduction of TEWL and increased FLG mRNA expression, and reduced mRNA NLRP3, ASC, caspase-1, IL-1β, TNF-α in skin lesions effects of spray containing β-glucan and panthenol on dry skin-induced chronic pruritus mouse model is reported [115]. Curcumin loaded octenyl succinic anhydride modified schizophyllan hydrogels promoted cell proliferation, anti-inflammatory response through reduction of TNF-α and IL-6 and proposed as potential wound dressings [116]. Schizophyllan-methacrylate hybrid nanogels investigated for topical delivery properties on porcine stratum corneum and dendritic cell (DC) maturation, IL-6 and improved DC internalization observed [117]. *Ceriporia lacerata exo*-pharmaceutical substance (CLEPS) reported to have skin barrier improvement through increased *FLG* synthesis, anti-aging activity through increased collagen synthesis and reduced collagenase activity and skin whitener activity with reduced melanin synthesis together with anti-inflammatory and wound healing

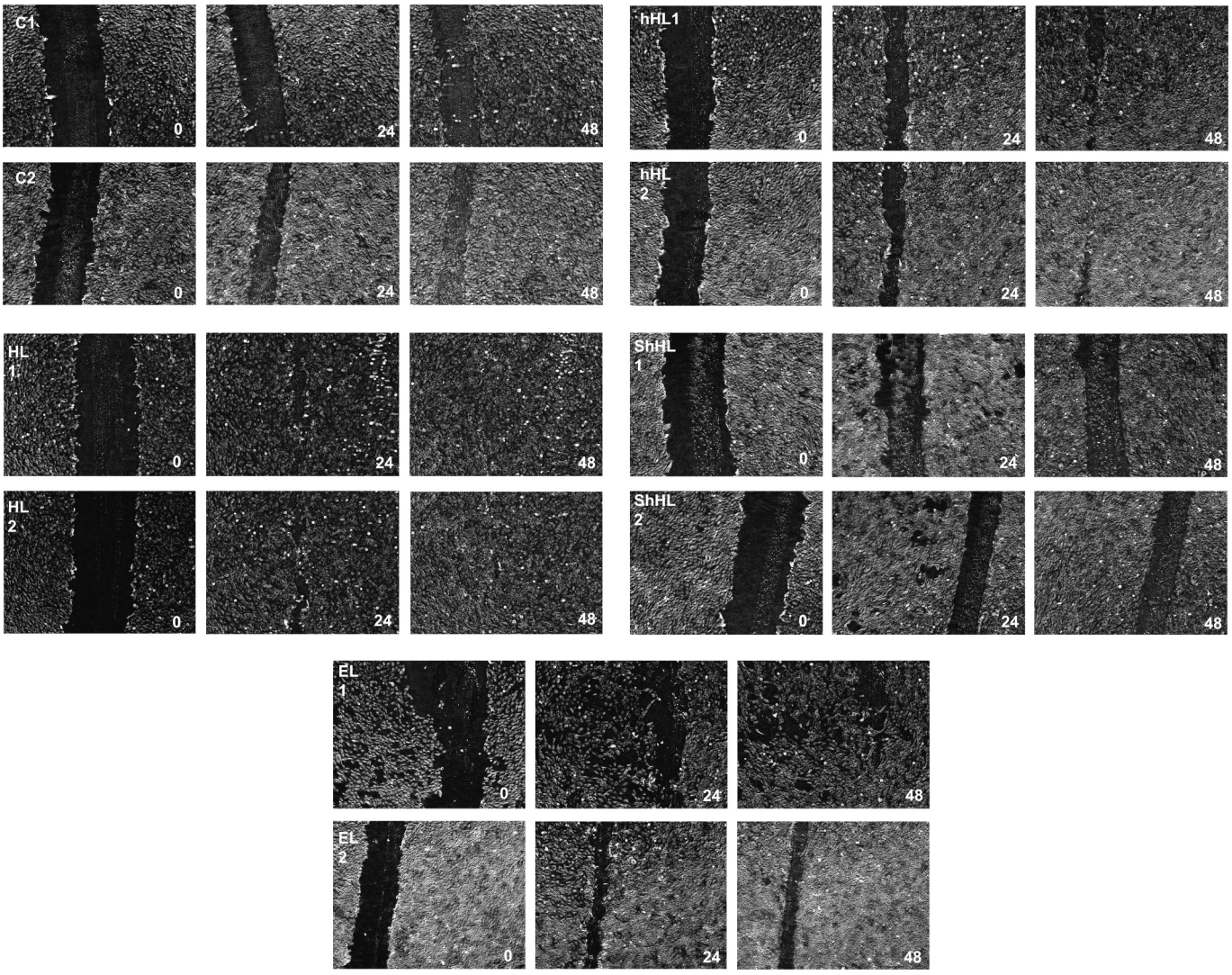

Fig. 7. Images of HaCaT Cells wound areas (0, 24 and 48 h) after incubation with samples.

effects on human dermal fibroblast and B16 melanoma cells [117]. *Tremella fuciformis* exopolymer is reported as candidate to be used in cosmetics previously with its high water holding property [118,119]. Heteropolysaccharide of *Pholiota nameko* mainly composed of β-glucan, protein, and uronic acid is reported to have high anti-oxidant and moisture preventing abilities for cosmetics [120]. Chen et al. [121] proposed (1 → 3)-β-D-glucan from *Cordyceps sinensis* as a moisturizer for food and cosmetic industries with high moisture absorption capability comparable to chitosan and urea [121].

Xu et al. [93] investigated the immunostimulatory effect of levan from *Bacillus subtilis* natto fermented soybean mucilage on two mouse macrophage cell lines (RAW 264.7 and J744.1), human embryonic kidney cell line (HEK 293), Spleen T cells and mice *via* toll like 4 receptor (TLR4) signaling, IL-12 production and suppression of T helper-2 response and Ig E production [93]. Significant production of IL-12 and TNF-α by macrophage cells was observed. TLR-4 was found to play a significant role in the recognition of levan and oral administration of levan was found to reduce serum Ig E levels significantly. Levan type branched fructan isolated from *Curcuma kwangsiensis* showed high Raw 264.7 murine macrophage cell proliferation (100 %) and concluded as an immune-stimulating activity based on macrophage proliferation [122]. Silver nanoparticles of levan isolated from *Bacillus amyloliquefaciens* PB6 were investigated for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* and immune stimulating activity with RAW 264.7 cells and significant microbial growth inhibition were observed while RAW 264.7 cells showed above 75 % viability [123].

Cosmetic industry seeks active ingredients that reduce the degradation of ECM components like HA, elastin and collagen through hyaluronidase, elastase and collagenase inhibition to be used in anti-aging formulations. Collagen is known to support fibroblasts' cell-cell and cell-ECM interactions and allows new tissue formation together with growth factors, cytokines, integrin and MMPs [84,124].

Wounded skin areas are a major concern worldwide, and HL, hHL, ShHL, and EL can be utilized as active ingredients in skin regeneration and wound healing products or scaffolds due to their quick wound healing abilities. According to the results obtained from q-RT PCR and ELISA, the sulfate-modified derivative of microbial linear levan (ShHL) can be proposed for use in wound healing scaffolds and formulation designs due to its CoL1A1 inducing abilities, together with its heparin mimetic activity, which can stabilize and prolong the half-life of growth factors secreted by the cells in the wound zone.

As described above, the sulfate group-related bioactivity of the polymers, such as angiogenesis, MMP, and FGF activities, could play vital roles in wound healing stages and may expedite the remodeling phase with the production of type I collagen. An increase in FLG expression could result in a strengthened skin barrier in newly formed tissue. A mixture of these polysaccharides could be used in wound healer products, where each of them has different bioactivities that induce fast

wound closure, type I collagen production, improved skin barrier function, and hyaluronic acid production, resulting in a formulation that supports skin renewal while inducing cells to produce ECM components and maintain hydration to the wound zone both from the inside with hyaluronic acid production and outside with their humectant abilities as well. In another approach, each levan polymer could be used in target-specific formulations. To sum up, samples of HL, hHL, ShHL, and EL can be introduced into the market to be used as active ingredients in skin renewal promising products, while samples of HL, ShHL, and EL can be proposed to be used in anti-aging products that stimulate collagen and hyaluronic acid production. As described previously, skin moisturization is closely related to healthy and strong skin barrier function controlled by natural moisturizing factors that are breakdown products of filaggrin proteins in the stratum corneum. Thus, samples of HL, ShHL, and EL could be strong candidates that repair or enrich skin barrier function-related skin moisturization by supporting the expression of these genes. HA is one of the main components of the extracellular matrix that supports the dermis and epidermis and maintains hydration when used topically on SC. Since the synthesis of HA is very important for healthy, younger, and hydrated skin, the sample of HL can be used in dermal fillers or scaffolds that target dermis support or repair or moisturizer formulations that maintain hydration.

Due to the limited studies on the use of levan as an active ingredient in cosmeceuticals, primarily due to yield and production costs, the outcomes of the present study may provide a foundation for further research and the development of novel formulations and products that can find a place in the global market. Such research could focus on the use of extremophilic *Halomonas symrnensis* or levansucrase enzymes for the non-sterile, large-scale production of levan, which could adapt to extreme conditions and overcome issues related to production and downstream processes, as well as improve stability. Further research is required to investigate the immunomodulatory effects and interleukin synthesis, UV protection, suitability for skin prebiotic applications, and skin whitening capabilities of levan using full-thickness skin models. 3D partial and full thickness wound models should also be used to explore the physiological effects of Halomonas levan on wound healing abilities.

4. Conclusion

Since the skin is the first line of defense in our body, any wound occurring in this tissue can have a severe impact on life due to secondary unwanted situations, such as infection. Therefore, it needs to be treated with minimal scar tissue formation and reduced pain in a short time. Thus, supporting skin renewal is one of the main goals of cosmetic producers, and many studies aim to find natural alternatives for cosmetics that have high moisturizing, antioxidant, anti-aging, and anti-wrinkle effects, which are closely related to high cell proliferation, skin repairing, improved barrier function, collagen, and HA synthesis. Therefore, the development of cosmeceuticals containing biologically active wound healing and cell proliferation-supporting polysaccharides is necessary. On the other hand, the results of this study clearly showed that the source and structural features of polysaccharides will also affect their bioactivity in natural cosmeceutical formulations. Hence, *Halomonas* levan, as well as its derivatives, can be proposed as active ingredients for cosmeceutical formulations to be used in moisturizers, including special formulations for atopic dermatitis, with their skin barrier function improver activities. Their collagen and HA production-inducing abilities may make them suitable for use in anti-aging formulations. Furthermore, their skin regenerative properties, such as cell proliferative and wound closing abilities, make them perfect candidates to be used in tissue regenerative formulations or scaffolds that can close wounds without scar tissue after further experiments to illuminate their true potential in the field of cosmetics.

CRediT authorship contribution statement

Merve Erginer Hasköylü: Conceptualization, Methodology, writing-Original Draft, Reviewing and Editing, **Barış Gökalsın:** Data curation, Software, Writing-Original draft preparation. **Selay Tornaci:** methodology, writing, **Cenk Sesal:** Supervision, **Ebru Toksoy Öner:** Supervision, writing-Reviewing and Editing,

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.124418.

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