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Plants as a cost-effective source for customizable photosynthetic wound dressings: A proof of concept study

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Abstract

Oxygen is essential for tissue regeneration, playing a crucial role in several processes, including cell metabolism and immune response. Therefore, the delivery of oxygen to wounds is an active field of research, and recent studies have highlighted the potential use of photosynthetic biomaterials as alternative oxygenation approach. However, while plants have traditionally been used to enhance tissue regeneration, their potential to produce and deliver local oxygen to wounds has not yet been explored. Hence, in this work we studied the oxygen-releasing capacity of *Marchantia polymorpha* explants, showing their capacity to release oxygen under different illumination settings and temperatures. Moreover, co-culture experiments revealed that the presence of these explants had no adverse effects on the viability and morphology of fibroblasts in vitro, nor on the viability of zebrafish larvae in vivo. Furthermore, oxygraphy assays demonstrate that these explants could fulfill the oxygen metabolic requirements of zebrafish larvae and freshly isolated skin biopsies ex vivo. Finally, the biocompatibility of explants was confirmed through a human skin irritation test conducted in healthy volunteers following the ISO-10993-10-2010. This proof-of-concept study provides valuable scientific insights, proposing the potential use of freshly isolated plants as biocompatible low-cost oxygen delivery systems for wound healing and tissue regeneration.

KEYWORDS
Marchantia polymorpha, oxygen, photosynthetic biomaterial, wound healing

1 | INTRODUCTION

Wound healing is a complex and highly regulated process that aims to restore the structure and function of damaged tissues. It involves four major overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling (Guo & Dipietro, 2010; Hamdan et al., 2017; Han & Ceilley, 2017). Given its clinical relevance, the development of novel wound dressings with enhanced bioactive properties is an active field of research (Han et al., 2023; Hawthorne et al., 2021).

Oxygen has been widely described as a critical molecule in wound healing due to its major role in key processes including cell metabolism, collagen deposition, epithelialization, angiogenesis, and immune response (Guo & Dipietro, 2010; Han & Ceilley, 2017). Therefore, several studies have addressed the need for local oxygen supply (Kimmel et al., 2016; Liu et al., 2023; Tejada et al., 2019). Consequently, various approaches have been explored to promote wound healing by developing oxygen-releasing biomaterials. For instance, some dressings have been

designed to provide oxygen through the decomposition of chemical reagents such as calcium peroxide (Steg et al., 2015), hydrogen peroxide (Ng et al., 2010), and sodium percarbonate (Ward et al., 2013), while other studies have employed carriers to release oxygen into wounds (Camci-Unal et al., 2013; Farris et al., 2016; Lim & Jang, 2021). Despite their promising properties, these approaches have shown issues such as local toxicity by hyperoxia and poor stability by failing to provide oxygen in a constant and controlled manner (Ashammakhi et al., 2020; Han et al., 2023). Hyperbaric oxygen therapy has also been investigated as a mean to provide oxygen to wounds; however, clinical results remain controversial, therefore, further studies are needed to obtain conclusive data (Al-Jalodi et al., 2022; Kimmel et al., 2016; Moreira et al., 2022).

As an alternative to the aforementioned strategies, the development of photosynthetic biomaterials has emerged as a promising approach for delivering oxygen to wounds (Ma et al., 2023). This concept relies on the combined use of conventional biomaterials and living photosynthetic microorganisms capable to release oxygen in a localized and controlled manner upon illumination (Hopfner et al., 2014). These biomaterials have shown promising results for wound healing, being validated in vitro (Hopfner et al., 2014), in vivo (Chávez et al., 2016; Schenck et al., 2015), and most recently in human patients (Obaíd et al., 2021, 2022). While the studies mentioned above focused on the use of scaffolds for dermal regeneration, recent articles have also described the potential use of photosynthetic dressings for wound treatment. For instance, hydrogel patches loaded with *Synechococcus elongatus* have shown to deliver significant levels of oxygen, thereby promoting cell division, migration, and tube formation in vitro, as well as enhancing skin graft survival and chronic wound healing in diabetic mice (Chen et al., 2020). Similarly, *S. elongatus* has been incorporated into hyaluronic acid, resulting in a topical gel that accelerated wound healing in ischemic wounds in mice (Zhu et al., 2022). In addition, alginate hydrogels containing *Chlamydomonas reinhardtii* has been shown to fulfill the metabolic oxygen requirements of zebrafish larvae and freshly isolated skin biopsies, while also exhibited high biocompatibility in in vitro and in vivo settings, as well as in healthy volunteers (Corrales-Orovio et al., 2023). Moreover, in vitro assays demonstrated that hydrogels with living *Chlorella* could produce oxygen, consume glucose, and reduce reactive oxygen species under light exposure, and being inactivated in darkness promoting diabetic wound healing (Wu et al., 2023). Similarly, chitosan-coated *Spirulina platensis* have shown to produce oxygen in infected wounds for wound healing (Li et al., 2021).

As described above, significant efforts have been made towards the development of photosynthetic biomaterials to promote wound healing. However, a central challenge in this field lies in optimizing and integrating biomaterials with living photosynthetic cells, which represents the need for extensive research efforts to develop and translate these dressings into clinical settings.

Considering that plants have evolved highly sophisticated photosynthetic structures that fit with some of the multiple requirements for an optimal oxygen-releasing wound dressing, this study explore for the first time the concept that freshly isolated photosynthetic explants could serve as a natural source for customizable and cost-effective photosynthetic dressings for the in situ delivery of oxygen to wounds.

In this study, *Marchantia polymorpha* was chosen because it represents a well-described model organism in plant biology and it is easy to grow in the lab (Bowman et al., 2017; Chang et al., 2016; Shimamura, 2016). Additionally, has a short and haploid life cycle, a small and sequenced genome, and several genetic tools are available to generate transgenic lines (Ishizaki et al., 2016; Pollak et al., 2019).

2 | MATERIALS AND METHODS

2.1 | *M. polymorpha* culture

M. polymorpha gemmalings were cultured aseptically in hydroponic medium using half-strength Gamborg's B5 medium (Gamborg et al., 1968) at 0.16% wt/vol (Phytotech labs), MES 0.05% wt/vol (Phytotech labs), and sucrose 1% wt/vol (Phytotech labs), adjusting pH to 5.5 with KOH 1 M (Winkler, Chile). Agar 1% wt/vol (Winkler, Chile) was used when required. After 2–3 weeks, haploid plants were transferred and cultured onto moist soil composed of peat, perlite, and vermiculite at a ratio of 3:1:1% vol/vol, respectively, for another 2–3 weeks and used for the experiments. All plant cultures were carried out at 22°C under light/dark cycles of 16/8 h, respectively, using a white led light (50–60 µmol m^{-2} s^{-1}), unless otherwise specified. All these protocols have been previously described and were slightly adapted for this study (Ishizaki et al., 2008; Pollak et al., 2019).

2.2 | Metabolic activity of *M. polymorpha*

For oxygen production and consumption measurements, ≈100 mg explants of *M. polymorpha* were placed in the electrode chamber of the Oxygraph+ system (Hansatech Instruments) and covered with 2 mL of either hydroponic media or saline (NaCl 0.9%). Then, the explants were subjected to dark/light cycles of 15 min with increasing intensities of light (455 nm) at three different temperatures: 25°C as the standard temperature, 37°C as the physiological wound temperature, and 31°C as their mean temperature. Oxygen metabolic rates were calculated from the slopes of the obtained curves and normalized using the explant weight. In all experiments, a custom LED equipment (Sky-Walkers Spa, Chile) was used for illumination (455 nm). Low, medium and high illumination intensities were studied applying 18.3, 36.6, and 54.9 µmol m^{-2} s^{-1}, respectively.

2.3 | Zebrafish breeding

Zebrafish embryos (*Danio rerio*, TAB5 strain) were obtained from our zebrafish facility as described before (Alvarez et al., 2015).

Briefly, all embryos were collected by natural spawning and were raised and maintained at 28.5°C in an incubator with 14–10 h of light-dark cycles in E3 media (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.3 mM $MgSO_4$) and adjusted to pH 7.0.

2.4 | Metabolic coupling assays

For the in vivo assays, 20 zebrafish larvae at 96 h post-fertilization (hpf) were introduced into the oxygraph chamber, covered with 2 mL of hydroponic media, and maintained at 25°C. After 10 min in darkness, light was applied for another 10 min. Then, ≈100 mg of *M. polymorpha* explants were introduced in the chamber and kept under illumination for 20 min followed by 20 min of darkness. For ex vivo assays, fresh skin biopsies (≈36 mm^2) were isolated from the abdomen of Wistar rats discarded from another experiment performed under an approved ethical protocol (CEC-CAA 190613020). Here, biopsies were introduced into the oxygraph chamber and covered with 1 mL of saline (NaCl 0.9%), maintaining a temperature of 31°C and subjected to 5 min of darkness followed by 5 min of illumination. Afterwards, *M. polymorpha* explants of equal surface (≈36 mm^2) were introduced and exposed to 10 min of light followed by 10 min of darkness. In both experiments, a 455 nm light was applied at 54.9 µmol m^{-2} s^{-1} of intensity using a custom-made illuminator (Sky-Walkers Spa, Chile) and oxygen metabolic rates were calculated from the slopes of the curves obtained.

2.5 | Cell co-culture with *M. polymorpha* explants

Embryonic mouse fibroblasts cells (3T3) were maintained under standard cell culture conditions (37°C, 5% CO_2) in high glucose Dulbecco's modified Eagle medium (DMEM) (Biological Industries) supplemented with 10% fetal bovine serum (Pan Biotech) and 1% of penicillin/streptomycin (Sigma). Twenty-four hours after seeding, culture media was replaced by a mixture of DMEM and hydroponic media at a ratio of 4:1, supplemented with 1% of penicillin/streptomycin (Sigma) and 2.5 µg/mL of amphotericin-B (Biological Industries). Then, ≈50 mg of *M. polymorpha* explants were placed on top in 8 µm transwell inserts for 24 h under standard culture conditions (37°C, 5% CO_2). For controls, 3T3 cells were subjected to the same conditions but in the absence of the explants.

2.6 | Metabolic assays

MTT metabolic assay was performed following the manufacturer's instructions (Abcam). Briefly, 2×10^5 3T3 cells were seeded on 12-well plates. After 24 h of culture, these cells were co-cultured with ≈50 mg of *M. polymorpha* explants as mentioned in 2.5. Then, *M. polymorpha* explants and cell culture media was removed and replaced by 1 mL of DMEM containing 500 µM MTT (Sigma). Then, cells were incubated for 2 h and 500 µL of dimethyl sulfoxide (DMSO) (Sigma) was added, followed by 45 min of incubation under agitation at room temperature. Finally, 200 µL of DMSO containing formazan blue were transferred to a 96-well plate, and absorbance was measured at 570 and 650 nm.

2.7 | Cell morphology

After 24 h of co-culture, *M. polymorpha* explantes were removed and 3T3 cells were washed once with phosphate buffered saline (PBS) Ca^{2+}/Mg^{2+}, fixed with 4% paraformaldehyde (Sigma), and then incubated with 0.1% Triton X-100 (Winkler, Chile) in PBS for 10 min. Next, cells were stained with 1 µg/mL Hoechst (Life Technologies) and 0.17 µM Phalloidin-AF546 (Life Technologies) for 40 min at RT in darkness. Images were obtained using a fluorescence microscope (Leica DM500) equipped with a standard digital camera (MShot MS60).

2.8 | In vivo biocompatibility assays

For acute assays, 10 larvae at 96 hpf were placed in a 12-well plate and covered with 5 mL of either E3 media, hydroponic media or a mixture of both at a 1:1 ratio, supplemented with 1% of penicillin/streptomycin (Sigma) and 2.5 µg/mL of amphotericin-B (Biological Industries). *M. polymorpha* explants were then added into the wells in direct contact with the zebrafish and co-incubated for 24 h. Afterward, the larvae were anesthetized in 4.2% (wt/vol) tricaine (Sigma), mounted in 1% low-melt point agarose (Winkler, Chile), and imaged using a stereoscope (Leica S6D) equipped with a digital camera. Survival was determined by observing the presence or absence of heartbeats during 30 s. For the chronic assay, the same approach was followed, but this time 10 embryos at 24 hpf were incubated with the explants in hydroponic media for 96 h and viability was daily measured.

2.9 | Human skin irritation test

The skin irritation test was conducted following previously established methods (Corrales-Orovio et al., 2023), in accordance with ISO-10993-10-2010 guidelines, and a protocol approved by the Scientific Ethics Committee for Health Sciences at Pontificia Universidad Católica de Chile (Approval No.: 220128002). Following ISO-10993-10-2010 guidelines, 5 healthy male and 5 healthy female volunteers, aged between 25 and 34 years, were selected for the skin irritation test. None of the female volunteers were pregnant or breastfeeding at the time of the test. Briefly, *M. polymorpha* explants (≈100 mg) or gauze moistened with bi-distilled water were placed in the forearms of the 10 healthy volunteers, and covered with a transparent commercial dressing,

capable of maintaining humidity to promote wound healing (Tegaderm Film, 3M) that was also used as control. After 4 h in contact with the skin, the explants or gauze were removed, and a qualified nurse assessed the Score of Primary Irritation (SPI) at 0, 1, 2, 24, 48, and 72 h. The Primary Irritation Index (PII) was calculated as the mean of the SPI values from all volunteers and categorized as follows: negligible (0–0.2), slightly irritating (0.3–0.9), moderately irritating (1–2.7), and severely irritating (2.8–4). Additionally, all volunteers completed a self-evaluation questionnaire to assess pain (0–10), itching (0–3), burning (0–3), and skin palpitation (0–3) at the contact sites. Furthermore, the recovered *M. polymorpha* explants were weighed to analyze dehydration and overall integrity.

2.10 | Statistical analysis

All experiments were performed in at least three independent assays. For graphics and statistical analyses, GraphPad Prism 8 software was used. Error graphs correspond to standard deviation (*SD*) and data comparison was analyzed by *t*-test, one-way or two-way analysis of variance with Tukey's multiple comparison as a secondary test. Significance was considered at $p \leq 0.05$ and all details are specified in each figure legend.

3 | RESULTS

3.1 | Oxygen metabolism of *M. polymorpha* explants

M. polymorpha explants in hydroponic media or saline were subjected to three different temperatures and light intensities, showing that under all these conditions the explants were able to produce oxygen during illumination and consume it in the darkness. At 25°C oxygen concentration ranged from 231.29 to 339.67 nmol mL^{-1} using hydroponic media, while using saline the oxygen concentration was slightly lower ranged from 224.48 to 280.31 nmol mL^{-1} (Figure 1a). At 31°C oxygen concentration decreased from 199.75 to 297.09 nmol mL^{-1} using hydroponic media, while the oxygen concentration using saline was also slightly lower ranged from 145.79 to 221.06 nmol mL^{-1} (Figure 1b), and, finally, at 37°C oxygen concentration ranged from 112.92 to 203.32 nmol mL^{-1} using hydroponic media, while using saline oxygen concentration ranged from 139.86 to 193.15 nmol mL^{-1} (Figure 1c), showing that oxygen concentration decreased while temperature rose as well described before in aqueous solutions (Xing et al., 2014). In addition, results showed that both at 25°C and 37°C production rates were significantly higher using hydroponic media than saline regardless the intensity of light (Figure 1a′ and 1c′). Similarly, at 25°C and 37°C the oxygen consumption showed a tendency to increase in hydroponic media, being significant at high light intensities at 25°C (Figure 1a″), and at medium and high light intensities at 37°C (Figure 1c″). In contrast, when experiments were performed at 31°C the oxygen production (Figure 1b′) and consumption (Figure 1b″) rates were not affected by the culture media or light intensity.

3.2 | Biocompatibility of *M. polymorpha* explants

After demonstrating the capability of *M. polymorpha* explants to produce and consume oxygen under different experimental conditions, their biocompatibility was studied in vitro and in vivo. To assess the in vitro biocompatibility of *M. polymorpha* explants, a co-culture with 3T3 cells was established, allowing for the exchange of soluble factors through trans-well inserts. After 24 h, morphological differences in the groups were observed, as co-cultured cells exhibited a more elongated shape compared to the control group (Figure 2a). However, no significant differences were observed in the metabolic activity of both groups (Figure 2b). Finally, macroscopic analysis revealed that the overall appearance of the *M. polymorpha* explants remained unchanged after co-culture, preserving their integrity and characteristic green color (Figure 2c).

Subsequently, the biocompatibility of *M. polymorpha* explants was assessed in vivo through acute and chronic assays using zebrafish larvae. In the acute assay, larvae at 96 hpf were incubated with *M. polymorpha* explants in either E3 or hydroponic media, as well as a 1:1 mixture of both. After 24 h, the general morphology of the zebrafish larvae remained unaffected, and no evident signs of damage, such as pericardial edema or changes in eye size, were observed (Figure 2d). Furthermore, no overall alterations in the morphology of *M. polymorpha* explants were detected after interaction with zebrafish larvae (Figure 2d). Additionally, the survival of zebrafish larvae remained unaffected across all conditions, indicating that *M. polymorpha* explants were biocompatible with zebrafish larvae (Figure 2e).

Finally, for the chronic assay, zebrafish embryos at 24 hpf were co-incubated with *M. polymorpha* explants for 96 h under both dark and light conditions. Similar to the acute assays, the morphology (Figure 2f) and viability (Figure 2g) of the zebrafish larvae, and the appearance of the explants were unaltered by the co-incubation.

3.3 | Functional oxygenation capacity of the *M. polymorpha* explants

Once the biocompatibility of the *M. polymorpha* explants was established, their capacity to fulfil the oxygen demand of highly metabolically active biological systems was evaluated. For in vivo assays, zebrafish larvae were placed within an oxygraph chamber, and oxygen concentration was measured both with and without light and explants (Figure 3a,b).

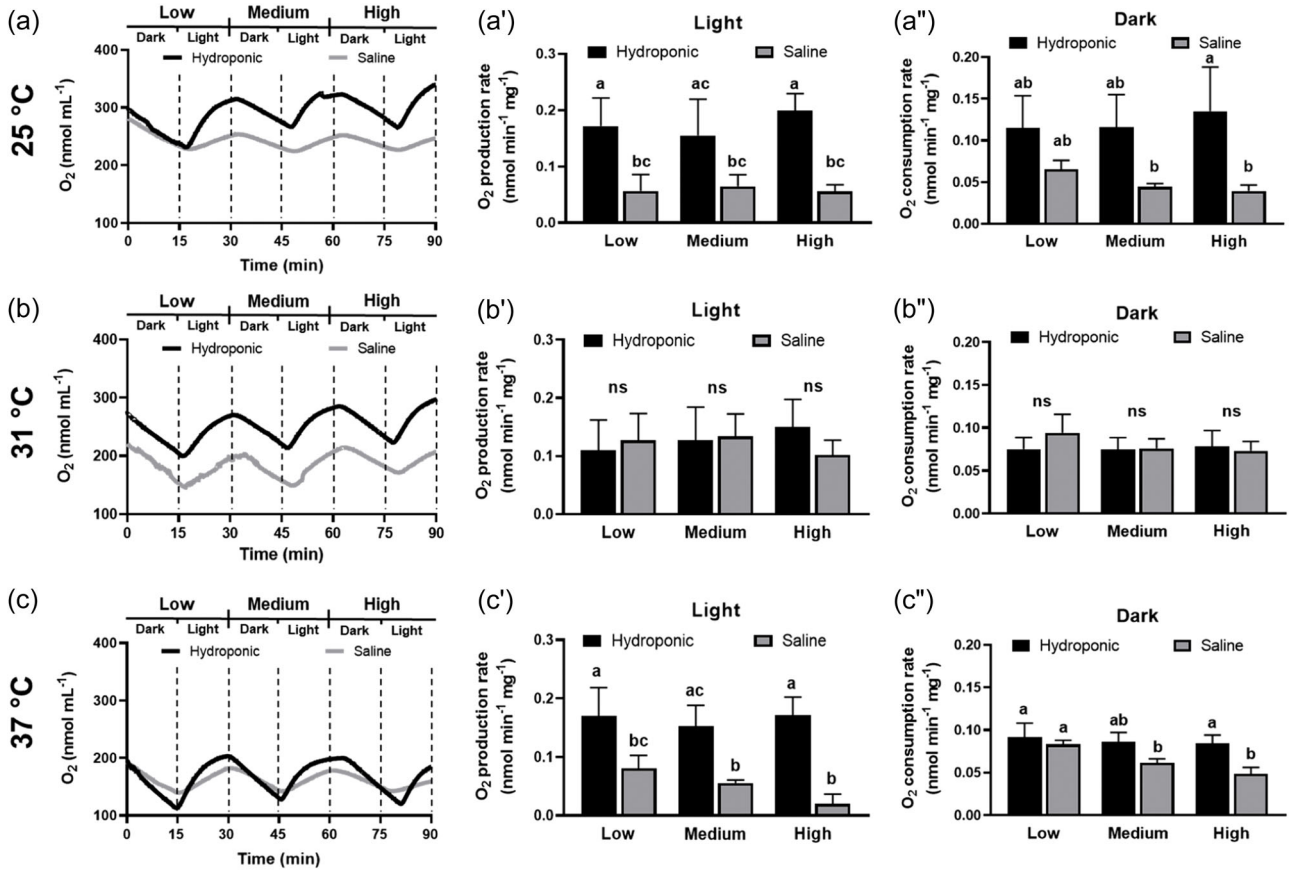


FIGURE 1 Oxygen metabolism of *Marchantia polymorpha* explants under different conditions. Oxygen production and consumption were assessed in explants exposed to temperatures of 25°C (a), 31°C (b), and 37°C (c), in hydroponic or saline media and three distinct light intensities (low, medium, and high) Oxygen production (a′, b′, & c′) and consumption (a″, b″, & c″) rates were quantified in the presence or absence of light. The data represent at least three independent experiments and are presented as mean + *SD*. Statistically significant differences ($p \leq 0.05$) between conditions were determined using a two-way ANOVA followed by Tukey's test, with different letters indicating significance. Low, medium, and high light intensities correspond to 18.3, 36.6, and 54.9 µmol m^{-2} s^{-1}, respectively. ANOVA, analysis of variance.

In the absence of explants, oxygen measurements showed a constant decrease, regardless of the light conditions (Figure 3c, I and II), and no significant differences were observed in the metabolic rates (Figure 3d, I and II). In contrast, in the presence of *M. polymorpha*, a steady increase in the oxygen concentration was observed in the presence of light (Figure 3c, III), while its absence led to a significant decrease in the oxygen metabolic rate (Figure 3d, IV).

Subsequently, a similar experimental setup was used to study the explants' ability to meet the oxygen demands of skin biopsies (Figure 4a,b). Here the metabolic interaction between freshly isolated rat skin samples and *M. polymorpha* explants of same area was studied. Similar to the experiments with zebrafish larvae, the skin samples showed constant oxygen consumption regardless of the presence or absence of light (Figure 4c, I and II), as confirmed by calculating the oxygen consumption metabolic rates (Figure 4d, I and II). Upon introducing the *M. polymorpha* explant into the chamber, its oxygen production capacity exceeded the metabolic requirement of the skin (Figure 4d, III), while oxygen concentration decreased in darkness (Figure 4c, IV). Interestingly, in contrast to the experiments performed with zebrafish larvae, no significant differences in the oxygen consumption metabolic rate were observed when comparing the skin sample alone or combined with the explants (Figure 4d, IV).

3.4 | Human skin irritation test

Given that the primary focus of this proof-of-concept study is the potential use of photosynthetic explants as bioreactors for oxygenating human wounds, the biocompatibility of *M. polymorpha* with human skin was assessed. Therefore, a human skin irritation test was conducted on healthy volunteers in accordance with ISO-10993-10-2010 guidelines (Figure 5a).

The results indicate that volunteers did not experience irritation at the different analyzed time points (Figure 5b). However, at the 0-h following removal, volunteers 1 and 3 showed irritation in the skin area that was in contact with gauze and a commercial dressing used as control. Additionally, volunteer 4 showed a very slight irritation to the gauze from 2 h until the end of the assay (Figure 5b). The calculated PIIs were −0.033 and 0.083 for *M. polymorpha* explants

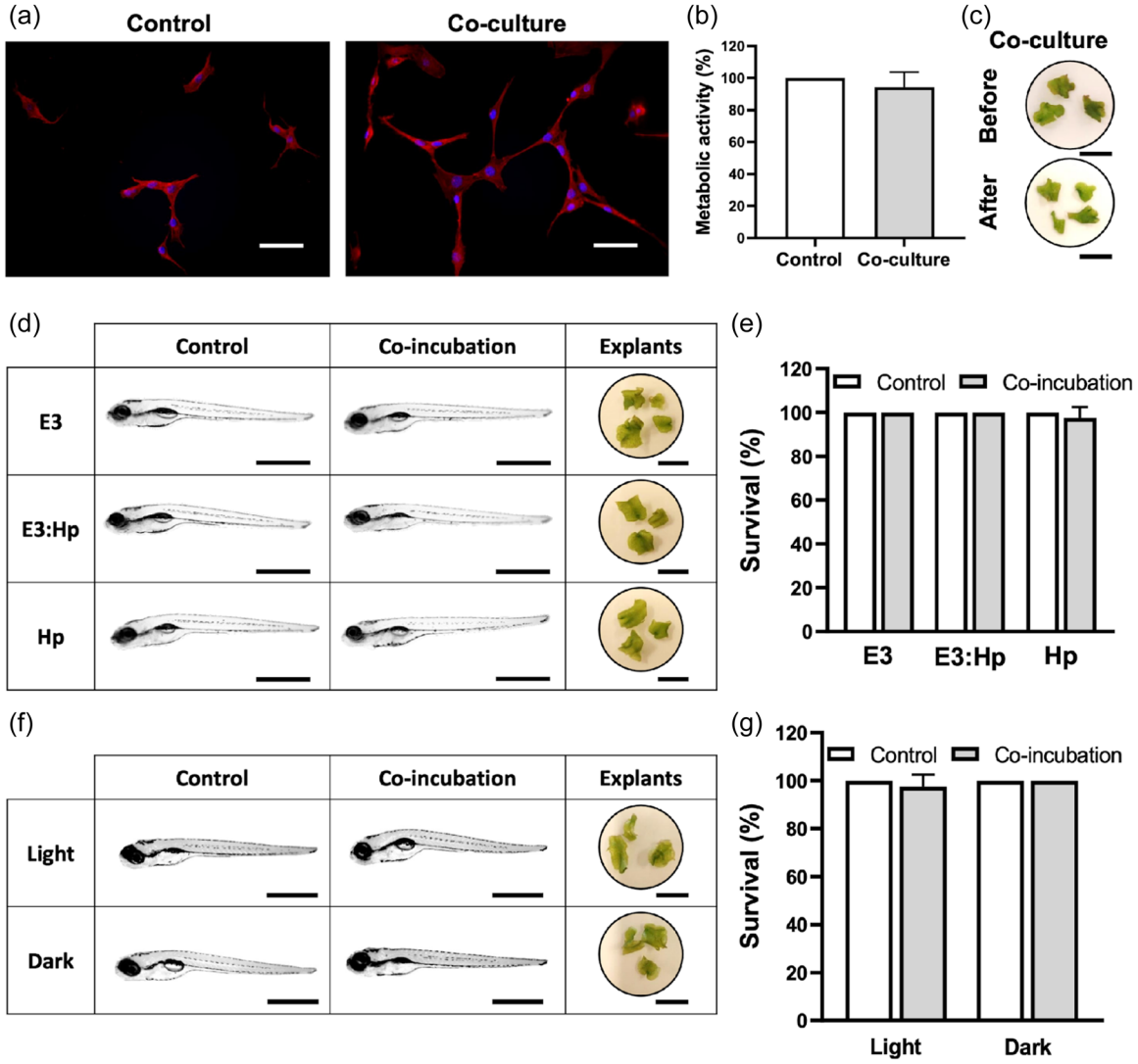


FIGURE 2 Biocompatibility of *Marchantia polymorpha* in vitro and in vivo: For the in vitro experiments, 3T3 cells were co-cultured with *M. polymorpha* (*M.p.*) explants, and cell morphology (a) and metabolic activity (b) were evaluated by cell staining (phalloidin/red and Hoechst/blue) and MTT assays, respectively, after 24 h of co-culture. Representative images of *M. p.* explants before and after co-culture are shown (c). For the acute in vivo assays, zebrafish (Z.f.) larvae were co-incubated with *M.p.* explants in larvae media (E3), E3 and Hydroponic media (E3: Hp) and hydroponic media alone. The overall morphology of the larvae and explants (d) and the viability of the Z.f. (e) were evaluated after 24 h of co-culture. For chronic exposure, similar experiments were performed in the presence or absence of light and explants, and the overall morphology of larvae and explant (f) and Z.f viability (g) were evaluated after 96 h of co-culture. The data represent at least three independent experiments and are presented as mean + *SD*. Statistically significant differences ($p \leq 0.05$) between conditions were determined using an unpaired *t*-test; $N = 3$ (b), one-way ANOVA followed by Tukey's test; $N = 8$, $n = 200$ (e) and $N = 4$, $n = 80$ (g). Scale bar represents 50 μm in (a) and (b), 1 cm for *M.p.* explants in (c), (d) and (f), and 1 mm for Z.f larvae in (d) and (f). ANOVA, analysis of variance.

and gauze, respectively (Figure 5b), indicating that the explant irritability was even lower than a standard traditional wound dressing. Furthermore, while most of the volunteers' self-evaluation for the *M. polymorpha* explants did not indicate any signs of pain, itch, palpitations, or burning sensations, volunteers 1 described itching at the skin site exposed to controls dressing and gauze and, similarly, volunteer 9 experienced itching at the gauze site (Figure 5c).

Finally, *M. polymorpha* explants were recovered showing a weight reduction slightly above 50% of their initial mass (Figure 5d), exhibiting a softer and pale appearance (Figure 5e).

4 | DISCUSSION

Photosynthetic therapies aim to provide oxygen to tissues in a blood-independent manner. This concept relies on the local implantation of photosynthetic microorganisms and has been previously studied for various clinical applications (Yang et al., 2023), including tumor treatment (Agarwal et al., 2021), organ preservation (Veloso-Gimenez et al., 2021), ischemic heart (Cohen et al., 2017), and peripheral artery disease (Zhu et al., 2022). Additionally, several independent research groups have proposed

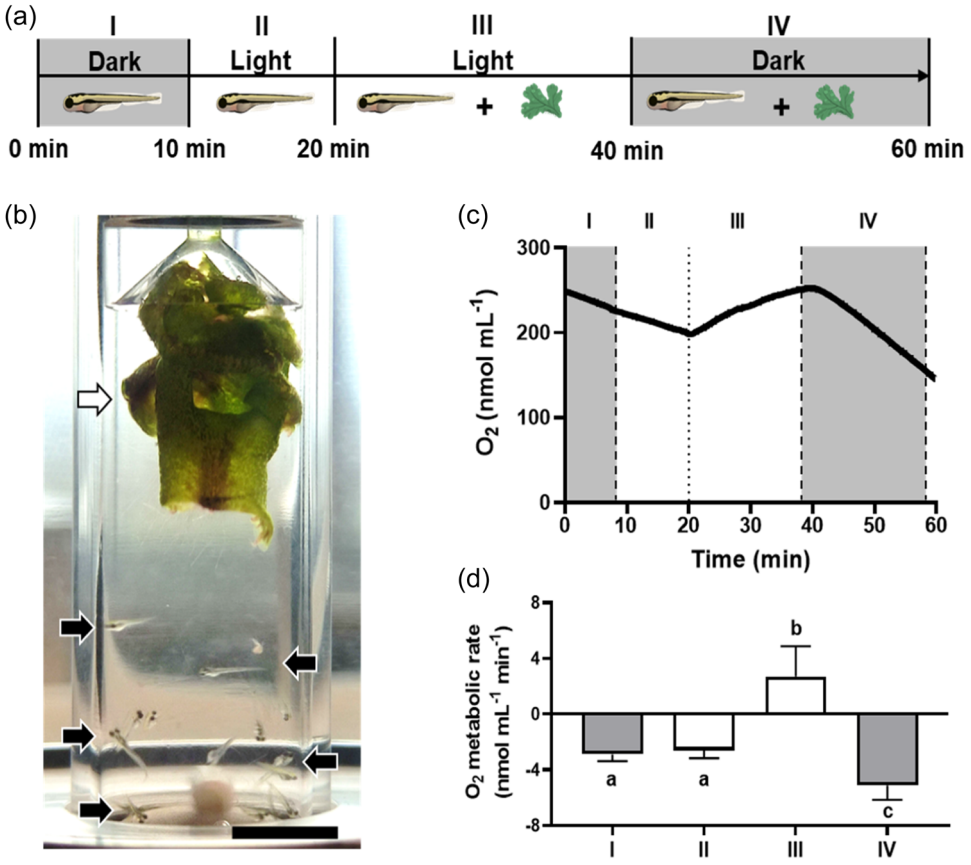


FIGURE 3 Oxygenation capacity of *Marchantia polymorpha* explants in vivo. Oxygen consumption of zebrafish larvae was measured for 10 min in the absence (I, Dark) or presence of light (II, Light). Then, explants were introduced, and oxygen evolution was measured for 20 min with (III, Light) or without (IV, Dark) light (a). An overall view of the experimental setting in the oxygraphy chamber is shown (b). Oxygen concentration was recorded over time (c), and the metabolic rates were compared (d). Data are presented as mean + *SD*, with $N = 10$. Different letters indicate significant differences at $p \leq 0.05$, determined by one-way ANOVA followed by Tukey's test. The white arrow indicates the explant, while black arrows indicate the larvae, with the scale bar representing 0.5 cm in (b). ANOVA, analysis of variance.

the use of photosynthetic biomaterials as bioactive dressings for wound healing (Ma et al., 2023).

An optimal dressing should exhibit certain characteristics for adequate wound regeneration, including preventing microbial contamination, maintaining proper moisture, and being comfortable, painless, and cost-effective (Deutsch et al., 2017). Therefore, the development of photosynthetic dressings requires a biocompatible scaffolding for the photosynthetic microorganisms (Chen et al., 2020; Corrales-Orovio et al., 2023; Li et al., 2023; Zhao et al., 2023). This requirement translates into additional preparation steps, increasing cost, and potentially affecting their clinical use.

Interestingly, some plant tissues resemble those photosynthetic dressings as they consist of photosynthetic cells, embedded in a natural biocompatible scaffold. Although plants have traditionally been used to promote wound healing through different means, to the best of our knowledge, no studies have described their potential use as an oxygen source for wound healing. For instance, banana tree leaves and potato peels have been employed as wound dressings in burn patients due to their mechanical properties and physical structure (Gore & Akolekar, 2003a, 2003b; Guenova et al., 2013; Mulukutla & Kale, 2020) and, during the First World War, sphagnum moss was widely used to cover wounds, due to its swelling capabilities (Hotson, 1921; Riegler, 1989). Moreover, from a chemical perspective, plants also contain bioactive molecules that have been locally applied for centuries to improve the wound healing process (Sharma et al., 2021; Yazarlu et al., 2021). In addition, several structural components of plants, such as cellulose, are commonly used for the fabrication of wound dressings (Tarrahi et al., 2022). Moreover, decellularized plant-based scaffolds have also been tested for tissue regeneration and regeneration (Bilirgen et al., 2021; Mutra et al., 2023).

Interestingly, the results presented here demonstrate that *M. polymorpha* explants could generate oxygen independently of the media and temperature. As expected, such oxygen concentration decreases while temperature rises, which is a well described phenomenon in for dissolution of gases in aqueous solutions (Xing et al., 2014). Nevertheless, oxygen also diminishes its solubility as electrolytes rise its concentration, which was not observed here, where hydroponic media showed a slightly higher oxygen concentration at 25°C and 31°C than saline. Further studies should be conducted to understand these differences among these medium to improve the oxygen production.

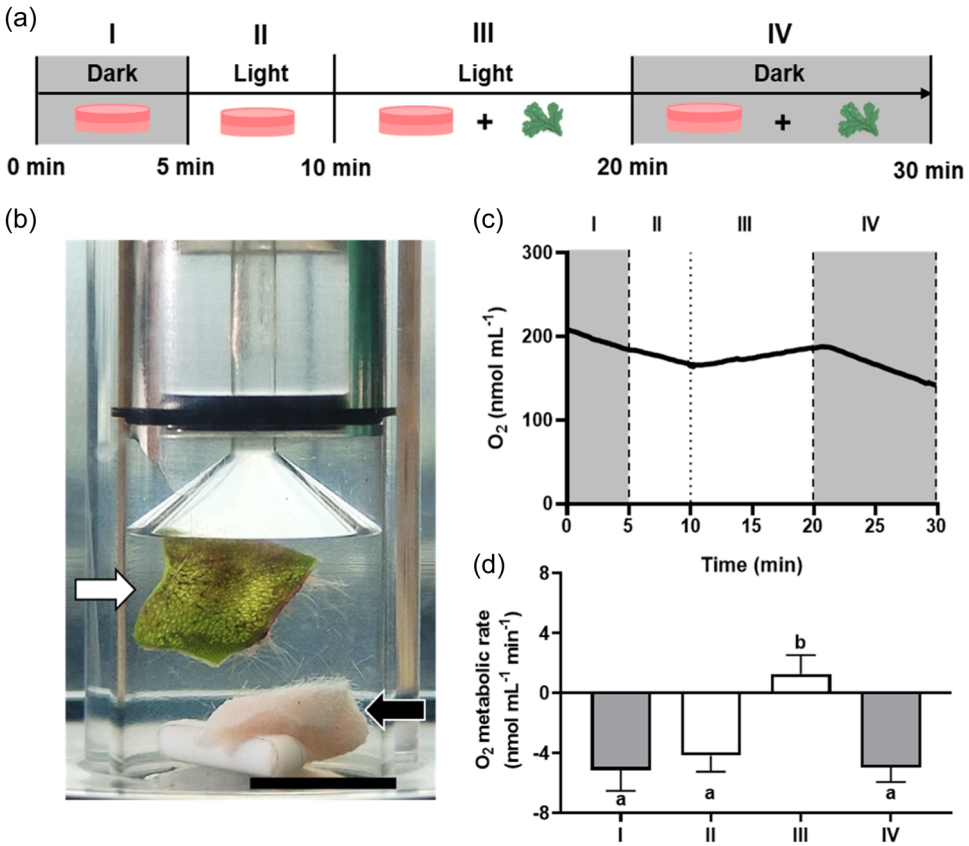
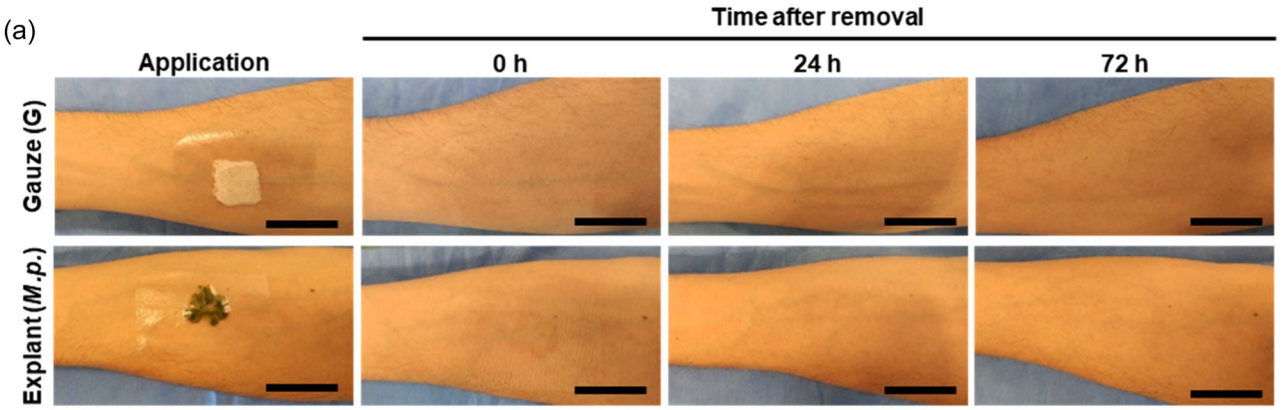


FIGURE 4 Oxygenation capacity of *Marchantia polymorpha* explants ex vivo. Oxygen consumption of freshly isolated skin biopsies was measured for 5 min in the absence (I, Dark) or presence of light (II, Light). Then, explants were introduced and oxygen evolution was measured for 10 min with (III, Light) or without (IV, Dark) light (a), using an oxygraph chamber (b). Oxygen concentration was recorded over time (c), and the metabolic rates were compared (d). Data are presented as mean + *SD*, with $N = 9$. Different letters indicate significant differences at $p \leq 0.05$, determined by one-way ANOVA followed by Tukey's test. White and black arrows in (b) indicate the explant and skin biopsy, respectively, with the scale bar representing 0.5 cm. ANOVA, analysis of variance.

Additionally, *M. polymorpha* explants could produce sufficient oxygen to fulfill the demands of highly metabolically active systems such as zebrafish larvae and fresh skin biopsies. This finding is intriguing, given that the explants maintained their functionality even when exposed to temperature and media conditions that significantly vary from their optimal requirements. However, these results align with other previously published works, where photosynthetic microorganisms demonstrated their capability to sustain the oxygen demands of other in vitro and in vivo systems (Chen et al., 2020; Zhao et al., 2023; Zhu et al., 2022) as well as mouse brain slides ex vivo (Voss et al., 2021).

A key outcome of this study is the observed biocompatibility of *M. polymorpha* explants in vitro, in vivo and in healthy volunteers. For the in vitro studies, fibroblasts were used as they represent one of the main skin cell types. In the in vivo tests, zebrafish larvae were chosen due to their well-established status as a model for biomedical toxicity (Choi et al., 2021), having been previously employed to assess the biocompatibility of other photosynthetic biomaterials such as hydrogels (Corrales-Orovio et al., 2023) and perfusable solutions containing the microalgae *C. reinhardtii* (Veloso-Giménez et al., 2021).

Once again, these results agree with previous studies on photosynthetic therapies where hydrogel patches were loaded with *Synechococcus elongatus* to deliver significant levels of oxygen, promoting cell division, migration, and tube formation in vitro, as well as enhancing skin graft survival and chronic wound healing in diabetic mice (Chen et al., 2020). Similarly, *S. elongatus* has been incorporated into hyaluronic acid, resulting in a topical gel that accelerated wound healing in ischemic wounds in mice (Zhu et al., 2022). In addition, microneedles loaded with *Chlorella vulgaris* promote cell proliferation, migration, and angiogenesis and enhance wound healing in diabetic mice effectively by providing oxygen directly into the wound (Zhao et al., 2023). However, the biocompatibility of *M. polymorpha* within a preclinical context had not been studied. Likewise, when the explants were tested in healthy volunteers using a previously established skin irritation protocol (Corrales-Orovio et al., 2023), no adverse effects were observed. In fact, despite the rough surface of *M. polymorpha* (Shimamura, 2016), results showed that the explants were even less irritating than a commonly used commercially available dressing. Nevertheless, it is important to note that it is well known that several plants can cause toxic effects, ranging from slight



(b)

	0 h			1 h			2 h			24 h			48 h			72 h			SPI		PII	
	G	*M.p.*	Ctrl	G	*M.p.*	Ctrl	G	*M.p.*	Ctrl	G	*M.p.*	Ctrl	G	*M.p.*	Ctrl	G	*M.p.*	Ctrl	G	*M.p.*	G	*M.p.*
V1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	-0.17		
V2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
V3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-0.17		
V4	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0.67	0		
V5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.083	-0.033
V6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
V7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
V8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
V9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
V10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

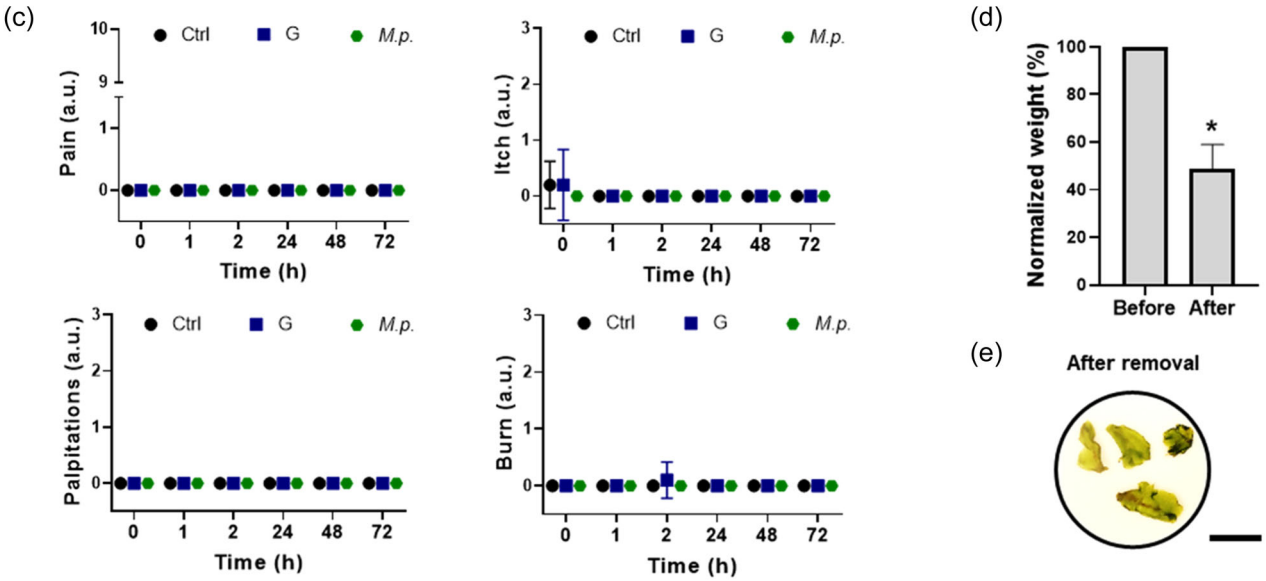


FIGURE 5 Skin irritation test of *Marchantia polymorpha* explants in healthy volunteers. Representative pictures of a single volunteer's forearms are shown immediately after application of gauze or the *M. polymorpha* explant (*M.p.*), and at 0, 24, and 72 h after removal (a). Summary table showing the skin reaction score for each volunteer at each time point after *M.p.* or gauze removal, categorized as absent (0), very slight (1), defined (2), moderate (3), or severe (4) erythema and/or edema. The Score of Primary Irritation (SPI) and Primary Irritation Index (PII) were calculated for all subjects (b). Volunteers self-assessed their level of pain (0–10), itching (0–3), palpitations (0–3), and burning sensation (0–3) at the sites in contact with gauze (G), explant (*M.p.*) and control (Ctrl) (c). Weight loss (d) and integrity (e) of explants were analyzed after application. Data are presented as mean + *SD*, with $N = 10$. Asterisk indicate significant differences at $p \leq 0.05$, determined by unpaired *t*-test (d) and two-way ANOVA followed by Tukey's test (c). Scale bar represent 5 cm in (a) and 1 cm in (e). ANOVA, analysis of variance.

allergies to other severe consequences (Sheehan, 2020), and therefore, before its clinical translation, further studies need to be done to determine the toxicological profile of *M. polymorpha* in open wounds.

Although the results presented here are promising, certain drawbacks of *M. polymorpha* indicate that further studies should explore the potential advantages of using explants obtained from alternative species to harness the immense biodiversity present

within the plant kingdom. For instance, the findings of this work revealed that the explants exhibited signs of dehydration after the irritation test, showing alterations in their overall appearance and a significant reduction in mass. This phenomenon may not be the case for explants derived from other species that are better adapted to drier environments or those containing higher contents of water, such as the *Aloe* family or other succulent species. Moreover, another concern regarding the potential clinical use of *M. polymorpha* is the small size of the explants, which contrasts with many other plant species characterized by significantly larger leaves.

While the results of this study clearly describe the capacity of the explants to provide oxygen in a physiologically relevant amount, the potential use of fresh explants as wound dressing may also offer the opportunity for local delivery of other bioactive molecules. This could be achieved by preloading the explants with the desired molecules or by generating transgenic lines capable of producing and releasing fresh recombinant molecules, such as growth factors or immunomodulatory peptides. On the other hand, further studies should also address the potential of the explants to actively remove metabolic waste, such as carbon dioxide, through the Calvin cycle, or passively remove other toxic compounds by absorbing wound exudate.

The results presented in this proof-of-concept study are promising; however, further research and developments will be key to translate this idea into clinics, ensuring the optimal conditions to further determine the safety of this approach and its efficacy to promote wound healing.

Altogether, this work provides key scientific evidence to support the development of novel photosynthetic dressings based on the use of living explants, which could be further used as a simple and customizable low-cost bioreactor for localized wound treatment. This concept capitalizes on the natural ability of certain plants tissues to act as physical barrier and, at same time, generate oxygen in a local and control manner, potentially offering a unique and sustainable wound healing approach.

5 | CONCLUSIONS

This proof-of-concept study suggest that photosynthetic explants may serve as a cost-effective biocompatible local oxygen delivery system for regenerative medicine, where explants could act as bioactive wound dressings. However, further studies are required to confirm the safety and efficacy of this approach in promoting wound healing in vivo. These studies should also analyze the capability of photosynthetic explants to be loaded with bioactive molecules and their ability to produce and secrete recombinant molecules with pro-regenerative properties.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All data associated with this study are presented in the paper and can be shared with approved outside collaborators under a materials transfer agreement. Requests should be sent to JTE.

ETHICS STATEMENT

Procedures were carried out in accordance with the ethical standards, and protocols were previously approved by the Scientific Ethics Committee for Health Sciences at Pontificia Universidad Católica de Chile (No.: 220128002). All the volunteers were informed and an informed consent were signed previous the experiments were performed.

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