



Unveiling Therapeutic Potential: A Systematic Review of Photobiomodulation Therapy and Biological Dressings for Diabetic Foot Ulcers

Sina Karimpour^{1,2*}, Mohammad Hussein Amirmotamed^{3*}, Fariborz Rashno⁴, Foozhan Tahmasebinia¹, Aliasghar Keramatnia¹, Fatemeh Fadaee Fathabadi², Hojjat Allah Abbaszadeh^{1,2,3*}, Shahram Darabi^{5*}

¹Laser Applications in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

²Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³Hearing Disorders Research Center, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁴Department of General Surgery, School of Medicine, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁵Cellular and Molecular Research Center, Research Institute for Non-Communicable Diseases, Qazvin University of Medical Sciences, Qazvin, Iran

*Correspondence to

Hojjat-Allah Abbaszadeh,
Email: Dr.Abbaszadeh@sbmu.ac.ir and
Shahram Darabi,
Email: shahram2005d@yahoo.com

*These authors contributed equally

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Abstract

Introduction: Diabetes poses a global health challenge, giving rise to various complications, including diabetic foot ulcers (DFUs). DFUs, marked by ischemic ulcers susceptible to infection and amputation, underscore the urgency for innovative treatments. This study investigated the impact of photobiomodulation therapy (PBT) and autologous platelet gel (APG) on DFUs recovery.

Methods: We systematically searched Web of Science, EMBASE, MEDLINE, Cochrane Library, Scopus, and Google Scholar (2015-2023) by using pertinent terms like “photobiomodulation therapy,” “low level light therapy,” and “platelet gel.” After meticulous data extraction and review, 57 articles were chosen and categorized. Among these, three randomized controlled trials involving 186 participants were selected for APG analysis.

Results: Findings demonstrate that APG application carries minimal risk and offers promising improvements in healing time, grade, pain reduction, and granulation tissue formation. Similarly, diverse PBT modalities involving distinct probes and wavelengths exhibit the potential to enhance tissue perfusion, expedite healing, and impede wound progression, reducing the need for invasive interventions.

Conclusion: PBT and APG emerge as valuable tools to augment wound healing, mitigate inflammation, and avert amputation, representing compelling therapeutic options for DFUs.

Keywords: Diabetes mellitus; Foot ulcer; Photobiomodulation therapy; Platelet gel.

Introduction

According to worldwide assessments, approximately 382 million individuals are affected by diabetes mellitus (DM), and by the year 2035, this figure is anticipated to rise to almost 592 million.^{1,2} In 2014, DM was identified as the cause of death for 4.9 million individuals globally.³ It is predicted that by 2030, DM could move up from being the ninth to the seventh most common cause of death worldwide.⁴ This chronic condition affects both the life expectancy and quality of life of individuals, while also restricting their ability to engage in daily activities and work-related tasks.⁵ DM causes a wide range of chronic complications, amongst which the most common are nephropathy, cerebrovascular disease, ischemic heart

disease and retinopathy. Diabetic foot ulcer (DFU) is one of the ischemic side effects of diabetes, characterized by damage to deep tissues, ulceration, and infection, often accompanied by neurological irregularities and peripheral vascular disease in the lower limbs. Apart from the substantial financial resources required and extended hospital stays, the indirect costs include the need for rehabilitation, home care, and the impact on productivity loss, individual expenses, and overall quality of life. Armed with knowledge about the disease's natural progression and epidemiology, healthcare professionals and managers possess the means to intervene at various stages, aiming to prevent or delay the progressive harm it inflicts.⁶ Even when considering meticulously controlled

clinical trials that assess advanced treatments for DFU and include highly specific patient groups in both control and treatment groups, only a range of 18% to 36% and 30% to 62% of DFUs, respectively, achieve complete healing.⁷⁻¹⁰ The financial burden associated with DFUs is estimated to range from \$9-13 billion on an annual basis.¹¹ The management of DFUs necessitates a comprehensive approach involving multiple disciplines. This includes surgical interventions and revascularization procedures, along with physiotherapeutic rehabilitation and infection treatment utilizing electric phototherapeutic resources to address co-morbidities, tissue malnutrition, pain, edema, metabolic disorders, as well as the precise treatment of the wound and biomechanical decompression. Consequently, providing specialized care for ulcers should be accessible to all patients and regarded as a priority in preventing amputations.¹² The use of red and near infrared light over wounds or lesions is known as photobiomodulation therapy (PBT), formerly known as low-level laser therapy (LLLT). It is a practical and efficient method of treating injuries or lesions, provided that essential factors such as dosage, strength, timing, and session intervals are carefully considered.¹³ PBT facilitates angiogenesis, the creation of extracellular matrix components, and their structure, all of which help to reduce the inflammatory phase. In addition to diminishing the size of the lesion and expediting the healing process, PBT is also user-friendly in terms of administration. These advantages contribute to enhanced quality of life for patients and help mitigate potential complications.^{14,15} The patient's own peripheral venous blood is used to create autologous platelet gel (APG). It produces a variety of growth factors after centrifugation and activation with calcium ions and thrombin, which help in the repair and regeneration of damaged tissue. Recent studies have demonstrated the utility of APG in plastic, orthopedic and maxillofacial surgery.¹⁶ The placenta's innermost layer, the amniotic membrane (AM), is clearly distinguishable from the other placental components. It possesses unique structural and biological properties, making it an attractive option as a biological dress.^{17,18} Elastin and collagen are present in the membrane, providing an appropriate texture for wound binding and adherence. Additionally, it is regarded as an immuno-privileged tissue that does not provoke immune rejection or cause tumor development.¹⁹⁻²¹ These features are attributable in part to its capacity to produce and release a wide range of chemicals, including cytokines, signaling molecules such as tumor necrosis factor (TNF- α), interferon, transforming growth factors alpha and beta (TGF- α and β), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), interleukins (IL-4, IL-6, IL-8), natural metalloprotease inhibitors, defensins, prostaglandins, and others.^{19,22} The AM serves as a great substrate for cellular migration and proliferation, allowing

epithelialization to occur. It is also anti-inflammatory, anti-fibrotic, antibacterial, and analgesic.^{17,23-26} APG has a high proportion of thrombocytes, and within the alpha-granules of platelets, numerous growth factors are found. Upon degranulation, platelets release these growth factors, which include thrombospondin-1, TGF- β 2, interleukin-1, fibrinogen, VEGF, insulin-like growth factor, osteocalcin, platelet-derived angiogenesis factor, vitronectin, fibronectin, TGF- β 1, platelet-derived growth factor (PDGF), EGF platelet factor IV, and thrombin. These factors have the ability to stimulate cell proliferation and differentiation, ultimately leading to tissue formation.²⁷⁻³⁸ Considering the positive effects of PBT and APG in the treatment of DFUs, as well as its synergistic effects and its methods, in this study, the effects of these methods on DFUs will be investigated.

Methods

Electronic Database Search

A thorough search was conducted across databases including MEDLINE, EMBASE, Cochrane Library, Web of Science, and Google Scholar, spanning from 2015 to 2023. The search terms used were: "Therapy photo-biomodulation (PBMT)" OR "therapy photo-biomodulation" OR "therapy PBM" (photo-biomodulation "OR" therapy PBM) (PBMT" OR "therapy PBM) photobiomodulation" OR "therapy photobiomodulation" OR "therapy photobiomodulation (PBM)" OR "therapy photobiomodulation (PBMT)" OR [All Fields] "low level light therapy" [MeSH Terms] OR ("All Fields] low level" AND [All Fields] "therapy light" AND [All Fields] "Fields] therapy") OR "Fields] light therapy" [All "Fields] low level" [All Fields] AND [All Fields] "laser level" [All Fields] AND [All Fields] "therapy laser" [All Fields] OR [All Fields] "therapy laser level" [All Fields] AND [All Fields] "therapy light" AND "therapy foot ulcer" AND [All Fields] "therapy light" AND "therapy mellitus diabetes" OR "therapy DM" AND "gel platelet" OR "PRP OR plasma rich Platelet" AND "factor Growth" AND [All Fields] "Chorion/Amnion."

Study Inclusion, Data Extraction, and Study Selection

Two separate reviewers evaluated the titles and abstracts to determine their relevance, and they subsequently assessed the eligibility of full-text articles. Data extraction was achieved by one reviewer and squared by another reviewer for correctness. Data were extracted from each study regarding study design, participant characteristics, interventions used, outcomes measured, and results obtained. The assessment of study quality was carried out using the Cochrane Risk of Bias tool. The studies included in this analysis were randomized controlled trials (RCTs) that explored the application of PBT as an intervention for DM.

Results

Search Results

This study examined 18 articles involving 630 participants from 2010 to 2022 (8 RCTs, 3 clinical trials, 4 case reports, 2 pilot studies, 1 comparative study) for PBT and APG. Initially, 193 articles were identified. After removing duplicates, 93 articles were screened further. Exclusions included non-human studies, reviews, and non-English articles, leaving 57 suitable articles. These were categorized into groups (Figure 1).

Characteristics of Included Studies

The laser setting used and the velocity of application was different in each study. A preliminary study demonstrated the effectiveness of an advanced class IV laser with four wavelengths in treating neuro-ischemic DFUs at Wagner stage 1 and 2. The trial included five patients with type 2 diabetes who had not responded to standard therapy. Among the laser-treated patients, four out of five (80%) achieved complete resolution of their ulcers, while the control group did not experience any ulcer healing by week 12.³⁹ In another case, a diabetic wound on the second, third, and fourth digits of the right foot caused prolonged pain and suffering, eventually leading to amputation. The wound exhibited poor healing factors. In this study, two probes were used to deliver laser therapy: a contact probe for the wound edges (820-nm laser diode, 140 mW/cm² power density, 2500 pps pulse repetition rate) and a non-contact cluster probe for the wound bed (pulse duration of 200 ns, 2 J/cm³ energy density). Eight illuminating diodes were employed in a multi-probe, with

four emitting at 660 nm and four at 880 nm (with a power density of 120 mW/cm², a repetition rate of 5000 pps, and an energy density of 4 J/cm³). According to the results, stump granulation started on Day 12 of the therapy, the wound's culture was negative for *Pseudomonas aeruginosa* and *Staphylococcus aureus* by Day 60, and the stump had fully recovered by day 120. Follow-up examinations revealed no recurrence of the ulcer, and measurements of the wound area revealed a decrease in the wound size during the two-month period from 13.74 cm² to 0.825 cm².⁴⁰ Low-intensity laser treatment with a 670 nm diode laser (30 J/cm²) was used on a 66-year-old man who had an ulcerous lesion with putrid discharge on the plantar side of his left first toe. The patient was discharged after 2 weeks of concurrent laser therapy, during which time the ulcer showed beginning granulation and the levels of C-reactive protein and fibrinogen returned to normal. The ulcer was entirely cured after a total of 16 laser treatments spread out over a 4-week period.⁴¹

Role of PBT in Healing Processes and Blood Flow

In addition, PBT was used to treat a pressure ulcer in the right calcaneus area of an 82-year-old woman with type 2 diabetes utilizing curcumin, LEDs, laser treatment (600 nm, 10 J/cm²), and a cellulose membrane. Epithelialization had fully completed after 30 days of therapy. PDT's bactericidal activity decreased the amount of germs on the ulcer surface, promoting healing and eradicating bacterial toxins. Before PDT therapy, microbiological samples from the wound revealed the presence of yeast and some Streptococcus species, but after one week of treatment, no contamination was found.⁴² PBT treatment employing combined He-Ne and infrared lasers (10 J/cm², 632.8 nm) was shown to enhance the average values of basal, maximum, and minimum skin blood perfusion in a study that sought to evaluate skin blood flow in diabetic individuals with disease-related skin lesions. In comparison to diabetic patients who got medication therapy or conventional treatment for skin lesions, this improvement was seen in those who received PBT.⁴³ Two additional studies examined the use of PBT in combination with topical hyperbaric oxygen (THO). One of these studies compared the effects of PBT (He-Ne 632.8 nm, 5 mW, and infrared laser 904 nm, 60 W) combined with THO versus THO alone. The findings revealed that patients who received laser irradiation along with THO experienced faster pain reduction and a decrease in leg edema compared to those who received THO alone.⁴⁴ The other literature examined the application of PBT (Helium-neon LEL laser 632.8 nm/5 mW and 904 nm/60 W, 4 J/cm²) combined with topical hyper baric oxygen (THBO) reported 81% (81 patients out of 100) were cured with this method. After 18 months, re-ulceration occurred in only 4% (3/81) and early retreatment with THBO/LEL led to ulcer healing in all cases. Amputations

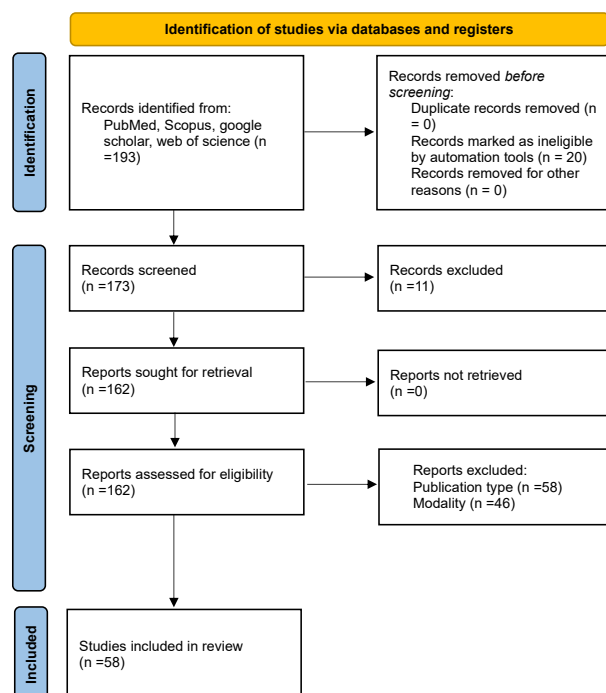


Figure 1. Identification of Studies Via Databases and Registers

were only performed on patients (19/19) who did not respond to treatment and had lower ankle-brachial index (ABI) values compared to those who showed a positive response.⁴⁵ Numerous studies have shown that transcutaneous LLLT irradiation significantly affects epidermal blood flow. One study found that one session of transcutaneous low-intensity laser irradiation with two helium-neon lasers (632.8 nm, 30 mW, 30 J/cm²) caused a statistically significant rise in skin temperature. The sham-irradiated control group, however, demonstrated a minor but substantial decrease in temperature. Following the radiation, at the conclusion of the radiation, and 15 minutes after the radiation was stopped, a rise in temperature was noticed. The decline in local skin temperature in the sham-irradiated feet became significant after the completion of the sham-irradiation procedure and 15 minutes afterwards. This suggests that a thermic laser irradiation can enhance skin microcirculation in patients with diabetic microangiopathy.⁴⁶ A different research examined the effectiveness of infrared laser treatment (904 nm, 20 mW, 6 J/cm²) with helium-neon laser therapy (632 nm, 20 mW, 5 J/cm²) and discovered that both groups saw a statistically significant decrease in ulcer surface area after the fourth and eighth weeks. Although the helium-neon laser treatment group saw a slightly higher reduction in ulcer area, this difference was not statistically significant (63.7% versus 56.8%; $P > 0.05$). According to the study's findings, treating DFUs with laser treatment for eight weeks can be advantageous.⁴⁷

Safety PBT in Clinic or Home

The effectiveness and safety of PBT usage at home has been the subject of two studies. In one report of four cases, patients self-administered the PBT (808 nm, 250 mW, 5 J/min) at the clinic or at home. One case achieved complete epithelialization after four treatments at the clinic, which was far quicker than the typical 1 to 3 months needed for recovery. Another case experienced improved skin texture, decreased neuropathic pain when wearing shoes, and a 50% reduction in the DFU size. After one treatment, the pain was greatly reduced, and after two, it totally vanished, improving mobility. The wounds were totally healed without scarring during the three-week check-up.⁴⁸ In the other trial evaluating at-home laser therapy, those who received laser treatment (808 nm Ga-Al-As laser, 250 mW, 8.8 J/cm²) saw a substantial decrease in the size of the wound. Wound closure exceeding 90% was achieved in seven out of ten patients receiving PBT, compared to only one out of ten patients in the sham group. The ulcers in the PBT group in a randomized clinical study examining the impact of PBT (685 nm, 10 J/cm²) on chronic DFUs healing showed a substantial reduction in size after the fourth week. Only three patients in the placebo group had fully healed their wounds after 20 weeks, compared to eight patients in the PBT group. The PBT group saw full

recovery in an average of 11 weeks as opposed to 14 weeks in the placebo group. Although 66.6% of ulcers in the PBT group fully healed, this percentage was not substantially higher than the figure observed in the placebo group (38.4%).⁴⁹ In another study, the effect of PBM (4 J/cm² for 20 min) and THBO (150 min x 2 to 3/wk at up to 1.04 atm) on the treatment of chronic DFUs in 100 consecutive patients was evaluated and the results showed that the simultaneous use of these two treatment methods, can be safe and appropriate treatment.⁵⁰ Another research examining the effects of PBT (660 nm, 30 mW, 6 J/cm²) on the healing of chronic lesions in diabetic foot found that the laser group significantly outperformed the control group in terms of the tissue repair index. Both groups experienced a reduction in the final wound area (control group = 1.63 cm², laser group = 0.32 cm²). With visible epithelial tissue forming in 55.5% of the lesions after four weeks, The PBT group demonstrated a successful response in treating DFUs. While there were no statistically significant differences in the visual analog scale (VAS) scores between the control and PBT groups on a weekly basis, there were statistically significant differences in how the wounds developed over the weeks using the Pressure Ulcer Scale for Healing (PUSH) scale, with an increasing intragroup difference seen by the fourth week.⁵¹

A study on 17 DFUs patients found that PBT (2 separate lasers: a helium–neon laser 632.8 nm, 3.1 J/cm² and 660 nm and 850 nm, 3.4 J/cm²) treatment reduced pain and increased vibration perception threshold in these patients. The wound exhibited gradual healing and achieved complete closure with a mean total closure time of 26 ± 11 days. The maximum healing rate, observed between days 10 and 20 after treatment, was 6 mm². After 28 days of initial PBT treatment, the DFUs showed continuous improvement and was entirely covered by new epithelium, accompanied by thick, vascularized granulation tissue.⁵² Significant reduction in the size of DFUs was observed following laser therapy using pulsed wave form, visible ray (632.8 nm, 30 mW, 4 J/cm²). Additionally, the treated group reported significant reductions in pain, with an average pain score decreasing from 9 to 5. This improvement may be attributed to the analgesic effect of the therapy.⁵³ Data analysis revealed that total hemoglobin concentration increased after PBT in DFU patients, but not in healthy controls. While no significant changes were seen in healthy controls, the very low frequency/low frequency (VLF/LF) ratio dramatically reduced during the PBT period in DFU patients, indicating increased autonomic nervous system activity. Furthermore, different PBT treatment intensities elicited varying responses in DFU patients. The increase in total hemoglobin concentration was more pronounced with the INT4 (830 nm, 80 J/cm²) configuration compared to the lower intensity setting (830 nm, 20 J/cm²). Additionally, the VLF/LF ratio significantly decreased

in DFU participants, particularly with the INT4 setting. These results show that in DFUs, PBT improves blood flow and autonomic nervous system function.⁵⁴

PBT and Biological Treatment

A specific study's goal was to compare the effects of human amniotic membrane (HAM) and PBT (660 nm, 30 mW, 6 J/cm²) on diabetic foot lesions. When comparing day 7 to day 28, the percent reduction in wound area was: control: 23.5% vs 65.3%; HAM: 25.3% vs 66.9%; and PBT: 28.1% vs 87.8%. According to the PUSH scale indicators, the HAM and PBT groups demonstrated statistically significant healing improvement. The control group's patients reported higher pain than the HAM and PBT groups. Within 7 days, those in the HAM group noticed tingling; nevertheless, after 7 days, they reported an improvement in pain. Macroscopic characteristics of the wounds improved after day 7 of PBT administration (ie, considerable decrease of the lesion and keratosis area, constriction of the margins, and development in the granulation tissue).⁵⁵ Another clinical experiment including 30 patients with type 2 diabetes and Meggitt-Wagner grade I foot ulcers investigated the benefits of laser treatment (particularly PBT at a wavelength of 660 ± 20 nm and energy density of 3 J/cm²). The outcomes showed that the PBT group had a considerably greater reduction in ulcer area than the control group (37 ± 9% vs. 15 ± 5.4%). Approximately 75% of wounds in the PBT group showed a decrease in area ranging from 30% to 50%. By day 15, however, only around 80% of the wounds in the control group had an area reduction of less than 20%. Additionally, it was observed that wounds with an initial size of 1000-2000 mm² tended to have better final outcomes compared to those with larger areas. Furthermore, the treated groups demonstrated a greater presence of granulation tissue compared to the control group.⁵⁶

APG Result and Analysis Effects

Three studies on this subject were examined, in the first study APG was applied weekly to the wound site whereas in the second and third study the velocity of application was based on wounds condition. In this study, the use of APG to treat DFUs was evaluated. According to the findings, the APG group received therapy for 4 weeks, but the control group required 8 weeks to recover. The experimental group's average spending and total hospital stay were much lower than the control groups. In comparison to the control group, the experimental group had considerably greater rates of sinus tract closure at the end of week 4. By the conclusion of the eighth week, there was, however, little difference between the two groups. The control group demonstrated a negative-converting rate of 95% at week 4 and 100% at week 8, whereas both the experimental and control groups had a bacteria turn

rate of 100% at both weeks four and eight. Analysis of hematoxylin and eosin staining revealed that in the APG group, the epidermis and dermis experienced thickening by the second week, collagen fibers were organized, and the glands exhibited regular presence. In the fourth week, the continuous structure was complete and the dermal layer was moderate.³⁶

In one research, which evaluates the application of APG in the treatment of chronic refractory cutaneous ulcers, it is found that the combination of APG with standard treatment is both safe and more successful compared to standard treatment alone for diabetic refractory cutaneous ulcers. The combination strategy improved wound healing grades, cut down on healing time, and increased the speed of healing. In the intent-to-treat (ITT) population, 85% (41 out of 48) of diabetic ulcers in the autologous platelet-rich gel (APG) group, compared to 69% (40 out of 58) and 67% (37 out of 55) in the control group, achieved healing grade 1 (complete healing), out of the total population. Furthermore, among the ITT population, 96.6% (57 out of 59) of patients in the APG group demonstrated wound area reduction rates of ≥ 80% (grades 1 and 2), while 72.4% (42 out of 58) achieved the same in the control group (98.2% (55 out of 56) vs 74.6% (41 out of 55) among the per-protocol (PP) population. The Kaplan-Meier analysis demonstrated a substantial disparity in time-to-healing between the two groups, with the APG group experiencing a median healing time of 36 days (interquartile range (IQR) 30-84) against 45 days (IQR 18-60) in the control group. In terms of infection management, 5 APG patients did not effectively resolve their infections, while 8 patients in the control group experienced advanced infection³⁷. Another study investigated the application of homologous platelet gel (PG) on lower extremity wounds. Results revealed that human endothelial cell line (ECV304) and human epidermal keratinocytes (HaCaT) demonstrated 40% and 50% healing, respectively, after 6 hours of using homologous PG. In contrast, the control group treated with fetal bovine serum (FBS) only exhibited a 20% recovery rate. By the 24-hour mark, wounds treated with homologous PG were fully recovered (100%), while those treated with FBS showed approximately 40% healing. Positive responses were observed in all cases, including the rapid formation of granulation tissue, pain reduction, and wound size reduction. The overall healing rate reached up to 86%. No major adverse effects were reported in all three studies.³⁸ However, in one trial, 18 subjects (six in the control group and twelve in the APG group) reported experiencing a formication or prickling feeling. Fortunately, this symptom went away on its own since the wounds' granulation tissue and epithelium were growing well.³⁷

Human Amnion/Chorion Membrane Result and Analysis Effects

Fourteen articles with a total of 733 patients from 2013 to 2020 were selected for the following topics which are summarized in Table 1.⁵⁷⁻⁷⁰ Two studies compared dehydrated human amnion/chorion membrane (dHACM) with various skin replacements, such as bioengineered living cellular construct (BLCC) and Apligraf.^{62,70} In these studies, various forms of amnion and chorion membranes, including dHACM, cryopreserved amniotic membrane (CAM), dehydrated amniotic membrane allograft (DAMA), cryopreserved amniotic suspension allograft (CASA), and granulated amniotic membrane and amniotic fluid (gAM-AF), were evaluated.

Most studies employed dHACA. According to a randomized, controlled multicenter clinical research, 70% of the ulcers treated with dHACM at the end of the 12-week treatment period had healed, as opposed to 50% in the non-dHACM group. In the group receiving dHACM, healing rates at 12 weeks were 81%, compared to 55% in the control group. At the week 16 follow-up, 36 of 38 (95%) healed wounds treated with dHACM were still closed, compared to 24 of 28 (86%) healed wounds treated without dHACM. According to a Kaplan-Meier plot of time to heal, dHACM-treated ulcers showed a better wound-healing trajectory than lower extremity ulcers that did not receive dHACM and were treated with conventional care.⁵⁷ In one research of 14 patients with

Table 1. Characteristics of Included Studies

Authors	Study Design	Population	Intervention
Saied et al ¹⁵	Prospective study	LILT group: 30 Conventional treatments=15, No treatment=15	He-Ne and infrared lasers
Xie et al ³⁶	RCT	APG treatment group=25 Conventional wound dressing control group=23	APG
Li et al ³⁷	RCT	APG treatment group=59 Control group=58	APG
Shan et al ³⁸	RCT	21 Patients	Homologous PG
Chandrasekaran et al ⁴⁰	Case report	1 Case	Laser (660 and 880 nm) and ultraviolet C (250 nm) radiations
Schindl et al ⁴¹	Case report	1 Case	Low-intensity laser therapy (670 nm)
Raizman et al ⁴³	Case report	4 Cases	Home-use PBM device (808 nm)
Landau ⁴⁵	RCT	THO alone=15 Low energy laser+THO=35	He-Ne (632.8 nm) and infrared laser (904 nm) and THO
Tantawy et al ⁴⁷	RCT	Helium-neon laser therapy (HNLT) and conventional therapy=31 ILT and conventional therapy=32	HNLT 632 nm Infrared laser 904 nm
Haze et al ⁴⁸	RCT	Active=10 Sham=10	At-home PBM device, 808-nm Ga-Al-As laser
Kaviani et al ⁴⁹	RCT	Placebo treatment=10 LLLT treatment=13	LLLT (685 nm)
de Alencar Fonseca Santos et al ⁵¹	RCT	Control=9 Laser treatment=9	LLLT (660 nm)
de Alencar Fonseca Santos et al ⁵⁵	Pilot clinical study	Control treatment=9 HAM treatment=9 LLLT treatment=9	HAM, LLLT (660 nm)
Tettelbach et al ⁵⁷	RCT	dHACM group=54 No dHACM group=56	dHACM
Valiente et al ⁵⁸	Case series	14 Patients	CAM
Abdo ⁵⁹	Case series	14 Patients	DAMA
DiDomenico et al ⁶⁰	RCT	dHACA-treated=40 Control group=40	dHACA
Carter et al ⁶¹	RCT	dHACA-treated=40 Control group=40	dHACA
Kirsner et al ⁶²	Comparative study	BLCC-treated=163 dHACM=63	BLCC and dHACA
Marcus ⁶³	Case series	5 patients	CASA
Penny et al ⁶⁴	Case series	5 patients	dHACM, EpiFix
Hawkins ⁶⁶	Case series	3 patients	dHACM
Thompson et al ⁶⁷	RCT	Human amniotic allograft group=7 Control group=6	Human amniotic allograft
Zelen et al ⁶⁹	RCT	Standard care group=12 EpiFix group=13	dHACM, EpiFix
Zelen et al ⁷⁰	Comparative study	BSS=33, EpiFix=32 Standard wound care=35	BSS and dHACM

APG, autologous platelet gel; PBM, photobiomodulation; THO, topical hyperbaric oxygen; ILT, intense laser therapy; LLLT, low-level laser therapy; HAM, human amniotic membrane; dHACM, dehydrated human amnion/chorion membrane; DAMA, dehydrated amniotic-derived tissue allograft; CAM, cryopreserved amniotic membrane; BLCC, bioengineered living cellular construct; CASA, cryopreserved amniotic suspension allograft; BSS, Bioengineered skin substitutes; LILT, low-intensity laser therapy.

persistent DFUs treated with CAM, all patients had full wound healing in 20 weeks (range: 7-56 weeks). There were no adverse events associated with the use of AM. A 64-year-old lady with a neuro-ischemic DFUs of 17.74 cm² and 18 months' duration in the heel of her left foot was described in the literature. Multiple pathogens were discovered during wound culture. The ABI value before AM administration was 0.4, indicating significant peripheral arteriopathy. The patient was treated with AM treatments at weekly intervals, and after 40 applications, a gradual wound closure was seen. Microorganisms in the wound were eradicated by multiple applications of AM and oral antibiotic medication. The entire healing process took 56 weeks.⁵⁸ The usefulness of DAMA on DFUs was investigated in a prospective case series. Patients in this study experienced full wound closure in 5 weeks (range: 1-14 weeks), with wound area and volume reduced by 58.3% and 74.1%, respectively, at the first and third weeks, and by a median of 100% at the fifth week and at all subsequent time points.⁵⁹ Another research that looked at the use of processed dHACM discovered that after 12 weeks, 85% (34/40) of dHACA-treated DFUs were healed as opposed to 33% (13/40) of DFUs treated just with the traditional method. The dHACA-treated group saw healing on average in 37 days as opposed to 67 days for the control group receiving normal treatment. Each cured wound required an average of 4.0 grafts, and the average cost of tissue to heal a DFUs was \$1771.⁶⁰ A health economics study calculated the cost-utility of standard of care (SOC) alone against SOC plus a dehydrated, aseptically processed dHACA. The study's findings showed that after one year, group 1 (dHACA) outperformed group 2 (SOC) by an estimated incremental cost effectiveness ratio of -\$4,373. According to probabilistic sensitivity analysis (PSA), group 1 had values with higher positive incremental effectiveness for 94.9% of values and values with 69.2% lower cost values. When \$50 000 was paid, a willingness to pay curve indicated that 92% of the treatments for group 1 were cost-effective.⁶¹ A comparative study that compared the effectiveness of a BLCC and a dHACM for the treatment of DFUs found that the BLCC significantly increased wound closure speed and incidence when compared to a nonviable DAM. Those who got dHACM submitted more applications (median 3.0 vs. 2.0) than those who received BLCC. The median time to closure for BLCC was 13.3 weeks compared to 26 weeks for dHACM, and the percentage of wounds healed was significantly higher for BLCC at 12 weeks (48% vs. 28%) and 24 weeks (72% vs. 47%) in a Cox model that was adjusted for important characteristics including area and duration. Treatment with a bioengineered live cellular technology increased the chance of recovery by 97% when compared to a DAM.⁶² A case series looked at CASA on five individuals with diabetic ulcers who were 39 to 86 years old. One

patient had a sacral pressure ulcer, three patients had surgical/radiation wounds, and one patient had a DFUs. Throughout CASA therapy, every patient's wound area and volume gradually improved. Surgery was necessary for one patient (post-mastectomy tissue necrosis) in addition to the removal of any residual sutures. CASA was especially well suited for use in these wounds with broad sinus tracts or undermining when administered topically and subcutaneously. Following one to two administrations of CASA over a period of five to twenty-two weeks, all five patients had completely healed wounds.⁶³ Another case study looked at dHACM on five individuals with DFUs who were over 45 years old and unresponsive to treatment. Among the wounds the patients had to deal with were post-surgical debridement, non-healing surgical wounds, trauma wounds, DFUs brought on by Charcot arthropathy, and pressure ulcers. When other treatment methods were ineffective, the administration of dHACM led to a full healing of the wound. The average healing time for a wound was 7.3 weeks (within a range of 2.5 to 11 weeks), and patients fully recovered in 2.5 to 11 weeks. The healing period was also sped up despite three patients' non-adherence.⁶⁴ A case series indicated that chronic, unresolved DFUs treated with DAMA recovered quickly and closed in 9.2 weeks. Those with the most DAMA applications had a shorter time to closure. At week 2, the wound area and volume had decreased by 48% and 60%, respectively, and by week 8, they had decreased by 89% and 91%.⁶⁵ In a case study on three wounds, dHACM were shown to be successful in treating DFUs. As a therapy for diabetic plantar ulcers, the dHACM allograft was simple to use, clinically efficacious, and well tolerated. All three lesions had healed within four weeks of the first application (after 2-4 applications). There were no adverse events observed, and the wounds were healed after 6 months.⁶⁶ Except for two patients who had Charcot foot, the majority of participants (92.3%) got wound closure during the trial, according to a study on 13 patients. The standard treatment group had a larger mean ulcer surface area than the HAA group (2.78 cm² vs 1.54 cm²). The standard care group had a recurrence rate of 83.33% (5/6) compared to 14.29% (1/7). In the conventional treatment group, the majority of patients (83.3%) had no drainage or only a tiny quantity (16.7%), but in the HAA group, 42.9% had considerable drainage, 28.6% had a small amount, and 28.6% had none.⁶⁷ In an RCT of 20 foot and ankle wounds, researchers determined that treating chronic diabetic foot wounds using gAM-AF is a potential option. During the 12-week monitoring period, 18 wounds (90%) healed, and gAM-AF appeared to eradicate sinus tracts and tunnels. The wound area and volume reduced by 0.935 ± 0.159 (93.5%±15.9%) and 0.977 ± 0.057 (97.7% ± 5.7%), respectively, from the baseline to the 12-week follow-up visit. All 20 wounds showed a clinically significant decrease in wound area, and none needed amputation.⁶⁸

In a randomized controlled trial (RCT), dHACM were used to treat DFUs in comparison to standard care. In the groups receiving conventional treatment ($n=12$) and EpiFix ($n=13$), the mean size of the wounds was decreased by $32.0\% \pm 47.3\%$ against $97.1\% \pm 70\%$ after four weeks, and by $-1.8\% \pm 70.3\%$ versus $98.4\% \pm 58\%$ after six weeks. EpiFix had an overall healing rate of 77% and 92% after 4 and 6 weeks of therapy, compared to 0% and 8% of the wounds being treated by normal care.⁶⁹ A case series of five patients with DFUs explored CASA. All patients achieved complete wound closure within 5 to 22 weeks with CASA applications, showcasing its potential for various diabetic ulcer cases.⁶³ Another case series involving five patients aged over 45 with unresponsive DFUs assessed dHACM application. Complete wound closure was achieved within 7.3 weeks, highlighting the potential effectiveness of dHACM in resistant DFUs.⁶⁴ Chronic DFUs treated with dHACM achieved closure in 9.2 weeks on average. The wound area and volume reductions were significant, suggesting the efficacy of dHACM in accelerating healing for chronic DFUs.⁶⁵ For diabetic plantar ulcers, dHACM allografts proved clinically effective, achieving complete healing within four weeks with 2 to 4 treatments. No adverse events were observed, showcasing the reliability of dHACM allografts for DFUs healing.⁶⁶ In a study involving 13 patients, the HAA group had smaller ulcer surface areas, lower recurrence rates, and better drainage management compared to standard care.⁶⁷ A Randomized Controlled Trial involving 20 foot and ankle wounds showed that gAM-AF healed 90% of wounds effectively over 12 weeks, addressing sinus tracts and tunnels without amputations.⁶⁸ In an RCT, DFUs treated with dehydrated HAMA were compared with conventional treatment. The HAMA group ($n=13$) showed a remarkable reduction of $97.1\% \pm 7.0\%$ in wound size at 4 weeks and $98.4\% \pm 5.8\%$ at 6 weeks, compared to the regular therapy group's reduction of $32.0\% \pm 47.3\%$ at 4 weeks and $-1.8\% \pm 70.3\%$ at 6 weeks. Healing rates were significantly higher in the HAMA group, reaching 92% at 6 weeks, while the standard care group achieved only 8%.⁶⁹ Comparing dHACA with Apligraf, dHACA demonstrated faster complete healing and greater cost-effectiveness. Apligraf achieved 73% complete healing in 12 weeks with an average of 47.9 days, whereas EpiFix achieved 97% healing in a much shorter average of 23.6 days. Graft costs were also significantly lower for EpiFix (\$1517) compared to Apligraf (\$8918) per healed wound (Table 1).⁷⁰

Discussion

The application of PBT and Advanced Wound Care Products such as APG and HAMA offers a safe and effective approach to treating DFUs. Given the complications of DFUs and their challenging healing process, PBT emerges as a promising strategy for

enhancing healing outcomes in DM. Studies reveal that PBT significantly accelerates wound healing, leading to faster recovery. Moreover, PBT correlates with improved wound healing grades, reflecting enhanced overall healing quality. Notably, it also contributes to shorter hospital stays for treated individuals. This underscores the potential of PBT to revolutionize DFUs management. The key advantage of PBT lies in its capacity to stimulate tissue repair and regeneration. By utilizing laser light energy, it deeply penetrates tissues, promoting cellular activity and enhancing blood flow to wounds. This improved circulation supplies crucial oxygen and nutrients for healing while eliminating waste products. The anti-inflammatory properties of PBT suppress pro-inflammatory agents and release anti-inflammatory substances, fostering an optimal environment for healing by minimizing excessive inflammation. Notably, PBT can be applied through different methods such as LLLT, Helium-neon laser therapy (HNLT), and intense laser therapy (ILT).²⁹ In a focused study, the effectiveness of LLLT was compared with the application of HAM to DFUs. During the comparison between day 7 and day 28, the reduction in wound area percentages unfolded: for the control group, reductions were 23.5% to 65.3%; in the HAM group, reductions were 25.3% to 66.9%; and in the PBT group, reductions were 28.1% to a notably higher 87.8%. This emphasizes the significant advantage of PBT over both control and HAM treatments in terms of wound area reduction within a specific timeframe. By utilizing the PUSH scale indicators, both the HAM and PBT groups displayed statistically significant healing improvements. Patients in the control group reported higher pain levels compared to those in the HAM and PBT groups. Within 7 days, the HAM group noted tingling, which later evolved into an improvement in pain. Macroscopic wound characteristics notably improved after 7 days of PBT administration, marked by substantial decreases in lesion and keratosis areas, margin constriction, and granulation tissue development.⁵⁵ Further research is needed to comprehensively compare the effects of PBT and HAM treatment. These studies would provide insights into their nuances, including their influence on short-term healing, ulcer reduction, healing grade, and potential enhancements in the patient's vascular and nervous system responses. These potential benefits are likely linked to the increased blood flow generated by these interventions.³⁰ Moreover, PBT boosts collagen synthesis, crucial for wound closure and tissue remodeling. Collagen is integral to connective tissues, offering structural support during healing. PBT spurs fibroblast activity, heightening collagen production and deposition at the wound site, ultimately enhancing the tensile strength of the healed tissue.³¹ Another positive effect of PBT is its antimicrobial properties. DFUs are prone to infections due to compromised immune function. Laser treatment

can help reduce the bacterial load by destroying or inhibiting the growth of bacteria present in the wound bed. This antimicrobial effect contributes to preventing infection and promoting faster healing. Additionally, PBT has been reported to alleviate pain associated with DFUs. Chronic wounds can be painful, affecting patients' quality of life and impeding their mobility. PBT helps reduce pain by stimulating nerve regeneration, releasing endorphins (natural painkillers), and decreasing nerve sensitivity.³²

Our findings emphasize APG as a valuable treatment avenue to address the complexities of healing DFUs. A key advantage lies in the capacity of APG to enhance tissue regeneration. Platelets, rich in growth factors such as PDGF, TGF- β , and VEGF, initiate vital processes like cell proliferation, angiogenesis, and collagen synthesis, significantly contributing to wound healing.³³ Moreover, APG showcases antimicrobial properties, crucial for combatting infections in vulnerable diabetic wounds due to compromised immunity. Antimicrobial peptides released by platelets thwart bacterial growth, facilitating faster healing. APG also orchestrates inflammation modulation at the wound site, and this is crucial as chronic inflammation can hinder diabetic wound healing. Anti-inflammatory cytokines released by platelets reduce inflammation, creating an optimal repair environment. Studies underscore that diabetic patients treated with APG experience accelerated wound closure rates compared to conventional treatments.³⁴ A safety highlight is APG use of the patient's blood components, mitigating allergic reactions or disease transmission risks. In conclusion, the benefits of APG encompass tissue regeneration, enhanced angiogenesis, infection defense, inflammation reduction, and accelerated wound closure rates. It holds promise for improving outcomes in DM with chronic wounds. Further research is warranted to refine protocols and assess long-term effectiveness.³⁵

Conclusion

In conclusion, studies suggest that using PBT, APG and HAM could be a promising and safe way to treat DFUs. These approaches might speed up healing, improve wound assessment scores, and shorten hospital stays. PBT and APG have potential as valuable treatments for DFUs, enhancing healing outcomes. However, more research is required to refine protocols and understand their long-term effectiveness in managing DFUs.

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Authors' Contribution

Conceptualization: Hojjat Allah Abbaszadeh, Sina Karimpour, Shahram Darabi.

Data curation: Sina Karimpour, Mohammad Hussein Amirmotamed.

Formal analysis: Fariborz Rashno, Foozhan Tahmasebinia.

Funding acquisition: Hojjat Allah Abbaszadeh.

Investigation: Hojjat Allah Abbaszadeh, Sina Karimpour, Aliasghar Keramatinia.

Methodology: Aliasghar Keramatinia, Fariborz Rashno, Foozhan Tahmasebinia.

Project administration: Hojjat Allah Abbaszadeh, Shahram Darabi

Resources: Aliasghar Keramatinia, Fatemeh Fadaee Fathabadi, Hojjat Allah Abbaszadeh.

Software: Sina Karimpour, Mohammad Hussein Amirmotamed.

Supervision: Hojjat Allah Abbaszadeh.

Validation: Shahram Darabi, Aliasghar Keramatinia, Fatemeh Fadaee Fathabadi.

Visualization: Hojjat Allah Abbaszadeh.

Writing—original draft: Sina Karimpour, Mohammad Hussein Amirmotamed, Hojjat Allah Abbaszadeh.

Writing—review & editing: Hojjat Allah Abbaszadeh, Foozhan Tahmasebinia, Sina Karimpour.

Competing Interests

The authors declare that there is no conflict of interest.

Ethical Approval

This study was approved by the Research Ethics Committee at Shahid Beheshti University of Medical Sciences, Tehran, under code No (IR.SBMU.LASER.REC.1402.002).

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