Acta Biomaterialia xxx (xxxx) xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Use of photosynthetic transgenic cyanobacteria to promote lymphangiogenesis in scaffolds for dermal regeneration

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ARTICLE INFO

Article history:
Received 14 December 2020
Revised 28 February 2021
Accepted 15 March 2021
Available online xxx

Keywords:
Oxygen
Hypoxia
Hyaluronic acid
Synechococcus sp. PCC 7002
Lymphatic endothelial cells
Biomaterials
Lymph vessels

ABSTRACT

Impaired wound healing represents an unsolved medical need with a high impact on patients´quality of life and global health care. Even though its causes are diverse, ischemic-hypoxic conditions and exacerbated inflammation are shared pathological features responsible for obstructing tissue restoration. In line with this, it has been suggested that promoting a normoxic pro-regenerative environment and accelerating inflammation resolution, by reinstating the lymphatic fluid transport, could allow the wound healing process to be resumed. Our group was first to demonstrate the functional use of scaffolds seeded with photosynthetic microorganisms to supply tissues with oxygen. Moreover, we previously proposed a photosynthetic gene therapy strategy to create scaffolds that deliver other therapeutic molecules, such as recombinant human growth factors into the wound area. In the present work, we introduce the use of transgenic *Synechococcus* sp. PCC 7002 cyanobacteria (SynHA), which can produce oxygen and lymphangiogenic hyaluronic acid, in photosynthetic biomaterials. We show that the co-culture of lymphatic endothelial cells with SynHA promotes their survival and proliferation under hypoxic conditions. Also, hyaluronic acid secreted by the cyanobacteria enhanced their lymphangiogenic potential as shown by changes to their gene expression profile, the presence of lymphangiogenic protein markers and their capacity to build lymph vessel tubes. Finally, by seeding SynHA into collagen-based dermal regeneration materials, we developed a viable photosynthetic scaffold that promotes lymphangiogenesis *in vitro* under hypoxic conditions. The results obtained in this study lay the groundwork for future tissue engineering applications using transgenic cyanobacteria that could become a therapeutic alternative for chronic wound treatment.

Statement of significance

In this study, we introduce the use of transgenic *Synechococcus* sp. PCC 7002 (SynHA) cyanobacteria, which were genetically engineered to produce hyaluronic acid, to create lymphangiogenic photosynthetic scaffolds for dermal regeneration. Our results confirmed that SynHA cyanobacteria maintain their photosynthetic capacity under standard human cell culture conditions and efficiently proliferate when seeded inside fibrin-collagen scaffolds. Moreover, we show that SynHA supported the viability of co-cultured

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https://doi.org/10.1016/j.actbio.2021.03.033

lymphatic endothelial cells (LECs) under hypoxic conditions by providing them with photosynthetic-derived oxygen, while cyanobacteria-derived hyaluronic acid stimulated the lymphangiogenic capacity of LECs. Since tissue hypoxia and impaired lymphatic drainage are two key factors that directly affect wound healing, our results suggest that lymphangiogenic photosynthetic biomaterials could become a treatment option for chronic wound management.

1. Introduction

According to the Global Burden of Disease Study 2013, skin and subcutaneous diseases are ranked as the 4th nonfatal leading cause of disability worldwide [1]. In particular, chronic skin wounds, which are those that fail to progress through the healing process over a physiologically appropriate length of time [2], affect over 1.5 out of 1000 population [3] and their incidence is projected to increase rapidly due to their association with aging and comorbid conditions such as diabetes, obesity, neuropathies and cardiovascular diseases [4,5]. Also, chronic wounds represent enormous direct and indirect costs to health care and society, reaching from 1% to up to 5.5% of the total expenditure of the health sector [6], where the costs of unhealed wounds may exceed by 135% the costs of healed wounds [7]. All this exemplifies the need for efficient therapeutic options to counteract excessive inflammation and the inability of the skin cells to respond to regenerative stimuli which are characteristic of chronic wounds [8].

Oxygen is a key mediator in wound healing, as it is involved in several processes including re-epithelization, oxidative immune response and collagen synthesis [9]. Poor wound oxygenation has been suggested as a parameter to predict chronic wound healing complications, it has been defined as a diagnostic criterion for wound treatment [10,11], and it has been proposed as a prospective therapeutic target [12–14]. On the other hand, the lymphatic vascular system has been recognized to play a significant role in skin wound healing, as it is responsible for the local removal of the extravasated fluid containing waste products, inflammatory mediators and immune cells after an injury [5]. The lymphatic vascular system consists of a network of vessels built from tubular-aligned lymphatic endothelial cells (LECs) that allow the transport and drainage of the interstitial fluid from the peripheral tissue into the lymph nodes and then back to the systemic circulation [15]. Disruption of this functional network results in lymphatic congestion, which in turn is associated with a wide range of diseases including lymphedema [16], autoimmune disease [17], susceptibility to infections and wound healing impairment [18,19].

Promoting lymphangiogenesis - the growth of lymphatic vessels from pre-existing ones - was shown to limit acute skin inflammation, prevent the formation of edema and hasten inflammation resolution [20], and hence is recognized as a promising therapeutic target to promote the wound healing process [21,22]. For instance, gene therapy approaches aimed to enhance lymphangiogenesis have significantly reduced chronic skin inflammation in healthy mice [9] and improved wound healing in diabetic mice [23]. Moreover, the use of dermal regeneration materials (DRM) to enhance lymph vessel growth in combination with therapeutically active gene-, protein- or cell-based approaches, has demonstrated the importance of restoring the lymphatic transport to improve the effect of dermal tissue substitutes [5,24,25]. However, the successful clinical application of artificial scaffolds is often limited due to their delayed vascularization and the inherent hypoxic environment, which is incompatible with the metabolic and proliferative requirements of a regenerating tissue [26,27].

To address this, we previously introduced the use of transgenic photosynthetic microorganisms, which could deliver both oxygen and key molecules that contribute to wound regeneration independent of blood perfusion [28–30]. Photosynthetic microorganisms, such as unicellular algae and cyanobacteria own a significant biomedical and biotechnological potential, due to their ease of cultivation, fast growth rates and amenability to genetic manipulation [31]. Their implementation as a strategic approach to locally increase oxygen supply was shown to be effective in skin, pancreas, heart and tumor tissues [32]. Also, their many advantages have allowed their exploitation as biotechnological platforms for the production of recombinant human therapeutics such as cytokines and growth factors [33], as well as biomaterials like spider silk [34] and glycosaminoglycans [35]. In line with this, we recently reported the potential of *Synechococcus* sp. PCC 7002, a photosynthetic unicellular euryhaline cyanobacterium, as a biotechnological platform to produce hyaluronic acid (HA) [36]. This transgenic strain (hereafter referred to as SynHA) was genetically modified to overexpress the *Pasteurella multocida* HA-synthase (pmHAS) and further engineered to produce and secrete more than 100 mg/L HA into the culture medium within 5 days. HA is a biopolymer that consists of alternating β-1,3-N-acetylglucosamine and β-1,4-glucuronic acid molecules and which has become a promising biomaterial for tissue engineering approaches [37]. More importantly, HA was previously reported to induce migration and proliferation of LECs, and as a result, to stimulate lymphatic vessel formation [38,39].

In this study, we provide evidence that SynHA cyanobacteria can promote the lymphangiogenic potential of LECs under hypoxic conditions by supplying both photosynthetic oxygen and recombinant HA. We show that SynHA cells can be seeded into a fibrin-collagen scaffold and maintain their proliferative, photosynthetic and HA-synthesis capacity. Finally, we show that these photosynthetic lymphangiogenic scaffolds can induce lymph vessel formation under hypoxic conditions. Given their unexplored potential, lymphangiogenic photosynthetic biomaterials could become a new therapeutic approach for impaired wound healing and the overall improvement of engineered tissue substitutes.

2. Materials and methods

2.1. Cell culture of lymphatic endothelial cells

LECs isolated from juvenile foreskin were purchased from PromoCell in passage 2. Cells were cultured in Endothelial Cell Growth Medium MV (ECGM MV, PromoCell, Heidelberg, Germany) supplied with a supplement mix (PromoCell, Heidelberg, Germany), 1% amphotericin B (Biochrom GmbH, Berlin, Germany) and 1% Penicillin-Streptomycin (Biochrom GmbH, Berlin, Germany) at 37 °C, 5% CO_2 in a standard cell culture incubator (Thermo Fisher Scientific, Waltham, MA, USA). For starvation, cells were incubated in supplement-free ECBM MV. The medium was changed every 2–3 days. For experiments under hypoxic conditions, cells were incubated in 1% O_2 and 5% CO_2 at 37 °C. Proliferation assays were performed in supplement-free ECBM MV with 2% FBS (Invitrogen; Carlsbad, CA, USA), 1% amphotericin B and 1% Penicillin-Streptomycin. LEC number was determined using Countess 2 FL (Invitrogen, Carlsbad, CA, USA). All experiments were performed in triplicates with LECs of the same batch in passages 3–5.

2.2. Cell culture of cyanobacteria

Wild-type (SynWT) and transgenic *Synechococcus* sp. PCC 7002 cyanobacteria (SynHA12, SynHA03) were cultivated on A-D7 medium agar-plates supplemented with glucose (1 g/L) and chloramphenicol (10 µg/mL) at 30–50 $\mu E \cdot m^{-1} \cdot s^{-1}$ and 25 °C–30 °C as previously described [36]. Plates were refreshed every 3 weeks. The strain SynHA12 was used for all co-culture experiments and the preparation of conditioned media since its genetic antibiotic resistance facilitated sterile culture conditions. SynWT and SynHA03 were used to replicate the scaffold characterization experiments to validate the approach. A liquid preculture was started before each experiment by inoculating agar-growing cyanobacteria in 50 ml A-D7 medium (supplemented with 1 g/L glucose) and incubating it for 3 days at standard culture conditions (30 °C, 150 rpm, 30–50 $\mu E \cdot m^{-1} \cdot s^{-1}$). Then, to induce HA production, 1 mM IPTG (Isopropyl β-D-thiogalactoside, Merck, Darmstadt, Germany) was added to the cyanobacteria cultures resuspended in fresh medium to OD750 = 1 (IMPLEN P300 Nanophotometer, Munich, Germany) and further cultured for at least 7 days (SynHA(+)). The control group (SynHA(-)) was cultured under the same conditions in absence of IPTG. For all cell-culture experiments, cyanobacteria cell number was determined by light-microscopy (Primovert, Zeiss, Oberkochen, Germany) using a Neubauer cell-chamber. Cyanobacteria were then cultured under standard mammalian cell culture conditions (37 °C, 5% CO_2) and constant illumination by placing them under a light source with the complete spectrum of white light at a distance of 25 cm above the samples (32.25 $\mu E \cdot m^{-1} \cdot s^{-1}$, LED, Sebson, Dortmund, Germany) to allow photosynthetic growth.

2.3. Production of conditioned media

Supernatants of induced SynHA(+) or uninduced SynHA(-) cyanobacteria were collected by centrifugation (5 min, 5000 g), sterile filtered (0.3 µm; Millex®-GP Filter Unit, Merck, Darmstadt, Germany) and stored at −20 °C. Conditioned cell culture media were prepared by diluting the collected supernatants of 1:100 in either LEC medium (ECGM MV) for tube formation assays and gene expression experiments or in supplement-free basal medium (ECBM MV) for proliferation assays.

2.4. Seeding of cyanobacteria in DRM

Dermal scaffolds composed of collagen and silicon layers (IDRT, Integra© Matrix Life Science Cooperation, Plainsboro, NJ, USA) were cut using a Ø12 mm biopsy punch (Pico Punch® P1225, Acuderm® inc., Ft. Lauderdale, FL, USA), air-dried for 20 min on sterile gauze and placed with the collagen-layer facing upwards in 6-well plates. For seeding, cyanobacteria cell-suspensions were mixed with fibrinogen (TISSEEL, Baxter GmbH, Unterschleißheim, Germany) in a ratio of 1:1. Then, 50 µl thrombin solution (TISSEEL, Baxter GmbH, Unterschleißheim, Germany) was pipetted into the scaffold, followed by 100 µl of the cyanobacteria-fibrinogen solution. Scaffolds were air-dried for 1 h and then cultured with A-D7 medium covering the scaffold at 30 °C and 5% CO_2 under constant illumination for the desired time. Unless stated otherwise, cyanobacteria were seeded at a final density of $1 \cdot 10^7$ cells per scaffold. Control scaffolds were prepared using A-D7 medium instead of the cell-suspension.

2.5. Oxygen release measurement

Dissolved oxygen concentrations were measured in Oxodishes (OD24, OD-1842-01, PreSens GmbH, Regensburg, Germany) every 10 min using the Sensor©DishReader system (PreSens GmbH, Regensburg, Germany) according to the manufactures instructions. This system monitors the percentage of dissolved oxygen content (% pO_2) in the culture medium using a fluorometric oxygen sensor. Co-cultures with $2 \cdot 10^7$ bacteria were prepared as described previously and cultured for 3 days under hypoxia (1% pO_2) and under constant illumination. To monitor oxygen release in scaffolds, $1 \cdot 10^8$ and $1 \cdot 10^7$ cyanobacteria were seeded in IDRT-collagen matrices.

2.6. Chlorophyll measurements

$1 \cdot 10^8$ bacteria were seeded on scaffolds as described previously and incubated at 30 °C under constant illumination. After 1 and 7 days, scaffolds were imaged (Stereomicroscope Stemi 508, Zeiss, Oberkochen, Germany), and then stored at −80 °C until further analysis. For chlorophyll measurement, a cell lysate from the defrosted scaffolds in methanol (Carl Roth GmbH, Karlsruhe, Germany) was prepared using Ø 7 mm stainless steel beads (Qiagen, Hilden, Germany) in a Tissue Lyser LT system (Qiagen, Hilden, Germany) at 50 Hz for 10 min. The lysate suspension was centrifuged (5000 g, 5 min) to remove cell debris and the chlorophyll content was determined by optical density at 666 nm using a reference wavelength of 720 nm (IMPLEN P300 Nanophotometer, Munich, Germany) following the formula (Chlorophyll in µg/ml) = (OD666–OD720) x 12.61.

2.7. Quantification of hyaluronic acid in bacteria supernatants by ELISA

The concentration of hyaluronic acid in the supernatants of IPTG-induced and uninduced cyanobacteria cultures was determined using the Hyaluronan Quantikine ELISA kit (DHYAL0, R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions. This colorimetric immunoassay uses recombinant human aggrecan to specifically detect hyaluronic acid in a range of 0 to 100 ng/ml with a sensitivity of <0.2 ng/ml and a detection limit of 0.068 ng/ml. The hyaluronic acid concentration in a sample is determined by comparing it to a serially diluted standard solution with a defined hyaluronic acid concentration by optical density using a plate reader (Infinite M Plex, TECAN, Männedorf, Swiss). For this, cyanobacteria cultures were prepared as in 2.2 and incubated in the presence or absence of IPTG for 7 days. Supernatant samples were sterile-filtered and diluted 1:1000 in A-D7 medium to produce samples with values within the dynamic range of the assay.

2.8. HABP assay

Scaffolds with $1 \cdot 10^8$ IPTG-induced cyanobacteria were prepared as described above and cultivated in A-D7 medium with IPTG for 3 days. Scaffolds were fixed in 4% formaldehyde solution at room temperature overnight and dehydrated in an ascending alcohol series according to the following scheme: 1-h 4% formaldehyde at 40 °C, 30 min 70% ETOH at 40 °C, 1-h 70% ETOH at 40 °C, 2 × 1-h 96% ETOH at 40 °C, 3 × 1-h 100% ETOH at 40 °C, 30 min paraffin at 60 °C, 2 × 1-h paraffin at 60 °C. The scaffolds were embedded in paraffin. 5 µm sections were obtained using a rotary microtome (Mikrom HM 355S, Thermo Fischer Scientific, MA, USA) and placed on slides (Superfrost®Plus, Menzel glasses, Thermo Fischer Scientific, MA, USA). Sections were deparaffinized and re-hydrated in xylol-alcohol series (2 × 10 min xylene, 2 min 100% ETOH, 2 min 96% ETOH, 2 min 80% ETOH, 2 min 60% ETOH, 5 min Aqua dest.). Each blocking and antibody incubation step was followed by washing 2–3 times with PBS-Tween (0.1%) for 3–5 min. Blocking of the endogenous peroxidase was carried out by Hydrogen Peroxide Block (HRP/DAB-Detection Kit, ab 64261) for 10 min at room temperature, followed by the treatment with Avidin-Solution and with Biotin-Solution (Avidin/Biotin-Blocking System, Biolegend, 927301,

San Diego, CA, USA) for 20 min each. Non-specific antibody binding sites were blocked with 0.2% Triton X-100 and 2% BSA in PBS for 1 h at room temperature. The sections were covered with 2 µg/ml of biotinylated hyaluronan binding protein (Amsbio, AMS.HKD-BC41, Abingdon, UK) in PBS-Tween and incubated at 4 °C overnight. The secondary antibody-specificity control was incubated at 4 °C overnight with only PBS-Tween in the absence of the primary antibody. Streptavidin peroxidase (HRP-Detection IHC-Kit, ab 64261, Abcam, Cambridge, UK) was pipetted onto the slices and incubated for 10 min at room temperature. For staining, the DAB staining solution (HRP-Detection ICH-Kit, ab 64261, Abcam, Cambridge, UK) was applied onto the sections and incubated for 5 min. The slides were dehydrated in an ascending alcohol series and covered with mounting medium (Tissue-Tek Glass® Mounting Medium, Sakura Finetek, CA, USA). Pictures of at least 6 randomly chosen microscopic fields per experimental group were obtained (Axio observer, Zeiss, Oberkochen, Germany). As a negative control, scaffold sections were treated with 20 mg/ml hyaluronidase from bovine testes (1000 U/mg, Serva, Heidelberg, Germany) diluted in 100 mM sodium acetate buffer pH 6.0 (Sigma-Aldrich, St. Louis, MI, USA) for 2 h at 60 °C before the blocking step.

2.9. Co-culture of lymph endothelial cells and cyanobacteria

Indirect co-cultures of LECs and cyanobacteria were established using 0.4 µm pore cell culture inserts (Greiner Bio-One, Kremsmünster, Austria), which allowed sharing media and oxygen diffusion but provided spatial separation between the two different cell types. For this, LECs were seeded in a 24 well plate at a density of 120 000 cells pro well and cultured for 8 h to allow them to attach to the culture plate. Then, cyanobacteria were added into the inserts at a 1:150 ratio. Co-cultures were maintained in ECGM MV medium mixed with A-D7 medium in a 2:1 ratio supplemented with 0.1% chloramphenicol (Sigma-Aldrich, St. Louis, MO, USA) and 25 mM/ml HEPES (Sigma-Aldrich, St. Louis, MO, USA) under hypoxic conditions (pO_2 1%) and constant illumination. As a control, LECs were cultivated in the same medium without cyanobacteria. All conditions were performed in triplicates, and the medium was changed after each measurement. Pictures were taken using a brightfield microscope (Axio Observer, Zeiss, Oberkochen, Germany) and the numbers of LECs were determined by blinded digital image analysis using the ImageJ Cell-counter (ImageJ V-1.52A, [40]). To determine the number of cyanobacteria in each well, cells were resuspended several times by pipetting up and down and counted using the Neubauer chamber as described before.

2.10. Alamar Blue assay

For quantification of the metabolic activity of LECs, the Alamar Blue assay (AlamarBlue Cell Viability Reagent, Invitrogen, Carlsbad, CA, USA) was performed. First, cells were seeded in triplicate in a 24-well plate at a density of $7–10^4$ cells/cm^2 and allowed to attach for 2 h. Conditioned media or indirect co-cultures were prepared as described before. At each experimental time point, cells were washed with PBS (Gibco^{TM}, Thermo Fischer Scientific, MA, USA) to avoid possible residues, and incubated for 2 h in Alamar Blue working solution (0.1 mg/ml in ECGM MV) under standard culture conditions. Triplicates of the Alamar Blue working solution were prepared as blank samples and treated under the same conditions. After incubation, 100 µl of the suspension was transferred to a 96 well plate and cell viability was determined using a fluorescence plate reader (Infinite M Plex, TECAN, Männedorf, Swiss) at Ex/Em 560 nm/590 nm. After the measurement, the Alamar Blue working solution was replaced by a medium suitable for the respective experiment for further incubation.

Table 1
Primer Sequences (5′–3′).

Gene	Fwd primer	Rev primer
HRPT1	TGACCTTGATTTATTTTGCATACC	CGAGCAAGACGTTCAGTCCT
LYVE-1	AGCTATGGCTGGGTTGGAGA	CCCCATTTTTCCCACACTTG
Podoplanin	AGGCGGCGTTGCCAT	GTCTTCGCTGGTTCCTGGAG
Prox-1	ACAAAATGGTGGCACGGA	CCTGATGTACTTCGGAGCCTG
VEGF-C	CACCACCAAACATGCAGCTG	TGAAAATCCTGGCTCACAAGC
VEGFR-3	TCTGCTACAGCTTCCAGGTGG	GCAGCCAGGTCTCTGTGGAT

2.11. Tube formation assay

To assess the lymphangiogenic potential of the HA secreted by SynHA cyanobacteria, 60 µl of Geltrex ^{TM} (LDVE-Free Reduced Growth Factor Basement Membrane Matrix; Gibco^{TM}, Thermo Fischer Scientific, Waltham, MA, USA) was polymerized in a pre-cooled 96-well plate for 1 hour at 37 °C. Serum-starved (8 h) LECs were resuspended in 100 µl growth factor free Basal Medium (PromoCell, Heidelberg, Germany) and seeded over the Geltrex^{TM}-coated wells at a density of $1.6{\cdot}10^4$ cells/well in triplicates. Cells were then stimulated with 100 µl of conditioned media, as described before, reaching a final ratio of 1:100 in culture wells and incubated for 24 h. To evaluate the lymphangiogenic effect of photosynthetic scaffolds seeded with SynHA-cyanobacteria, Ø 5 mm DRM-scaffolds were seeded with $1{\cdot}10^8$ SynHA cyanobacteria as described before but using proportional volumes of thrombin, fibrinogen and A-D7 medium. Photosynthetic scaffolds were placed over the seeded LECs floating on top of the culture medium and incubated for 8 h under hypoxia (1% O_2) and continuous light exposure, as described previously. LECs cultured in pure ECGM MV without scaffold were included as the negative control. Tube formation was imaged using a brightfield microscope (Axio Observer, Zeiss, Oberkochen, Germany). The total tube length, number of nodes, junctions and segments were quantified by analysis of blinded images using the ImageJ Angiogenesis Analyzer software (ImageJ V-1.52A, [40]) with fixed parameters and presented relative to the negative control (fold change). The total tube length describes the sum of the length of segments, isolated elements and branches in the analyzed area. Nodes stand for pixels with 3 neighbors as a circular dot, whereas junctions detected as a group of fusing nodes describe the number of branching points in the formed lymphatic vascular network. Segments are elements delimited by two junctions and represent the number of connections between individual lymphatic cells. The unit of area and length is pixel. One pixel = 264.58 µm.

2.12. qPCR

For each experimental condition, $1{\cdot}10^6$ LECs were cultured in triplicates as described above until they reached 80% confluence. Cells were starved for 8 h and then stimulated for 4 days in conditioned media SynHA(+) and SynHA(-) and compared to a reference condition where cells were incubated in ECGM MV only. RNA extraction was performed using the RNAeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purity of the RNA was assessed by a NanoDrop spectrometer (IMPLEN P300 Nanophotometer, Munich, Germany). cDNA was generated from 1 µg RNA using Transcriptor First Strand Kit (Roche, Rotkreuz, Swiss) according to the manufacturer's protocol. The collected cDNA was stored in aliquots at −20 °C until further analysis. SYBER-GREEN based qPCR was performed using the InnuMIX DS Green Standard Kit (Analytic-Jena, Jena, Germany). The primer sequences used for this experiment were taken from Hirakawa et al., 2003 [41] and are provided in Table 1. The qPCR primers were designed and synthesized using Sigma-Aldrich's OligoArchitect^{TM}

Primer software. The amplification conditions were: 95 °C for 2 min, followed by 50 cycles of 95 °C for 30 s, 60 °C for 1 min, 68 °C for 30 s. The expression data were normalized based on the level of expression of the house-keeping gene hypoxanthine phosphoribosyltransferase 1 (HRPT1). The entire experiment was performed with biological triplicates.

2.13. Immunofluorescence staining

To analyze the presence of VEGFR-3 and LYVE-1 in LECs, CultureSlides (Falcon® Corning, NY, USA) were seeded with $8{\cdot}10^4$ cells per chamber and incubated overnight under standard culture conditions. Serum-starved (8 h) cells were then incubated in conditional medium for 4 days. LECs were washed in PBS (Gibco™, Thermo Fischer Scientific, MA, USA) and fixed in 3.5% formaldehyde (Otto Fischer GmbH, Saabrücken, Germany) for 10 min at room temperature. Cells were permeabilized for 15 min with 0.1% Triton X-100, blocked with 0.1% Tween-20, 10% BSA, 1% FBS) for 1 h and then incubated with the primary antibody (anti-LYVE-1 receptor, 1:500, Abcam; ab14917; Cambridge, MA, USA; or Anti-VEGFR-3-receptor, 1:500, BIOZOL; A01276-3; Eching, Germany)) in PBS-T (0.1% Tween-20, 5% BSA, 0.5% FBS) at 4 °C overnight. Then, slides were incubated with goat anti-rabbit IgG-Alexa 488 Fluor (1:1000, Invitrogen™, Thermo Fischer Scientific, MA, USA) and DAPI (1:1000, 4′,6-Diamidin-2-phenylindol; Invitrogen™, Thermo Fischer Scientific, MA, USA) at 4 °C protected from light overnight. Pictures of 10 randomly chosen microscopic fields were obtained for each condition using the same acquisition settings in all experimental groups (Axio observer, Zeiss, Oberkochen, Germany). The area covered by the signal corresponding to the protein of interest was quantified by image analysis (ImageJ V-1.52A, [40]) and normalized to the cell surface area using fixed signal-intensity thresholds. Quantitative image analysis was performed using blinded images.

2.14. Statistical analysis and illustrations

All assays were performed in at least 3 independent experiments with at least 2 technical replicates in each experimental group. All data are presented as mean ± standard deviation. Student's *t*-test was performed for comparison between two different groups. Differences among groups were considered significant if $p \leq 0.05$ (ns: not significant; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}\ p \leq 0.001$). All data were tested for normal distribution before performing a *t*-test. Schematic representations were created using the platform www.BioRender.com.

3. Results

The lack of vascularization in the wound bed of chronic skin injuries and the artificial dermal substitutes used for their treatment hinders oxygen perfusion and the transport of immunoregulatory mediators required for adequate wound healing. In this work, we introduced the use of photosynthetic SynHA cyanobacteria to improve the oxygenation and lymphatic vascularization of DRM through the continuous release of oxygen and the secretion of hyaluronic acid.

3.1. Photosynthetic oxygen produced by SynHA improves LEC viability under hypoxia

To confirm the biocompatibility of SynHA cyanobacteria with LECs, we first established a protocol for indirect co-culture of SynHA and LECs, which allowed the physical separation between both cell populations, but at the same time, culture medium solute exchange between them. LECs metabolic activity in the co-culture was assessed on days 1, 4 and 7 and showed no significant differences to LEC-monocultures, suggesting no negative effect of the cyanobacteria on LECs viability and growth (Fig. 1A). Moreover, we assessed the proliferation of the cyanobacteria under the same culture conditions as the LECs (37 °C, 5% CO_2) and observed a strong increase in their cell number after 7 days of cultivation (Fig. 1B), thus supporting their capacity for cell division under standard human cell culture conditions. To evaluate the photosynthetic capacity of the cyanobacteria, we next placed the co-culture under hypoxic conditions (1% pO_2) as an approximation to the microenvironment of non-perfused chronic wounds [42] and quantified the oxygen released by the cyanobacteria under constant illumination. After 3 h, co-cultures showed a sharp increase of dissolved oxygen over time, which reached the saturation of the measurement device (50% pO_2) after 10 h and was maintained for at least 20 h. Meanwhile, the oxygen concentration in the LEC-monocultures decreased steadily (Fig. 1C). To test the effect of photosynthetic oxygen release on LECsśurvival under hypoxic conditions, we placed the co-culture at 1% pO_2 and under constant illumination for 7 days. While hypoxic incubation led to a marked decrease of LECs metabolic activity in the monocultures, LECs in co-culture were capable to retain 77% of their activity after 4 days and 55% after 7 days, suggesting an attenuation of the hypoxic microenvironment through the photosynthetic oxygen (Fig. 1D). Microscopic images supported the aforementioned findings, as only a few cells were found in the monoculture after 4 days under hypoxic conditions, while co-cultures revealed a ~60% confluent well-plate. Quantification of the number of cells per observation field revealed a significantly increased number of LECs in the co-cultures after 4 and 7 days compared to the monocultures (Fig. 1E). Thus, these results demonstrate the biocompatibility between cyanobacteria and LECs and provide evidence that photosynthetic oxygen released by cyanobacteria benefits human LEC viability under hypoxic conditions.

3.2. HA produced by transgenic SynHA(+) induces LECs lymphangiogenic gene expression profile

Through a genetic modification, SynHA cyanobacteria are capable to produce and secrete HA when induced with the compound isopropyl β- d-1-thiogalactopyranoside (IPTG), which triggers the transcription of the inserted transgenes required for the synthesis of HA. Having established the impact of photosynthetic oxygen on LEC viability, we next investigated the effect of HA on the LECs' lymphangiogenic potential. For this, we first estimated the concentration of HA in the supernatants of SynHA induced with IPTG (SynHA(+)) compared to uninduced cultures (SynHA(-)) as culture conditions in this study differed from the original characterization (smaller culture volume, high light intensity, vigorous agitation and CO_2 supplementation). According to our estimation, SynHA(+) released 10.7 µg/ml HA after 7 days of induction with IPTG compared to the undetectable amounts of HA released by uninduced SynHA(-) (Fig. 2A), thus confirming the inducibility of the system. We next evaluated whether the secreted HA affected the expression of lymphangiogenic genes coding for the vascular endothelial growth factor C (VEGF-C), VEGF receptor 3 (VEGFR-3), podoplanin (PDPL), Prospero homeobox 1 (PROX-1) and the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1). These cell-lineage specific differentiation gene markers have been used to validate lymphatic tube-formation *in vivo* [41], and in the case of LYVE-1 and VEGFR-3, to correlate with lymphangiogenesis in skin wound healing [43]. For this, LECs were treated with conditioned media for 4 days and gene expression was evaluated by qPCR. Stimulation of LECs with SynHA(+) supernatant induced the expression of all five lymphangiogenic genes, though the difference was only found to be significant for Prox 1 (Fig. 2B). However, protein levels of two

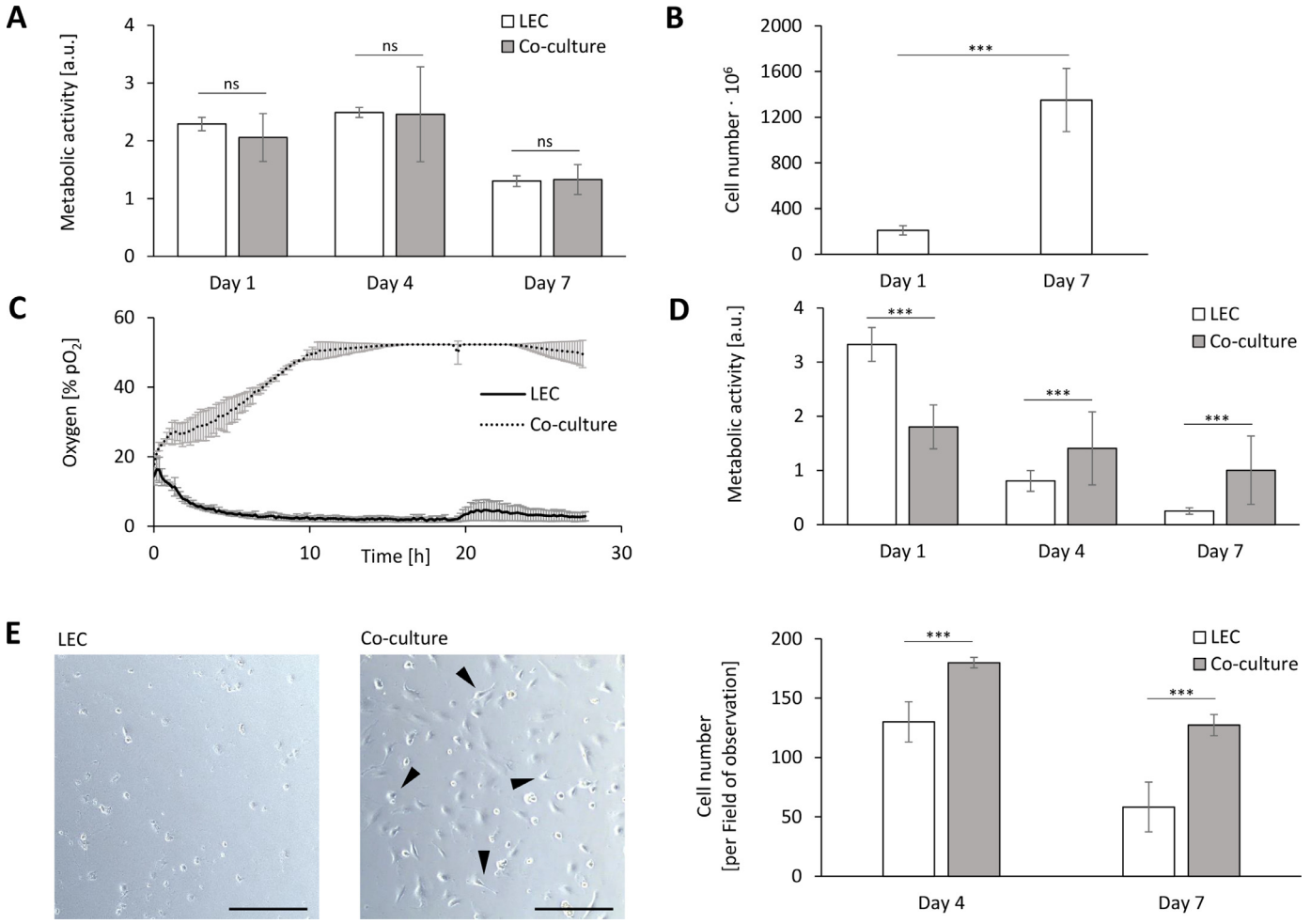

Fig. 1. Photosynthetic oxygen produced by SynHA cyanobacteria improves cell vitality under hypoxia. A. Cell vitality under normoxic culture conditions (21% pO_2) was evaluated using an Alamar Blue assay and showed no differences between the metabolic activity of LECs cultured alone (LECs) or in indirect co-culture with SynHA cyanobacteria (Co-culture) for 7 days. **B.** The significant increase in the total number of cyanobacteria from day 1 to day 7 confirms the sustained proliferative capacity of cyanobacteria under co-culture conditions with human cells ($N = 3$). **C.** Photosynthetic oxygen production by SynHA in co-culture with LECs was measured under hypoxic conditions over time and compared to the dissolved oxygen concentration in the LECs monoculture. **D.** Photosynthetic oxygen was sufficient to sustain the LECs viability under hypoxia (1% pO_2) as shown by a less pronounced decrease in the LECs metabolic activity from day 1 to day 7 in co-culture conditions. **E.** The number of LECs (arrowheads) after 7 days of cultivation under hypoxic conditions was significantly increased by the presence of photosynthetic cyanobacteria. Representative images of the culture wells are shown. All experiments were repeated at least three times ($N \geq 3$, ns = not significant; $^{*}p < 0.5$; $^{**}p \leq 0.01$; $^{***}\ p < 0.001$). The scale bar represents 300 µm.

of these genes, LYVE-1 and VEGFR-3, were markedly increased by 11-fold and 6-fold respectively, as shown by immunofluorescence staining of these membrane receptors (Fig. 2C, D). Altogether, these results suggest that LECs are susceptible to stimulation with HA produced by SynHA(+) and respond by increasing the amount of lymphangiogenic cell surface receptors.

3.3. HA produced by transgenic SynHA(+) enhances LEC proliferation and tube formation capacity

We next investigated the impact of the cyanobacteria-secreted HA on lymphangiogenic processes such as LECs proliferation and lymph tube formation. For this, LECs were subjected to starvation conditions and then stimulated with conditioned media from SynHA(-) or SynHA(+) cultures for up to 7 days (Fig. 3A). SynHA(-) was chosen as the negative control condition as it shares the genetic background of SynHA(+). Cultivation of LECs with SynHA(+)-conditioned medium significantly increased the metabolic activity of LECs after 2, 4 and 7 days when compared to the control condition (Fig. 3B). The capacity of LECs to build lymph vessels was evaluated in a tube formation assay after 24 h upon stimulation with SynHA(+)-conditioned media (Fig. 3C). Quantification of the lymph vessel network built by the LECs showed a strong increment in tube length, mesh area and mesh number when the supernatant of SynHA(+) cyanobacteria was used, concordantly suggesting that cyanobacteria-derived HA induces the lymphoproliferative potential of human LECs.

3.4. SynHA-photosynthetic dermal scaffolds stimulate lymphangiogenesis in vitro

Having demonstrated the lymphangiogenic effects of oxygen and HA released by SynHA(+) cyanobacteria, we then evaluated whether SynHA(+) could be incorporated into a collagen scaffold commonly used for dermal repair in clinical practice. Cyanobacteria were seeded into the scaffold by encapsulating them in a fibrin hydrogel, as this strategy was previously shown to efficiently encapsulate microalgae [29]. We observed a significant increase in the green color of the scaffolds suggesting the viability and proliferative capacity of the cyanobacteria inside the scaffold over 7 days (Fig. 4A). Cross-sectional images of a seeded scaffold showed a gradual distribution of the cyanobacteria with most of them grow-

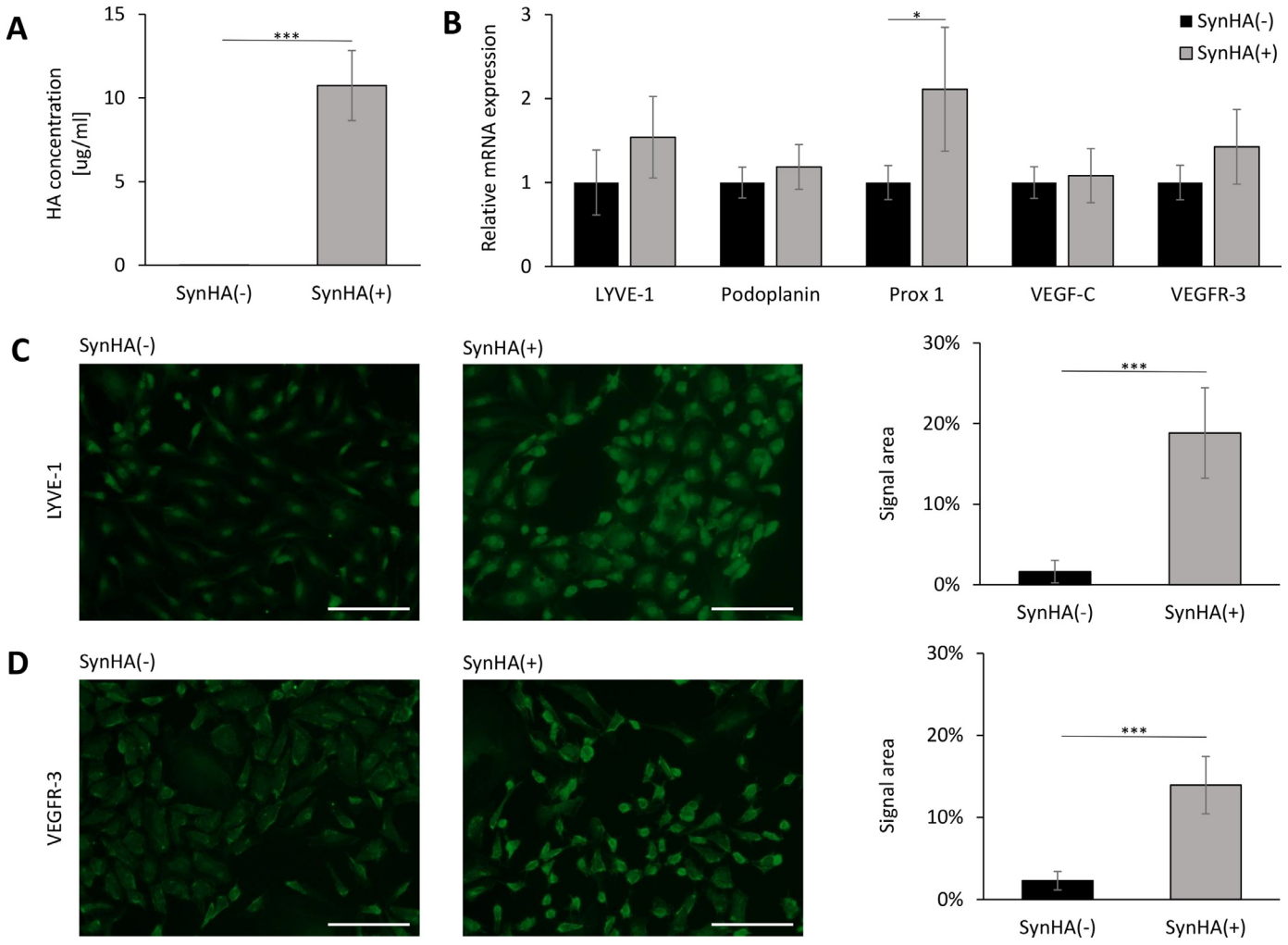

Fig. 2. Hyaluronic acid produced by SynHA cyanobacteria stimulated the lymphangiogenic gene expression profile of LECs. A. The concentration of hyaluronic acid in the supernatant of SynHA-cyanobacteria cultures induced with 1 mM IPTG for 7 days (SynHA(+)) was compared to uninduced cyanobacteria (SynHA(-)) **B.** Relative gene-expression of VEGF receptor 3 (VEGFR-3), podoplanin (PDPL), Prospero homeobox 1 (PROX-1), vascular endothelial growth factor C (VEGF-C) and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1) measured by qRT-PCR upon stimulation of LECs with SynHA(+)-supernatant conditioned media for 4 days compared to LECs cultured in unconditioned ECGM MV medium. **C.** Immunofluorescence-staining of LYVE-1 shows a higher LYVE-1 synthesis in LECs treated with SynHA(+)-supernatant compared to the supernatant of uninduced cyanobacteria. **D**. Immunofluorescence staining of VEGFR3 demonstrates an increased synthesis of VEGFR3 by LECs when stimulated with cyanobacteria-derived hyaluronic acid. Representative examples of 3 independent experiments are shown. ($N \geq 3$, $^{*}p < 0.05$; $^{**}p \leq 0.01$; $^{***}p < 0.001$). Scale bare represents 200 µm.

ing on the scaffold's surface (Fig. 4B). Then, to determine their capacity for photosynthetic oxygen production, we placed scaffolds seeded with different SynHA-cell numbers under hypoxia (1% pO_2) and monitored the dissolved oxygen concentration for 24 h upon stimulation with light. We found that photosynthetic scaffolds released increasing amounts of oxygen in correlation to the cell density (Fig. 4C), where a 10-fold increase in the number of seeded cells resulted in a 4-fold increase of pO_2 after 10 h of incubation (10^8 cells = 43.74% pO_2; 10^7 cells = 14.50% pO_2; Control = 2.43% pO_2). To quantify the growth of SynHA(+), we measured the chlorophyll content in the scaffold over time and observed a five-fold increase between days 1 and 7 (Fig. 4D). Further, we quantified the concentration of secreted HA in the SynHA(+)-scaffold culture media by ELISA and determined that the scaffolds sustained a release of 30 ng/ml–40 ng/ml HA for 7 days (Fig. 4E). The HA-content in the scaffold was detected through immunohistological staining and showed an accumulation of HA in the scaffold's upper half (Fig. 4F), which correlated with the distribution of the cyanobacteria cells inside the scaffold (Fig. 4B). The colorimetric detection of HA was reduced in scaffolds treated with hyaluronidase and absent in control scaffolds incubated without the HA-specific primary antibody (Fig. 4F), thus corroborating the specificity of the assay.

Finally, we analyzed the potential of the seeded scaffolds to stimulate lymphangiogenesis in an *in vitro* tube formation assay, where SynHA(+)-photosynthetic scaffolds and empty scaffolds were placed over the LECs and incubated under hypoxia and constant illumination. After 8 h, SynHA(+)-seeded scaffolds induced the formation of a more extent lymphatic network as measured by the mesh area, vessel length and the number of junctions (Fig. 4G). Altogether, these results indicate that bioactivation of DRMs with SynHA-cyanobacteria is a feasible strategy to generate photosynthetic lymphangiogenic scaffolds with the potential of releasing both oxygen and HA to treat chronic dermal wounds.

4. Discussion

Chronic wounds are considered an emerging epidemic and a major challenge to healthcare systems worldwide [44]. Their healing is heavily impaired by the hypoxic environment and persistent inflammatory state of the wound [45,46], and while strategies to

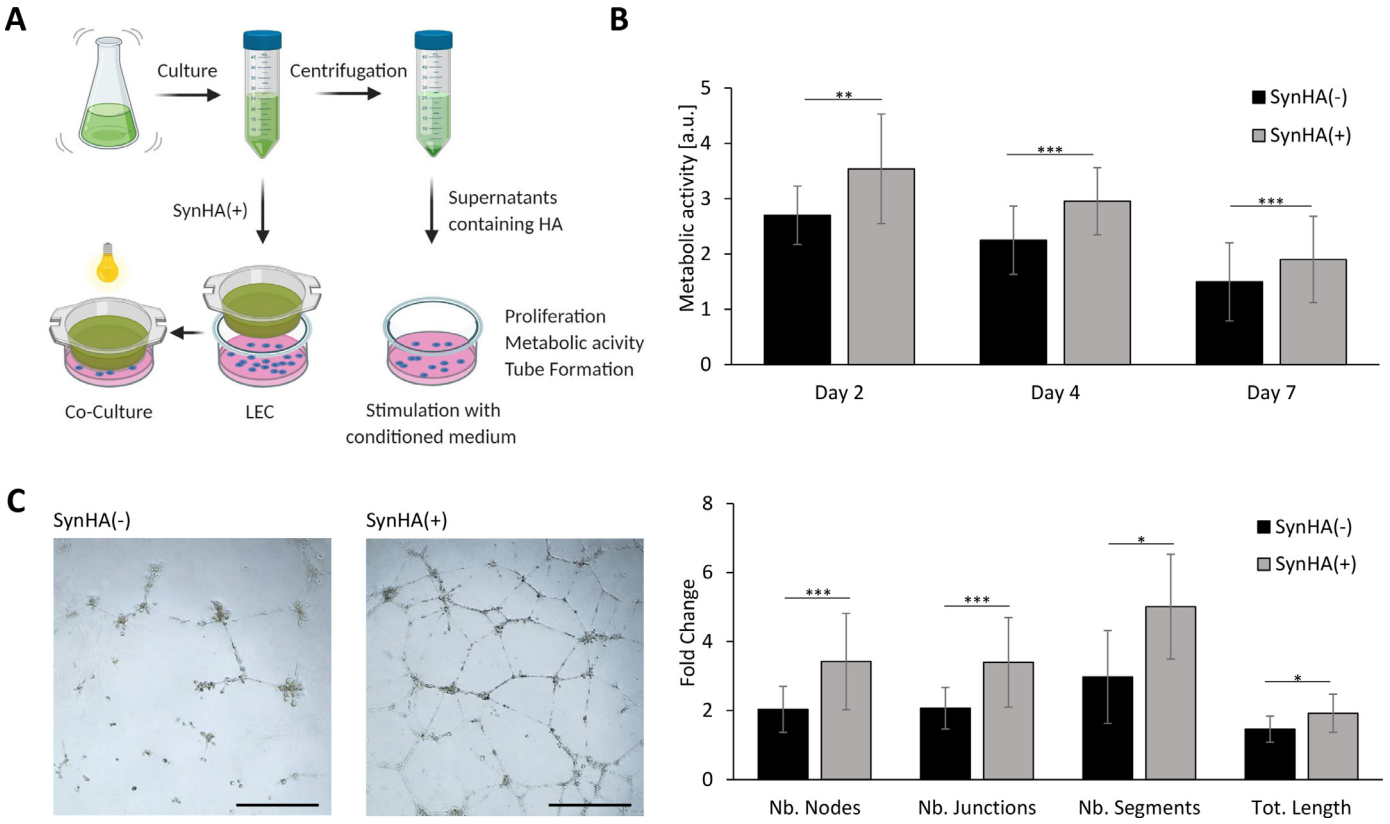

Fig. 3. Transgenic SynHA stimulates lymphangiogenesis. A. Schematic representation of the experimental workflow. Living cyanobacteria were used for indirect co-cultures with human LECS for biocompatibility and hypoxia experiments, whereas HA-containing SynHA(+)-supernatants were used to stimulate LECs in proliferation, gene expression and tube formation assays. **B.** LECs metabolic activity was significantly improved by the presence of hyaluronic acid under conditions of starvation. **C.** LECs treatment with SynHA(+)-supernatant containing HA significantly increased the lymph vessel formation capacity of LECs. Representative pictures of the results obtained from a tube formation assay after 24 h (left panel). Quantifications of tube length, area and number of the vascular network formed by the LECs (right panel) were evaluated regarding uninduced cyanobacteria (SynHA(-)). All experiments were performed in triplicate and are presented relative to the positive control. ($N \geq 3$, $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p < 0.001$). Scale bare represents 500 µm.

improve oxygenation and tackle lymph congestion in wounds have been proposed to address these issues, their limitations are still significant and their clinical success disputed [5,47,48]. Exemplary, the effect of hyperbaric oxygen therapy (HBOT) to increase levels of dissolved oxygen in the bloodstream and reduce systemic and local hypoxemia [49] is limited by the time spent in the pressure chamber, and thus on the patient's accessibility to the device. On the other hand, the use of compression and negative-pressure wound therapy brings momentary palliative-effects but does not target the pathophysiology of lymphedema, while microsurgical tissue transfer to re-route damaged lymphatic vessels greatly depends on the health of the patient's donor tissues [19,50].

Our group previously introduced the use of *C. reinhardtii* microalgae to generate photosynthetic DRMs which produce oxygen and recombinant growth factors when stimulated with light [28–30]. Building on this approach, in this study we explored the use of photosynthetic transgenic cyanobacteria capable of releasing oxygen and hyaluronic acid to improve the oxygenation and lymphangiogenic potential of dermal tissue substitutes. Compared to microalgae, *Synechococcus* sp. PCC 7002 cyanobacteria have several advantages that make them an ideal organism for potential clinical applications. For instance, their small size (ø 2 µm) and optimal growing temperature (38 °C) are cornerstones for higher biocompatibility with mammalian hosts. Also, by nature, *Synechococcus* sp. PCC 7002 can withstand and even profit from high light intensities [51] to couple with salinity and temperature changes, and greatly tolerate oxidative stress, thus suggesting their capacity to tolerate the wound microenvironment [52]. Also, their division time of approximately 4 h (two times shorter than *C. reinhardtii*) and established tools for gene modifications have promoted their use as a biotechnological platform for the production of bioactive molecules [53], including pro-regenerative extracellular matrix molecules such as hyaluronic acid [36]. All these characteristics could facilitate the incorporation of higher numbers of viable photosynthetic cells into biomedical materials and thus maximize their capacity of both oxygen and recombinant biomolecule supply into regenerating tissues.

Our results confirmed that SynHA exerted no toxic effect on human LECs (Fig. 1a) since the presence of the cyanobacteria did not affect the metabolic activity of the LECs in the co-culture. Still, both mono- and co-cultures showed a decay from day 4 to 7, which we attributed to the limited supply of nutrients in the co-culture medium (LECs culture medium mixed 2:1 with SynHA medium). In line with our results, previous studies have demonstrated that photosynthetic cyanobacteria evoke no immune response, are non-toxic and non-pathogenic *in* vivo [54], while systematic screening of the endotoxicity of cyanobacterial LPS has demonstrated that it does not elicit strong gastrointestinal, dermatological, or allergic reactions [55,56]. Moreover, *Synechococcus* sp. PCC 7002 has been used to produce postbiotics to treat and prevent inflammatory gastrointestinal diseases [57], thus supporting the use of cyanobacteria as a safe organism in biomedical applications.

The photosynthetic capacity of SynHA-cyanobacteria was sufficient to sustain both proliferation and viability of human LECs under hypoxic conditions (Fig. 1C–E), where the positive effect of

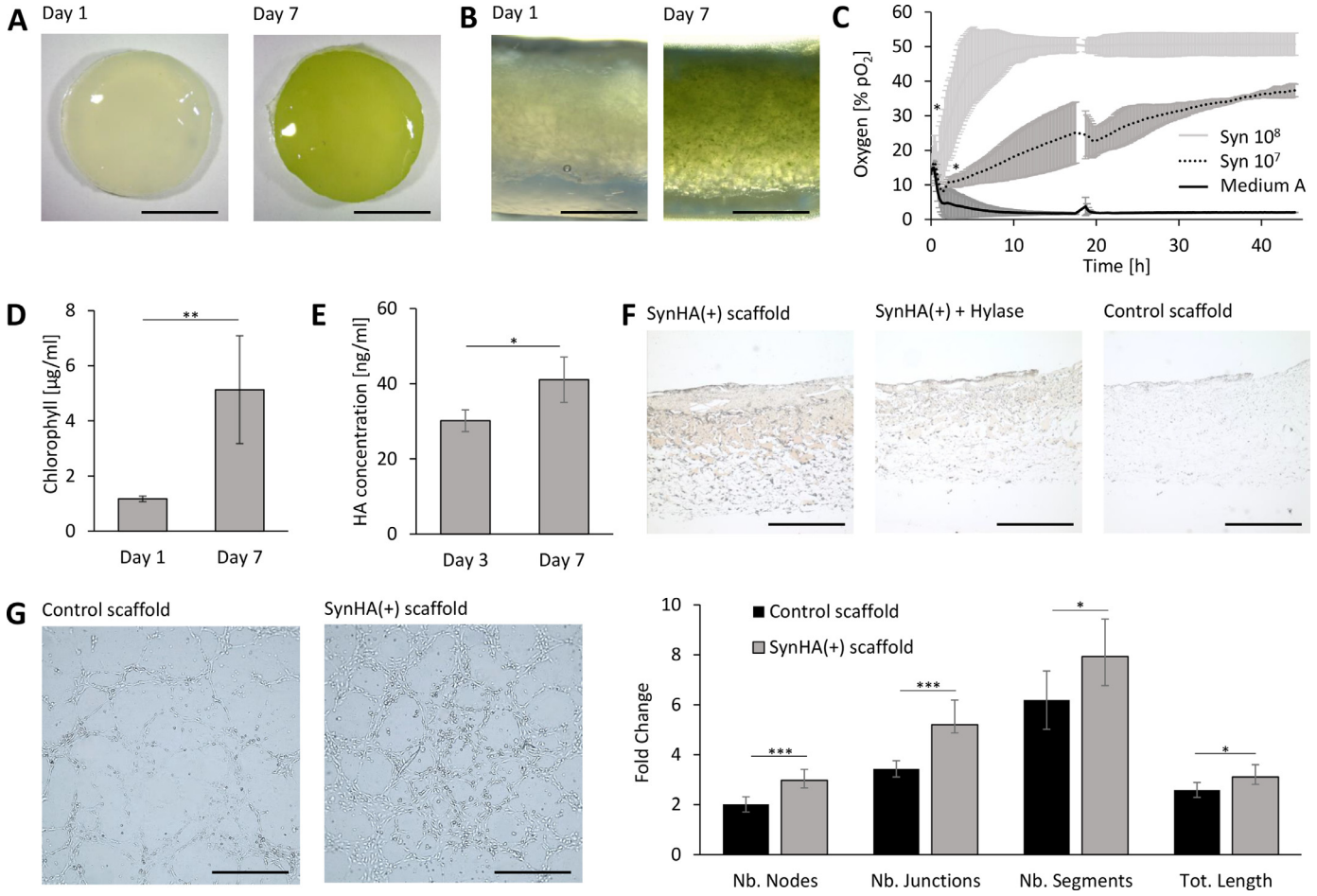

Fig. 4. Bio-compatibility of photosynthetic cyanobacteria with dermal scaffolds. A. The proliferation capacity of SynHA cells when seeded inside fibrin-collagen scaffolds was evaluated for 7 days. The overall green color of the scaffold at day 1 demonstrates a homogeneous distribution of the seeded of cyanobacteria throughout the scaffold-area. Further, the increase in the green color intensity over time suggests the proliferation of the cyanobacteria when encapsulated in the scaffold. Scale bar represents 5 mm. **B.** Cross-sectional images of a seeded scaffold after 1 and 7 days show a cell-density gradient along with the scaffold's depth, with most of the cells growing on the scaffold's surface, which is intended to be in contact with the wound. Scale bar represents 1 mm. **C.** Quantification of the chlorophyll content in the cyanobacteria-seeded scaffolds shows a significant increase after 7 days, supporting the proliferation of SynHA inside the fibrin-collagen scaffold **D.** Photosynthetic scaffolds released oxygen in correlation with the number of cyanobacteria seeded in the scaffolds, when cultured under hypoxic conditions and constant illumination ($n = 3$)al. **E.** The concentration of HA in the culture medium of SynHA(+)-seeded scaffolds was determined by ELISA after 3 and 7 days of cultivation. **F.** Immunohistological staining of HA confirmed the accumulation of hyaluronic acid in SynHA(+)-seeded photosynthetic scaffolds. Hylase: hyaluronidase-treated scaffolds. Scale bare represents 500 µm. Representative pictures are shown. **G.** LECs stimulated directly with HA released by SynHA(+)-seeded scaffolds were capable to form a more extensive lymph-vessel network compared to a control group without scaffold. Representative pictures are shown. Scale bare represents 500 µm. All experiments were performed in triplicate and are presented relative to the positive control. ($N \geq 3$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

photosynthetic oxygen supply on LECs viability was especially evident the longer the cells were incubated under hypoxic conditions (Fig. 1D). This could be a significant finding when translated into the setting of wound healing, as studies have shown that hypoxic stress leads to a temporary increase in cell proliferation [58], immune response activation [59] and contributes to the initiation of the healing process in wounds [60], but prolonged oxygen deprivation (as found in chronic wounds) has a net inhibitory effect on regeneration [60,61]. The consistent oxygen supply through photosynthetic SynHA could reinstate the normoxic environment in a wound and permit the progress of wound healing. However, since cell proliferation and the rate of collagen-synthesis are highly dependent on the skin oxygen levels [12], it will be necessary to optimize the SynHA-density in the scaffold to best match the requirements of the regenerating dermal tissue.

HA is a naturally occurring biopolymer that performs several functions in the frame of skin wound healing including moisture retention, space-filling and constitution of a framework for cell migration [62,63], and it is a key element for scar-free skin regeneration [23]. Also, it is involved in the response regulation of immune and epithelial cells, required for lymphocyte activation and homing [64], and systematic review studies support its effective use in the treatment of diabetic foot ulcers, burn injuries and chronic wounds [65–67]. Our results show that when cultured under standard human cell culture conditions (37 °C, 200 ml), SynHA can produce over 10 µg·ml^{-1} HA in 7 days, which, although less than was previously reported (100 µg/ml when supplemented with 1% CO_2, under high light intensity and vigorous shaking) [36], and the HA-concentrations used in commercially-available dermal fillers (20 mg/ml) [68], it approximates the range that is used to study LEC-stimulation [39]. Furthermore, the production and direct secretion of HA by SynHA-cyanobacteria provides the advantage of circumventing the need for extraction and purification, which is the case of animal and bacterial-derived HA [69], while decreasing the risk for animal pathogen contamination in the first case.

Remarkably, supplementing the LEC-culture medium with cyanobacteria-derived HA for 4 days potentiated the expression of lymphatic-vessel markers (Fig 2B, C) such as Prox-1, a transcription factor required for the development of the lymphatic system and budding of LECs from existing lymphatic vessels; LYVE-1, a lym-

phatic receptor that mediates HA-induced lymphangiogenesis; and VEGFR-3, considered a key signaling receptor for LECs survival, proliferation and migration as well as lymphatic sprouting [15,41,70]. Furthermore, cyanobacteria-derived HA supported the viability of LECs for 7 days when cultured under limited nutrient conditions, which to some extent might mimic the ischemic conditions of chronic wounds conditions (Fig. 3B) and reinforced the LECs' capacity for lymph vessel formation in an *in vitro* assay (Fig. 3C). These results corroborate the angiogenic potential of HA described before [71], even though the molecular weight of the HA produced by SynHA was estimated to be greater than 1 MDa [36], thus exceeding the size in HA molecules reported being angiogenic [39]. Considering the effect of the HA molecular-weight on inflammatory [64,72], angiogenic [73] and repair responses [74–76], the effect of cyanobacteria-derived HA on immune cells, blood endothelial cells and tissue-building dermal and epidermal cells needs to be addressed to better assess the regenerative potential of *Synechococcus* sp. PCC 7002-derived HA.

Our results demonstrated that SynHA cyanobacteria could grow inside a collagen-based DRM, which is approved for dermal repair by the US Food and Drug Administration (FDA), thus supporting the potential application of live cyanobacteria-photosynthetic biomaterials. SynHA retained their proliferation and photosynthetic capacity when encapsulated inside the scaffold, as demonstrated by the increase in chlorophyll content inside the scaffold (Fig. 4A, B) and the significant amount of oxygen (50% pO_2, 350 mmHg at 37 °C) released upon illumination. Thus, our measurements suggest that SynHA-seeded photosynthetic scaffolds could start promoting cell proliferation after 2 h (≥160 mmHg) and collagen synthesis after 4 h post-implantation (≥250 mmHg), according to the evidence-based recommendations for the use of topical oxygen therapy [12]. More importantly, SynHA-seeded scaffolds were capable to constantly release more than 30 ng/ml HA into the cultivation medium for up to 1 week (Fig. 4E) and promote the formation of lymph tubes *in vitro* after 24 h, which confirmed the continuous production of HA by living cyanobacteria and its efficient release through the fibrin-collagen scaffold into the medium.

Since lymphangiogenesis has been described as a transient process that peaks at around day 8 in the course of skin wound healing [43], SynHA(+) scaffolds would be a unique approach to stimulate and support the growing lymphatic vascular network through the simultaneous release of hyaluronic acid and oxygen at this early stage of the wound healing process. As SynHA(+) photosynthetic scaffolds already function as a production-delivery system for bioactive molecules, they could overcome the drawbacks related to hyaluronic acid concentration and purification processes that other approaches face. Also, it would be very interesting to explore the combination of cyanobacteria cells with three-dimensional bioprinting technologies [77,78], which could grant geometric versatility to photosynthetic approaches and have been shown to maximize the growth and cell viability of microalgae [79]. In addition, using SynHA-cyanobacteria in cell-laden bio-ink approaches could facilitate their application as tissue fillers in complex or large wounds along with other cells such as fibroblasts and keratinocytes, which have already been used in bioprinting systems [80].

Still, *in vivo* studies will need to address the immune response towards cyanobacteria-based photosynthetic scaffolds, as well as their net-effect on wound oxygenation to demonstrate their feasibility and effectivity. In this regard, increasing evidence supports the biocompatibility of photosynthetic cells with vertebrate organisms [32]. For instance, the immunogenicity of the lipopolysaccharides of *Synechococcus sp*. cyanobacteria has been described as very low [55,81], which supports their use as a safe host for biotechnological applications. Also, studies employing cyanobacteria to oxygenate internal organs like the heart, have demonstrated that systemic exposure to these microorganisms does not induce a significant immune response in rats [54,82]. If proven viable, cyanobacteria bioactivated biomaterials could offer a means for the controlled release of lymphangiogenic factors and oxygen-supplementation directly to the wound bed (Fig. 5). These biomaterials could promote the healing of chronic wounds and other skin pathologies related to hypoxia and pronounced inflammation, but also support other approaches such as lymph node transfer and lymphovenous anastomosis to promote the restoration of lymphatic vasculature and accelerate wound healing.

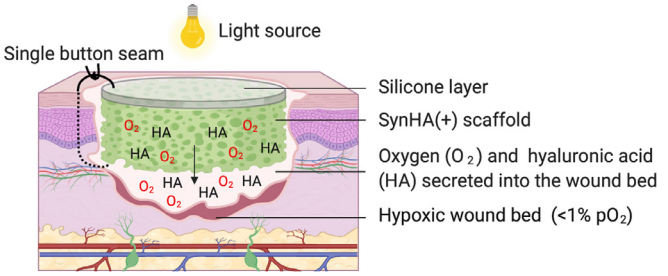

Fig. 5. Schematic representation of the pursued clinical application of photosynthetic SynHA(+)-scaffolds in the treatment of chronic wounds. Upon implantation, SynHA(+)-scaffolds may support the wound healing process by providing oxygen to the hypoxic wound bed and promoting the growth of lymph vessels (green) through the secretion of hyaluronic acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Disclosures

JTE is the founder and VP of Technology at SymbiOx Inc. This startup did not provide any financial support to this work but is closely related to some topics of this manuscript. Also, he is currently supervising an ongoing clinical trial (NCT03960164) to prove the safety of the implantation of photosynthetic scaffolds in wounds that result from trauma injuries.

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

The authors would like to thank Alexandra Birt for her technical assistance with primary cell culture and gene expression analysis and Lars Leibrock for proofreading this manuscript. This work was funded by the Deutsche Forschungsgemeinschaft (FOR2092 Ni390/9-2; SFB TRR175-A06).

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