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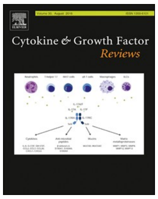

Role of the PI3K/AKT (mTOR and GSK3β) signalling pathway and photobiomodulation in diabetic wound healing[☆]

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ABSTRACT

Activated phosphatidylinositol 3 kinase/Protein kinase B (PI3K/AKT) signalling with increased or reduced mTOR and GSK3β activity influences the wound repair process. Diabetic wounds, usually ulcerated, are characterised by reduced growth factors and cellular performance. The occurrence of diabetic ulcers is linked to peripheral arterial disease, neuropathy, and wound contamination. Lasers or light emitting diodes (LEDs) provide photon energy with therapeutic benefits (Photobiomodulation-PBM), and has been broadly commended to quicken diabetic wound healing. PBM is efficient in the visible red and near-infrared electromagnetic spectrum, and fluencies ranging from 2 to 6 J/cm^2. However, cellular and molecular mechanisms induced by PBM are not fully understood. In this review we discuss PBM and the PI3K/AKT pathway with specific focus on the mTOR and GSK3β downstream activity in diabetic wound healing.

1. Background

Cell proliferation, differentiation and migration is critical for physiological outcomes including growth, wound repair and development of cancer. Wounds develop as a consequence of an injury to the living tissue, usually caused by a blow, a cut or any impact that can break the tissue. Injury initiates a coordinated process for wound repair, typically regulated by growth factor/cytokine stimulated signalling [1]. During wound healing, growth factors/cytokines orchestrates inter- and intracellular signalling for cell proliferation, differentiation, migration and protein synthesis. In normal circumstances, this process results into a predictable repair of tissue structural design and function, where cells including fibroblasts, platelets, immune cells, keratinocytes and microvascular endothelial cells play a significant role [2]. Immediately after injury, a rapid immune reaction, the accumulation of the extracellular matrix (ECM) and coordination of all cellular mechanisms of the epidermis are triggered to restore local tissue homeostasis. Wound repair is split into four interrelated phases of haemostasis, inflammation, proliferation and remodelling. For this process to be complete, it requires effective coordination of molecular, cellular, and physical events amongst haematopoietic, immunologic, and local skin cells [3,4]. Nonetheless, this process may be compromised, and can be aggravated

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Abbreviations: 3′-OH3, prime hydroxyl group; AGEs, advanced glycation end products; AKT, protein kinase B; ATP, adenosine triphosphates; BAD, Bcl2 associated death promoter; BAK, Bcl2 homologous antagonist killer; BAX, Bcl2-like protein 4; Bcl2, B cell lymphoma 2; BH3, Bcl2 homology 3; BMP, bone morphogenetic protein; cAMP, cyclic adenosine monophosphate; CO$_2$, carbon dioxide; COX, cytochrome c oxidase; DM, diabetes mellitus; DNA, deoxyribonucleic acid; DOK, oral dysplastic cells; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial mesenchymal transition; ERKs, extracellular signal regulated kinases; ERK-Sp1, ERK-specificity protein 1; FAK, focal adhesion kinase; GS, glycogen synthase; GSK3β, glycogen synthase kinase 3 beta; HIF-1α, hypoxia-inducible factor-1 alpha; IGF-I, insulin-like growth factor I; IκBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; JAK, janus kinase; JNK, C-JUN N-terminal kinase; KGN, immortalised granulosa cells; LEDs, light emitting diodes; LLLT, low level light therapy; MAPK, mitogen-activated protein kinase; miRNAs, micro-RNAs; MMPs, matrix metalloproteinases; mRNA, messenger ribonucleic acid; mTOR, mammalian target of rapamycin; mTOR1, mammalian target of rapamycin complex 1; mTOR1, mammalian target of rapamycin complex 2; NF-Kb, nuclear factor kappa-light-chain-enhancer of activated B cell; NFκB, nuclear factor κB; NIR, near infrared; NO, nitric oxide; p, phosphorylated; PBM, photobiomodulation; PDK1, protein serine/threonine kinase-3′-phosphoinositide-dependent kinase 1; PEMFT, pulsed electromagnetic field therapy; PH, pleckstrin homology; PI3,4,5-P$_3$, phosphatidylinositol-3,4,5-triphosphate; PI3K, phosphatidylinositol 3 kinase; PI4,5-P$_2$, phosphatidylinositol-4,4-bisphosphate; PTEN, phosphatase and tensin; ROS, reactive oxygen species; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; Ser-473, Serine-473; Ser-9, Serine-9; SH2, Src homology 2; STAT, signal transducers and activators of transcription; TGF-β, transforming growth factor beta; TLR, toll-like receptor; VEGF, vascular endothelial growth factor; α, alpha; β, beta

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in the presence of contamination and underlying pathologies, including diabetes mellitus (DM) [5]. Wounds that have failed to successfully progress through the normal phases of wound healing results in chronic wounds, which are often seen to stall in the inflammatory phase [6]. Chronic wounds are classified into diabetic, vascular (venous and arterial) and pressure ulcers, representing a significant concern in the medical field. This burden is expected to rise due to an upsurge in illnesses such as DM, and an increase in the aged population [7].

Wound chronicity is a critical complication commonly seen in DM, and is typified by disordered repair processes leading to the development of diabetic ulcers. Although the precise pathophysiological conditions for the progression of DM are partially understood, insulin resistance is known as the initial detectable defect. Recent findings suggest that various molecular mechanisms, including the cellular signalling pathways, play a critical role in the initiation and development of DM [8]. Khorami et al. [9], implicated the impairment of PI3K/AKT signalling in the development of DM and its subsequent complications. Ulceration of the lower limb in patients with DM is a distressing complication, and is worsened in the presence of peripheral neuropathy and vascular disease, and the development of advanced glycation end products (AGEs). Diabetic wounds occur in approximately 15% of all diabetic patients, attributing to 75% of all non-traumatic lower limb amputations [10]. Abnormal cell migration, proliferation, differentiation, apoptosis and the consequences of electrophile toxicity, oxidative stress, low-grade chronic inflammation and senescence are some of the factors that have been related to the development of diabetic ulcers [11]. Additionally, the presence of increased levels of proteases exceeding the levels of their corresponding inhibitors play a critical role in the development of these ulcers. The increased and continued presence of proteases leads to excessive degradation of the ECM, including growth factors and growth factor receptors [6]. Although associated with development and progression of cancer, PI3K/AKT signalling is crucial in normal tissue repair processes, and plays a critical role in the regeneration [12], remodelling [13] and reepithelialisation [14] of injured tissue. Deregulated PI3K/AKT signalling have significant effects on wound healing [9].

PI3K/AKT signalling turns on and off several downstream regulating proteins, including the mammalian target of rapamycin (mTOR) and glycogen synthase kinase 3 (GSK3). Phosphorylation of mTOR and GSK3 by AKT influences a wide range of biological activities such as proliferation, growth and survival. Deregulated mTOR activation results in complications related to DM and cardiovascular diseases [15], whereas phosphorylation dependent inhibition of GSK3 isoforms; GSK3 beta (β) and GSK3 alpha (α) by AKT, regulates several downstream proteins including glycogen synthase (GS) that is involved in glucose metabolism and storage [16]. GSK3 phosphorylation at its N-terminal (S21 of GSK3α and S9 of GSK3β) reduces the activation of GS, inhibiting the negative regulation of its downstream substrates. Additionally, the GSK3β pathway controls biological processes including the inflammatory response, cell migration, proliferation and apoptosis [17]. Inhibition of GSK3β promotes pro-apoptotic activities, an intrinsic cell death pathway in which GSK3β regulates B cell lymphoma 2 (Bcl2) protein activity [18]. Indirectly, the activity of GSK3β signalling can similarly be initiated via p70S6K, a downstream protein of the AKT/mTOR pathway, which phosphorylates the N-terminus of both GSK3 isoforms [19].

2. PI3K/AKT signalling

Growth factors/cytokines are upstream extracellular signalling proteins for many pathways that influence a range of biological processes. In reaction to these extracellular regulating proteins, signalling pathways including PI3K/AKT, modulates several cellular activities, representing an essential factor for processes involved in acute wound healing and maintenance of tissue homeostasis [14]. AKT, a serine/threonine kinase, is a vital signalling center for a wide-range of cellular functions. PI3K dependent activation of AKT further affects the activity of several downstream pathways involved in cell proliferation, angiogenesis, senescence, apoptosis and cellular survival [15,4]. PI3K is comprised of a family of lipid kinases that are categorised by their ability to activate the inositol ring of the hydroxyl (3′-OH) group in inositol phospholipids. Binding of mitogenic proteins to receptor tyrosine kinases (RTKs) activates auto-phosphorylation of the tyrosine residues on its cytosolic domain. This is followed by the recruitment of PI3K to the membrane, which binds to the phosphotyrosine residues of the receptors through the src homology 2 (SH2) domains present in the adaptor subunit. Activated PI3K controls the production of phosphatidylinositol-3,4,5-triphosphate (PI3,4,5-P_3) from phosphatidylinositol-4,4-bisphosphate (PI4,5-P_2). PI3,4,5-P_3 then recruits protein serine/threonine kinase-3′-phosphoinositide-dependent kinase 1 (PDK1) and AKT/PKB that contains the pleckstrin homology (PH) domain [20,21,22]. AKT activation is negatively regulated by antagonists of PI3K signalling, including the phosphatase and tensin homolog (PTEN), which converts PI3,4,5-P_3 to PI-4,5-P_2 to restrain AKT activation [5]. With PTEN phosphatase dependent and phosphatase independent roles, its genetic modifications can lead to deregulated DNA repair, protein synthesis, and cell migration, cycle and survival. Initially, PTEN was identified as a transformed or deleted tumor suppressor gene in human cancers [9], and its down regulation is crucial for appropriate cutaneous wound healing. PTEN deletion has been seen to increase epithelial cell proliferation, migration and acceleration of wound healing [5].

3. AKT/mTOR and GSK3β pathways in wound healing

A network of signalling pathways stimulated by a variety of alert signalling peptides are initiated in response to tissue injury [5]. This response is not completely regulated by cellular intrinsic signalling, but rather as a result of coordinated signalling within and outside the wound milieu. In the wounded skin of mice, Gao et al., [23], observed a diversified alteration in the expression of PI3K, AKT, phosphorylated (p)-PI3K and p-AKT, with the expression of p-PI3K and p-AKT peaking during the inflammatory and proliferation phase, and the expression of PI3K and AKT peaking during the remodelling phase. This observation demonstrates the significant link of PI3K/AKT signalling to wound healing. Although AKT1 is regarded a critical isoform, and can adequately maintain cellular growth by itself as compared to AKT2 and AKT3 isoforms, they all are essential for proper cell growth and performance [18].

The PI3K/AKT/mTOR pathway is critical for cell migration and its reduced function prevents epithelial mesenchymal transition (EMT), cell proliferation and wound healing [24]. mTOR forms two unique complexes; mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 controls protein synthesis, which is followed by cell proliferation, migration, differentiation and survival. Apart from phosphorylated PI3K that has effects on mTORC2, other factors that lead to its activation remains unclear [25,26]. Phosphorylation of T308 and S473 amino acid residues by PDK1 and mTORC2, respectively, activates AKT [19], and unaided, AKT controls cellular activities concerned with cell cycle progression and survival [27]. Deregulated PI3K/AKT/mTOR signalling initiates the development of several diseases including cancer and its progression, obesity, cardiovascular diseases and diabetes [28]. Apart from regulating the expression of growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF) [14], stimulated AKT/mTOR promotes cellular growth and migration, angiogenesis and collagen synthesis [29]. During normal wound healing, a progressive up-regulation of p-AKT at serine-473 (ser-473) has been observed at the wound edge when related to the intact adjacent skin [14,5]. Xiao et al., [13], demonstrated that during wound healing, ozone oil enhance the migration of fibroblasts and the process of EMT via the PI3K/AKT/mTOR pathway. The activation of PI3K/AKT/mTOR signalling is characterised by the raised expression of p-AKT in the wounded tissue. As the activation of PI3K/AKT/mTOR accelerates wound healing, irreversible blocking of cell cycle progression by tumor suppressor p53 causes senescence of epithelial stem cells, and prevents PI3K/AKT/mTOR pathway induced proliferation [5,30]. Hoke et al. [4], observed a significant increase, at either a messenger ribonucleic acid (mRNA) or protein level, of the PI3K/AKT/mTOR pathway in a murine wound model during the various phases of wound healing.

Another AKT signalling substrate, the GSK-3β pathway, regulates various biological activities for cellular metabolism, migration, apoptosis, spreading and inflammation. This pathway, also plays a critical role in the development of diseases including diabetes and alzheimer's [17]. Inhibition of GSK3β via its phosphorylation at position Ser9 by AKT is essential for cutaneous wound healing [16]. GSK-3β substrates such as β-catenin and cyclin D1, are key proteins for promoting cell proliferation and survival. In DM reduced expression of β-catenin and cyclin D1 is one of the critical mechanisms of reduced process of wound healing [23,31]. PI3K/AKT signalling stimulates cellular functions via the inhibition of GSK3β, and this inhibition allows β-catenin to stabilize and translocate into the nucleus for gene transcription [23]. PI3K/AKT dependent GSK3ß (Ser9) phosphorylation, and hence GSK3ß inhibition, is critical in wound healing. Karrasch et al. [16], implicated PI3K/AKT/GSK3ß signalling in the intestinal epithelial cell wound healing response. Furthermore, Chen et al. [32], observed a significant increase of phosphorylated AKT-ser473 and GSK3β-ser9 in response to injury in normal fibroblast cells. Phosphorylated GSK3β, stimulates cellular migration [16], and its deficiency elevates collagen production, decrease apoptosis, and enhances wound resolution [33]. Moreover, GSK3β, in the regulation of cell survival and death, activates nuclear factor κB (NFκB) signalling, and controls the pro- and anti-apoptotic Bcl2 family of proteins in the intrinsic apoptosis pathway [34].

Apoptosis is a critical factor for the appropriate development and performance of the immune system, cell turnover, embryo development, and chemically induced cell death [35]. In tissue injury, loss of cellular signalling, injury of the DNA, energy stress and hypoxia triggers apoptosis, and concludes the inflammatory phase of wound repair. In the later phases of wound healing, apoptosis is responsible for clearing out macrophages, endothelial cells and myofibroblasts, leaving the wound with few cells, collagen and ECM proteins that are responsible for tensile strength of the healing wound [36,37]. Increased expression of the Bcl2 protein inhibits apoptosis thereby enhancing cellular survival [28]. Localised to the outer part of the mitochondrial membrane, Bcl2 plays a crucial role in the inhibition of pro-apoptotic activities. Pro-apoptotic proteins, such as Bcl2-like protein 4 (BAX) and Bcl2 homologous antagonist killer (BAK), promote mitochondrial membrane permeabilisation, discharge of cytochrome c and release of reactive oxygen species (ROS), critical signals for apoptosis. BAX and BAK are sequentially stimulated by Bcl2 homology 3 (BH3) only proteins, and are obstructed by Bcl2, a PI3K/AKT signalling dependent regulation of cellular apoptosis [38–41]. Cells undergoing apoptosis have raised cytoplasmic and reduced mitochondrial cytochrome c, and overexpression of Bcl2 prevents the release of mitochondrial cytochrome c and the induction of apoptosis [28].

4. AKT/mTOR and GSK3β pathways in diabetic wound healing

Diabetic wounds are associated with complications including continuous tissue loss, tissue and bone wound infection, limb amputation and increased mortality rate [4]. While activated RTKs promotes the stimulation of several signalling pathways for cellular glucose uptake, proliferation and migration [9], their capacity to stimulate wound healing is compromised in the presence of large wounds that are further aggravated by the presence of infection and disease such as DM [5]. Reduced activation of PI3K/AKT signalling diminishes cellular responses, including insulin uptake and the development of DM [15].

DM alters the activation of several signalling pathways, including epidermal growth factor receptor/PI3K/AKT and extracellular signal regulated kinases (EGFR/PI3K/AKT and ERKs), and downstream Bcl2 associated death promoter (BAD) signalling. This results in increased cellular apoptosis and decreased proliferation, leading to delayed wound healing [42]. Emanuelli et al. [43], elucidated that decreased fibroblast proliferation and migration in DM is due, not only to hyperglycemia, but similarly, to oxidative stress that is generated by the deposition of AGEs and oxidative altered proteins. Furthermore, they suggested that the inhibition of fibroblast proliferation, migration and collagen expression is enhanced through contact with AGE modified collagen and the stimulation of stress-activated kinase p38 and mitogen-activated protein kinase (MAPK). This process is facilitated via the down-regulation of extracellular signal regulated kinase 1 and 2 (ERK1/2) and AKT/PKB. Deregulated signalling pathways in diabetic wounds is linked to altered concentrations of miRNAs linked to several signalling pathway proteins including those of the PI3K/AKT/mTOR pathway [44]. Reduced expression of micro-RNA (miRNA) proteins related to a particular signalling pathway leads to a significant increase in the expression of signalling proteins involved in that particular pathway, and is followed by increased cell proliferation, migration and reduced apoptosis. In DM, increased levels of miRNA proteins related to PI3K/mTOR signalling is accompanied by reduced cell proliferation, migration, and accelerated apoptosis [45]. The mTOR pathway attracts a wider interest in scientific and clinical disciplines as a prospective therapeutic target in the treatment of various diseases related to metabolic abnormalities, aging, oxidative stress, proliferative disorders and diabetic wounds [46]. Diabetic wounds express varying levels of total and phosphorylated proteins related to AKT/mTOR and AKT/GSK3β signalling, leading to reduced cell proliferation, migration and survival (Fig. 1). In the wounded tissue of both non diabetic and diabetic rats, the AKT/mTOR signalling pathway is stimulated with a significant increase in total and phosphorylated major proteins related to the pathway [47]. Hoke and colleagues [4] found a significant increase in the expression of PI3K, AKT, GSK3β, total mTOR, phosphorylated (p)-mTOR, NF-κB and caspase 3 proteins related to the PI3K/AKT, NF-κB and β-catenin pathways in diabetic wounds. However, these proteins were significantly decreased in diabetic wounds as compared to non-diabetic wounds. In DM the PI3K/AKT pathway is impaired due to reduced 2-deoxyglucose uptake and reduced expression and regulation of GLUT-1 transporter, resulting in reduced glucose metabolism [48].

Deregulated regulation of apoptosis is one of the crucial contributing factors in the development of diabetic ulcers. Due to diminished stimulatory factors for cell survival, diabetic wounds display higher levels of apoptosis and diminished granulation tissue formation when compared with non-diabetic wounds [49]. Chronic wounds display increased cellular apoptosis originating from increased ROS that raises the expression of pro-apoptotic proteins such as caspases, CD95 L (FAS ligand) and BAX, with reduced expression of Bcl2 [36]. Jiang et al. [50], observed an increased level of inflammation and collagen matrix disruption with increased apoptosis and reduced growth factors/growth factor receptors in chronic pressure ulcers. Furthermore, Frykberg and Banks [6] observed that oxidative stress contributes significantly to the development of senescent cells, deoxyribonucleic acid (DNA) damage, cell cycle arrest and apoptosis in chronic wounds. This has been related to irregular metabolic changes and deregulated intracellular biochemical pathways in diabetic patients. Furthermore, cell apoptosis or cellular adaptation to survive can also be stimulated by the gravity of hypoxia. Oxygen performs a leading role during the wound healing processes, and poor oxygen supply to the site of injury determines the success of wound healing. Hypoxia-inducible factor-1 (HIF-1) and hypoxia-inducible factor-1 alpha (HIF-1α) stimulates angiogenesis, cellular proliferation, migration and reepithelialisation. Hyperglycaemia alters HIF-1α signalling, suppressing the expression of target genes related to HIF-1, the main controller of oxygen homeostasis [51,52]. HIF-1 α induction is mediated by the AKT/mTOR pathway, and Jing et al.,

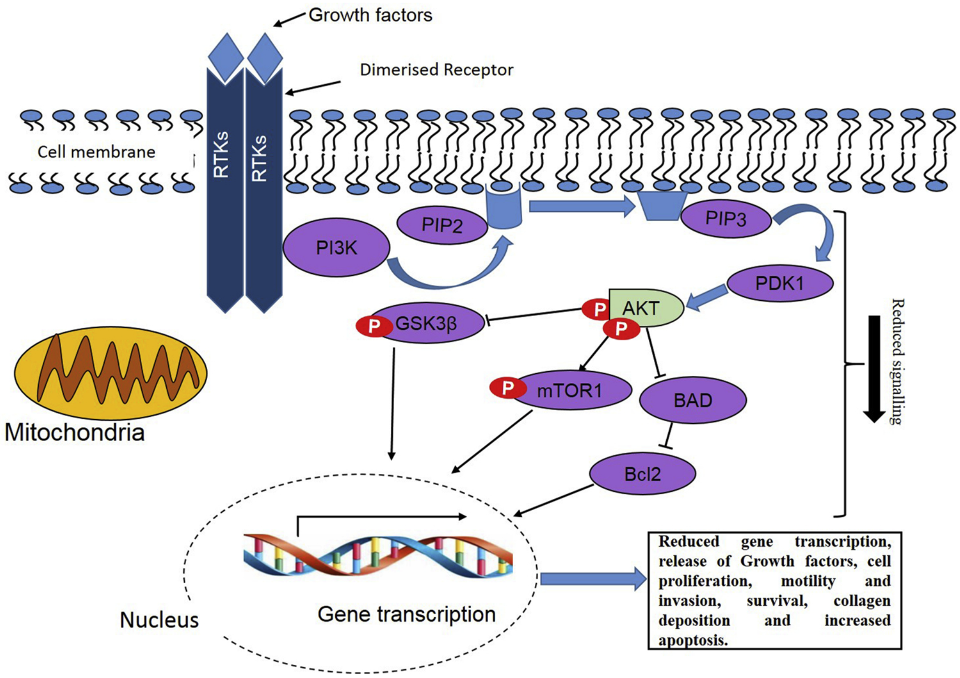

Fig. 1. Deregulated phosphatidylinositol 3 kinase/Protein kinase B (PI3K/AKT) signalling in diabetic wounds. PI3K/AKT signalling affects protein translation and transcription. The phosphatidylinositol-4,4-bisphosphate (PI-4,5-P_2) is phosphorylated by PI3K to generate phosphatidylinositol-3,4,5-triphosphate (PI3,4,5-P_3), which recruits kinase-3′-phosphoinositide-dependent kinase (PDK) and AKT. PDK phosphorylation activates AKT. At the AKT level, the signalling branches into several pathways including glycogen synthase kinase 3 beta (GSK3*β*), mTOR and Bcl2 that regulates downstream proteins controlling translation and transcription. In diabetes mellitus (DM), the activity of this signalling cascade is reduced, affecting downstream proteins that control translation and transcription leading to the reduced release of growth factors, cell proliferation, motility and invasion, survival, and collagen deposition, and increased cellular apoptosis.

[53] observed a significant decrease in wound healing and angiogenesis in diabetic mice in the presence of insufficient AKT/HIF-1*α* signalling activity.

5. Treatment of diabetic wounds

The treatment of diabetic ulcers remains a worldwide clinical challenge as the available treatment methods have been associated with limited efficacy, are very expensive, and their side effects are not completely evaluated [43]. In this case, preventing the development of diabetic ulcers is a major concern for wound management professionals. Currently, the healing rate of diabetic ulcers using the available therapeutic modalities stands at 50%, and mostly their effects are temporary with a high rate of relapse [6]. In this regard, treatment of diabetic ulcers demand a multidisciplinary linked attempt to diversify strategies that can be adopted. Treatment decisions and options are dependent upon the prognosis of the wound, including the use of invasive procedures for limb amputation, and at the same time avoiding the risks of unreasonable belligerent management of the patient [54]. Some treatments are based on the stimulation of cellular signalling pathways using synthetic growth factors to exert mitogenic effects on epithelial cells, fibroblasts, and endothelial cells, by targeting tyrosine kinase membrane receptors [55]. However, the response is diminished with the persistent presence of matrix metalloproteinases (MMPs), a common feature in diabetic ulcers. Furthermore, as mentioned earlier, most of these available treatment techniques lack substantial evidence to demonstrate therapeutic efficacy, and their use is therefore limited [6,56].

Recent advances in cellular and molecular science have significantly increased the knowledge pertaining to biological activities involved in wound healing, and diversified research into pharmacological and non-pharmacological treatment techniques to enhance chronic wound repair is currently an emergent part of biomedical science. Presently, innovative strategies, including pharmacological agents, tissue engineered structures, bio-active dressings, stem cell-constructed treatments, and non-pharmacological non-invasive therapeutic techniques using light energy (photobiomodulation-PBM or low level light therapy-LLLT) from lasers and LEDs are being investigated [57,58].

6. The effect of PBM on wound healing

PBM, previously known as "low-level laser therapy (LLLT)", was discovered by Endre Mester more than 50 years ago, and a ruby laser at a wavelength of 694 nm, and a helium neon (HeNe) laser at a wavelength of 633 nm were the initial instruments used. The mechanism of action for PBM has been unclear since its discovery, but recently there has been much progress made in clarifying the involvement of chromophores and signalling pathways [59]. PBM is a non-invasive, pain-free treatment methodology with no reported side effects, and is viewed as a potential treatment modality for diabetic wounds. PBM enhances cellular proliferation, viability and migration of fibroblasts, augments reepithelisation, enhances micro-circulation, and has anti-inflammatory and anti-bacterial effects through the production of ROS within the wound [60]. The biologic mechanism of PBM includes the absorption of photons by photo-acceptors or chromophores at the cellular, molecular and tissue level, resulting in the activation of cellular activities including recruitment of cytokines and growth factors, and activation of cellular signalling, leading to protein synthesis and deposition of the ECM, cellular proliferation, differentiation and migration [61]. Furthermore, PBM is known to promote tissue renewal, reduce inflammation and offer pain relief [62]. Photon energy (Fig. 2) from lasers or LEDs increases adenosine triphosphates (ATP), cyclic adenosine monophosphate (cAMP), ROS, nitric oxide (NO) and calcium (Ca^{2+}), that all play a critical role in initiating cellular signalling [63]. PBM employs coherent (lasers) and non-coherent (LED) light sources, and sometimes both lasers and LEDs combined, in the visible red and near infrared (NIR) electromagnetic spectrum [64,57].

According to the Arndt–Schulz law, on which PBM is based, a weak stimulus adjusts a certain biological function, and as the stimulus becomes stronger, the effect is further elevated. Eventually, a maximum response is achieved, and additional stimulus effects an undesirable response. Thus, in PBM, light parameters and doses used are essential [63]. At a molecular level, PBM modulates the electrochemical system within the cell, including mitochondrial cytochrome c oxidase (COX) signalling. This facilitates the removal of electrons from cytochrome c to molecular oxygen [65]. It is reported that PBM yields a shift in cellular redox potential towards greater oxidation with increased ROS generation. The cell redox status controls cellular signalling pathways which regulates gene expression, and alteration of cell redox state

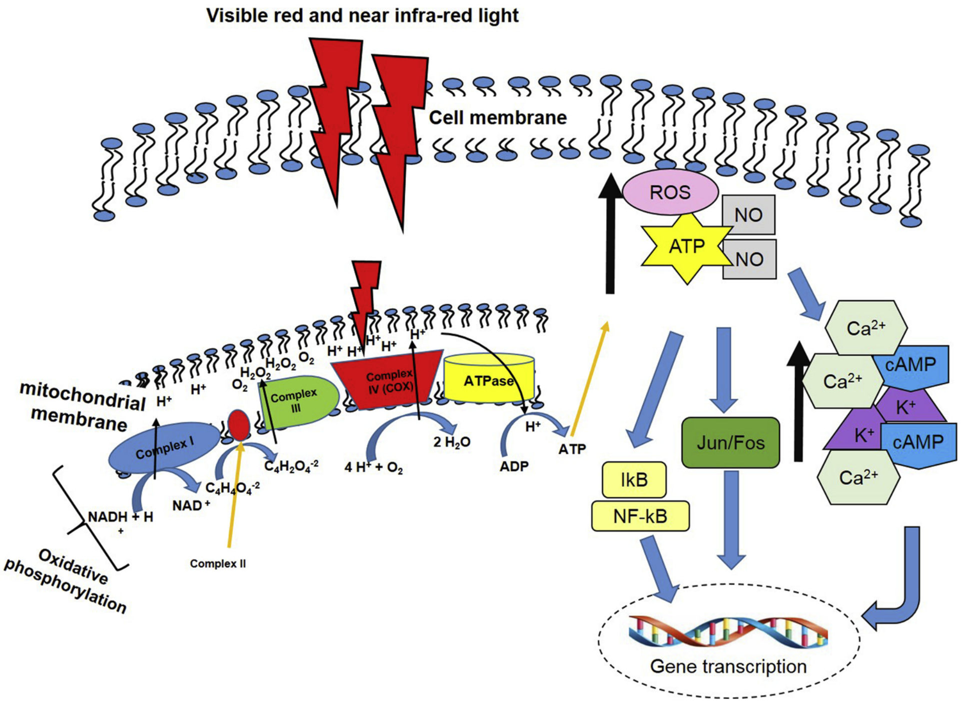

Fig. 2. Cellular molecular mechanisms after photobiomodulation (PBM) in chronic wounds. Cytochrome C oxidase - COX (unit iv), present on the mitochondrial inner membrane, absorbs photons from lasers or LEDs that increases its capability to pump hydrogen ions (H^+), leading to increased production of adenosine triphosphate (ATP), nitric oxide (NO) and reactive oxygen species (ROS) through oxidative phosphorylation. This initiates gene transcription leading to growth factors, cytokines and chemokine secretion, collagen deposition, cell metabolism, proliferation, migration and tissue reepithelialisation.

activates or obstructs numerous intracellular signalling pathways involved with enzyme activation and cell survival [66].

Shingyochi et al. [67] studied the effect of PBM with a carbon dioxide (CO_2) laser on fibroblast proliferation and migration in wound healing. They observed increased fibroblast cell proliferation and migration, and an eventual wound size reduction induced via activated AKT, ERK and JNK signalling pathways. In another study, Kiyosaki et al. [68] observed that the expression of runt-related transcription factor 2 (Runx2) and phosphorylation of ERK in irradiated osteoblasts influenced the mineralisation of osteoblasts through the synthesis of insulin-like growth factor I (IGF-I) and bone morphogenetic protein (BMP). ERK signalling is a significant activator of the MAPK pathway, and plays a critical role in controlling cellular processes such as proliferation, development, differentiation, survival and apoptosis [69]. MAPKs are comprised of three key signalling members, ERK, C-JUN N-terminal kinase (JNK) and p38. El-Makakey et al. [70] examined the effects of pulsed electromagnetic field therapy (PEMFT) and PBM using a pulsed wave diode laser at a wavelength of 905 nm with a maximum power output of 25 W and impulse duration of 100 ns on the human body. The MAPK expression level was adopted as an indicator via evaluation of activated levels of ERK, p38 and JNK in lymphocytes of patients before and after treatment. They observed that both PEMFT and PBM increased the activation of ERK, while PEMFT increased the activation of all the MAPK signalling members in human skin.

Cells irradiated with a HeNe laser at a wavelength of 632.8 nm and a power density of 12.74 mW/cm^2, displayed a significant increase in the activation of the ERK-specificity protein 1 (ERK-Sp1) signalling pathway [71]. ERK-Sp1 signalling is involved in the expression of VEGF and proliferation of vascular endothelial cells. Effective wound healing is achieved with restoration of blood flow to maintain the supply of nutrients and oxygen to the injured tissue for growth and function of cells. Neoangiogenesis is critical in the wound repair process for the development of new blood vessels. VEGF is the most potent pro-neoangiogenic growth factor that significantly influences wound healing [72]. In the ERK-Sp1 pathway, activation of ERK leads to increased interaction and phosphorylation of Sp1, resulting in the increased regulation of VEGF [73]. Kawano et al. [74], elucidated that PBM at a wavelength of 830 nm and an energy output of 60 mW (60 s irradiation time) stimulates MAPK and ERK induced synthesis of VEGF in immortalised granulosa (KGN) cells. In our previous study, we reported that PBM at a wavelength of 660 nm and a fluence of 5 J/cm^2 activated cell proliferation, viability and migration of diabetic wounded fibroblast cells *in vitro* through the release of EGF and subsequent phosphorylation of EGFR and the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling proteins [75]. Dang et al. [76], observed that PBM at a wavelength of 800 nm and a fluence of 40 J/cm^2 is effective in enhancing skin structure and expression of new collagen. They established that collagen synthesis was stimulated via activation of the TGF-β/Smad signalling pathway. Furthermore, Sperandio et al., [77] showed that PBM used at the wavelength of 660 nm or 780 nm, power output of 40 mW, and energy density of 2.05, 3.07 or 6.15 J/cm^2 alters the growth of oral dysplastic cells (DOK) and cancer cells (SCC9 and SCC25) via the modification of AKT/mTOR/CyclinD1 signalling.

Several signalling pathways, including EGF/EGFR, STAT, focal adhesion kinase (FAK) and MAPK are modulated by Src non-receptor tyrosine kinases [78,79]. The increased cellular ROS production post-PBM explains the initiation of signalling pathways in wound healing. Src kinases are triggered as a consequence of increased intracellular ROS levels, and are critically involved in essential cellular processes involved in wound healing, including proliferation and migration [80]. Song et al. [81], analysed the influence of a HeNe laser at a wavelength of 632.8 nm and a power density of 64.6 mW/cm^2 on activated microglia cells, and subsequent induction of signalling pathways involved in neuroprotective and phagocytic responses. They observed that PBM decreased toll-like receptor (TLR) mediated pro-inflammatory reactions in microglia, suggesting that Src tyrosine kinases are activated as a result of an altered MyD88-dependent pro-inflammatory signalling cascade. Activated PI3K/AKT/mTOR and PI3K/AKT/GSK3Kβ signalling is related to several activities that are linked to the positive influence of PBM including proliferation, migration, angiogenesis and cell survival and wound healing [82].

7. Conclusion and future directions

As a non-thermal phototherapy, PBM is used in a number of medical applications, including pain reduction and wound healing achieved via direct photon action on COX and indirect cellular alteration of tyrosine

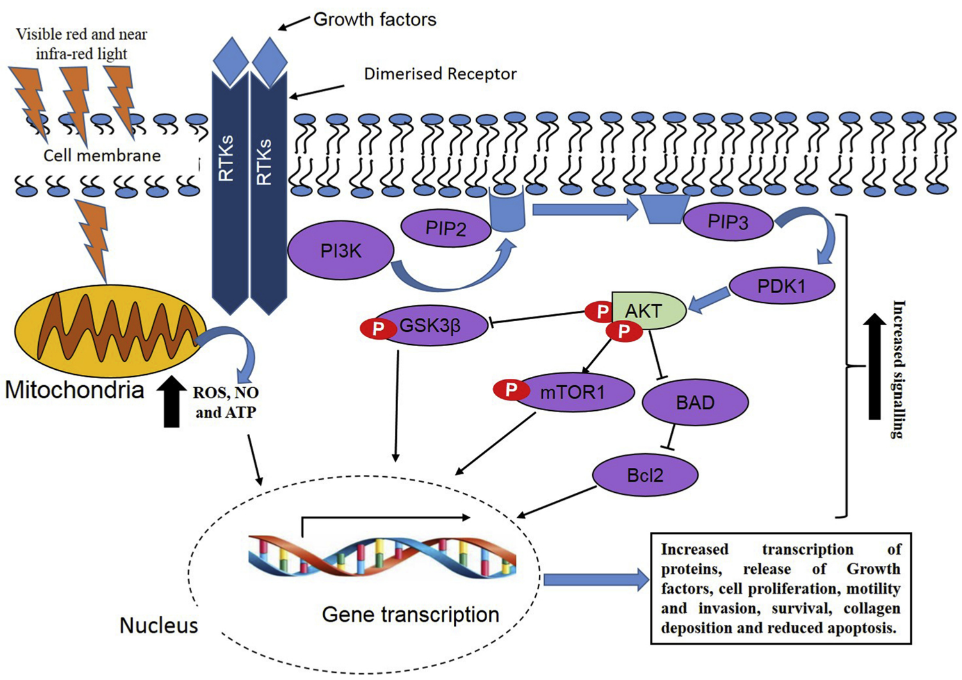

Fig. 3. The presumed effect of photobiomodulation (PBM) on the activation of the phosphatidylinositol 3 kinase/Protein kinase B (PI3K/AKT) signalling pathway in diabetic wounded cells. PI3K/AKT signalling affects protein translation and transcription. The phosphatidylinositol-4,4-bisphosphate (PI-4,5-P_2) is phosphorylated by PI3K to generate phosphatidylinositol-3,4,5-triphosphate (PI3,4,5-P_3), which recruits kinase-3′-phosphoinositide-dependent kinase (PDK) and AKT. PDK phosphorylation activates AKT. At the AKT level, the signalling branches into several pathways including the glycogen synthase kinase 3 beta (GSK3*β*), mTOR and Bcl2 that regulates downstream proteins controlling translation and transcription. In diabetes mellitus (DM), the activity of this signalling cascade is reduced affecting downstream proteins that control translation and transcription leading to the reduced release of growth factors, cell proliferation, motility and invasion, survival, collagen deposition and increased cellular apoptosis. PBM is presumed to revive PI3K/AKT signalling in diabetic wounds, resulting in the increased release of growth factors, cell proliferation, motility and invasion, collagen deposition and reduced apoptosis.

kinases and tyrosine kinase receptors [83]. The attribution of PBM in the ignition of the AKT/mTOR and AKT/GSK3*β* pathways has been reported in cell migration and survival [63,82,84,85]. PBM increases proliferation and migration of fibroblast cells during the wound healing process. Yet, the precise underlying mechanisms at a molecular and cellular level have not been formerly defined.

Diabetic wounds have increased levels of pro-inflammatory cytokines, ROS, proteases, and senescent cells, and reduced growth factors/receptors and cellular signalling activity, and are a major challenge for patients, wound care specialists and clinicians. With recent advances in the management of diabetic wounds and the identification of the underlying pathophysiology, innovative strategies, such as non-pharmacological, non-invasive therapeutic techniques, including PBM should be investigated. Although the PI3K/AKT pathway is among the commonly dysregulated signalling pathways linked to cancer, the activated PI3K/AKT pathway is involved in many activities that are related to PBMs beneficial effects on wound healing. PBM is believed to activate reepithelialisation in diabetic wounded cells via a complex of signalling pathways including PI3K/AKT that triggers downstream signalling responses, including the release of growth factors (Fig. 3). For PBM to be completely accepted and used as a treatment methodology, there is a need to understand the molecular biology which initiates cellular functions after irradiation. Work is in progress in our laboratory (Laser Research Centre, LRC, Faculty of Health Sciences, University of Johannesburg) regarding the effect of PBM on cell molecular mechanisms, specifically the activated cell signalling pathways in diabetic wound healing.

Authors' contributions

SWJ created, prepared and wrote the initial draft of the article. NNH is the postgraduate supervisor of lead author. She played an oversight and supervisory responsibility, including mentorship, planning and execution of the research. She was involved in critical revision of drafts of the manuscript for intellectual content. HA provided management and coordination responsibility for the research activity. She provided professional guidance and supplied editorial input and is a co-supervisor of the lead author. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Not applicable

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Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] K.A. Bielefeld, S. Amini-Nik, B.A. Alman, Cutaneous wound healing: recruiting developmental pathways for regeneration, Cell. Mol. Life Sci. 70 (2013) 2059–2081.

[2] T.N. Demidova-Rice, M.R. Hamblin, I.M. Herman, Acute and impaired wound healing: pathophysiology and current methods for drug delivery, part 1: normal and chronic wounds: biology, causes, and approaches to care, Adv. Skin Wound Care 25 (2012) 304–314.

[3] B.K. Sun, Z. Siprashvili, P.A. Khavari, Advances in skin grafting and treatment of cutaneous wounds, Science 346 (2014) 941–945.

[4] G.D. Hoke, C. Ramos, N.N. Hoke, M.C. Crossland, L.G. Shawler, J.V. Boykin, Atypical diabetic foot ulcer keratinocyte protein signaling correlates with impaired wound healing, J. Diabetes Res. (2016) 1586927, https://doi.org/10.1155/2016/1586927.

[5] R.M. Castilho, C.H. Squarize, J.S. Gutkind, Exploiting PI3K/mTOR signaling to accelerate epithelial wound healing, Oral Dis. 19 (2013) 551–558.

[6] R.G. Frykberg, J. Banks, Challenges in the treatment of chronic wounds, Adv. Wound Care 4 (2015) 560–582.

[7] S. Demirci, A. Doğan, H. Apdik, E.C. Tuysuz, S. Gulluoglu, O.F. Bayrak, et al., Cytoglobin inhibits migration through PI3K/AKT/mTOR pathway in fibroblast cells, Mol. Cell. Biochem. 437 (2018) 133–142.

[8] S.Z. Safi, R. Qvist, S. Kumar, K. Batumalaie, I.S. Ismail, Molecular mechanisms of diabetic retinopathy, general preventive strategies, and novel therapeutic targets, Biomed Res. Int. (2014) 801269.

[9] S.A.H. Khorami, A. Movahedi, K. Huzwah, A.M.M. Sokhini, PI3K/AKT pathway in modulating glucose homeostasis and its alteration in diabetes, AMBS 1 (2015) 46–55.

[10] M. Narres, T. Kvitkina, H. Claessen, S. Droste, B. Schuster, S. Morbach, et al., Incidence of lower extremity amputations in the diabetic compared with the non-diabetic population: a systematic review, PLoS One 12 (2017) e0182081.

[11] M.S. Bitar, The GSK-3β/Fyn/Nrf2 pathway in fibroblasts and wounds of type 2 diabetes: on the road to an evidence-based therapy of non-healing wounds, Adipocyte 3 (2012) 161–163.

[12] T.H. Peiris, Ramirez D, P.G. Barghouth, N.J. Oviedo, The Akt signaling pathway is required for tissue maintenance and regeneration in planarians, BMC Dev. Biol. 16 (2016) 7.

[13] W. Xiao, H. Tang, M. Wu, Y. Liao, K. Li, L. Li, et al., Ozone oil promotes wound healing by increasing the migration of fibroblasts via PI3K/Akt/mTOR signaling pathway, Biosci. Rep. 37 (2017) BSR20170658.

[14] C.H. Squarize, R.M. Castilho, T.H. Bugge, J.S. Gutkind, Accelerated wound healing by mTOR activation in genetically defined mouse models, PLoS One 5 (2010) e10643, , https://doi.org/10.1371/journal.pone.0010643.

[15] T. Li, G. Wang, Computer-aided targeting of the PI3K/AKT/mTOR pathway: toxicity reduction and therapeutic opportunities, Int. J. Mol. Sci. 15 (2014) 18856–18891.

[16] C.P. Daniel, M.R. Johann, S.H. Jan-Hendrik, Regulation of glycogen synthase from mammalian skeletal muscle – a unifying view of allosteric and covalent regulation, FEBS J. 280 (2013) 2–27.

[17] T. Karrasch, T. Spaeth, B. Allard, C. Jobin, PI3K-dependent GSK3ß (Ser9)-Phosphorylation is implicated in the intestinal epithelial cell wound healing response, PLoS One 6 (2011) e26340.

[18] U. Maurer, F. Preiss, P. Brauns-Schubert, L. Schlicher, C. Charvet, GSK-3 – at the crossroads of cell death and survival, J. Cell. Sci. 127 (2014) 1369–1378.

[19] J.L. Armstrong, S.M. Bonavaud, B.J. Toole, S.J. Yeaman, Regulation of glycogen synthesis by amino acids in cultured human muscle cells, J. Biol. Chem. 276 (2001) 952–956.

[20] J.S.L. Yu, W. Cui, Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination, Development 143 (2016) 3050–3060.

[21] H.J. Lim, P. Crowe, J.L. Yang, Current clinical regulation of PI3K/PTEN/Akt/mTOR signalling in treatment of human cancer, J. Cancer Res. Clin. Oncol. 141 (2015) 671–689.

[22] L. Wang, J. Wu, J. Lu, R. Ma, Sun D, J. Tang, Regulation of the cell cycle and PI3K/Akt/mTOR signaling pathway by tanshinone I in human breast cancer cell lines, Mol. Med. Rep. 11 (2015) 931–939.

[23] Y.L. Gao, C.S. Liu, R. Zhao, L.L. Wang, S.S. Li, M. Liu, et al., Effects of PI3K/Akt pathway in wound healing process of mice skin, Fa Yi Xue Za Zhi 32 (2016) 7–12.

[24] H.L. Yang, Y.C. Tsai, M. Korivi, C.T. Chang, Y.C. Hseu, Lucidone promotes the cutaneous wound healing process via activation of the PI3K/AKT, Wnt/β-catenin and NF-κB signalling pathways, Biochim. Biophys. Acta 1864 (2017) 151–168.

[25] P. Zarogoulidis, S. Lampaki, J.F. Turner, H. Huang, S. Kakolyris, K. Syrigos, et al., mTOR pathway: a current, up-to-date mini-review (Review), Oncol. Lett. 8 (2014) 2367–2370.

[26] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell. 149 (2012) 274–293.

[27] C. Porta, C. Paglino, A. Mosca, Targeting PI3K/Akt/mTOR signaling in cancer, Front. Oncol. 4 (2014) 64.

[28] A.C. Hsieh, Y. Liu, M.P. Edlind, N.T. Ingolia, M.R. Janes, A. Sher, et al., The translational landscape of mTOR signalling steers cancer initiation and metastasis, Nature 485 (2012) 55–61.

[29] C. Huang, S. Sheng, R. Li, X. Sun, J. Liu, G. Huang, Lactate promotes resistance to glucose starvation via upregulation of Bcl-2 mediated by mTOR activation, Oncol. Rep. 33 (2015) 875–884.

[30] E. Strozyk, D. Kulms, The role of AKT/mTOR pathway in stress response to UV-Irradiation: implication in skin carcinogenesis by regulation of apoptosis, autophagy and senescence, Int. J. Mol. Sci. 14 (2013) 15260–15285.

[31] J. Peng, G. Ramesh, L. Sun, Z. Dong, Impaired wound healing in hypoxic renal tubular cells: roles of hypoxia-inducible Factor-1 and glycogen synthase kinase 3β/β-catenin signaling, J. Pharmacol. Exp. Ther. 340 (2012) 176–184.

[32] Z. Chen, X. Wang, T. Jin, Y. Wang, C.S. Hong, L. Tan, et al., Increase in the radioresistance of normal skin fibroblasts but not tumor cells by mechanical injury, Cell Death Dis. 8 (2017) e2573.

[33] M.H.M. Lima, A.M. Caricilli, L.L. de Abreu, E.P. Araújo, F.F. Pelegrinelli, A.C.P. Thirone, et al., Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: a double-blind placebo-controlled clinical trial, PLoS One 7 (2012) e36974.

[34] U. Maurer, F. Preiss, P. Brauns-Schubert, L. Schlicher, C. Charvet, GSK-3 – at the crossroads of cell death and survival, J. Cell. Sci. 127 (2014) 1369–1378.

[35] Y.S. Wu, S.N. Chen, Apoptotic cell: linkage of inflammation and wound healing, Front. Pharmacol. 5 (2014) 1.

[36] A.N. Hata, J.A. Engelman, A.C. Faber, The BCL-2 family: key mediators of the apoptotic response to targeted anti-cancer therapeutics, Cancer Discov. 5 (2015) 475–487.

[37] A.K. Arya, R. Tripathi, S. Kumar, K. Tripathi, Recent advances on the association of apoptosis in chronic non-healing diabetic wound, World J. Diabetes 5 (2014) 756–762.

[38] J.M. Hardwick, L. Soane, Multiple functions of BCL2 family proteins, Cold Spring Harb. Perspect. Biol. 5 (2013) a008722.

[39] G. Song, G.L. Ouyang, S.D. Bao, The activation of Akt/PKB signalling pathway and cell survival, J. Cell. Mol. Med. 9 (2005) 59–71.

[40] T. Liu, L. Zhang, D. Joo, S.C. Sun, NF-κB signaling in inflammation, Signal Transduct. Target. Ther. 2 (2017) e17023.

[41] I. Vancurova, A. Vancura, Regulation and function of nuclear IκBα in inflammation and cancer, Am. J. Clin. Exp. Immunol. 1 (2012) 56–66.

[42] K. Xu, F.S. Yu, Impaired epithelial wound healing and EGFR signalling pathways in the corneas of diabetic rats, Invest. Ophthalmol. Vis. Sci. 52 (2011) 3301–3308.

[43] T. Emanuelli, A. Burgeiro, E. Carvalho, Effects of insulin on the skin: possible healing benefits for diabetic foot ulcers, Arch. Dermatol. Res. 308 (2016) 677–694.

[44] J. Moura, E. Borsheim, E. Carvalho, The role of MicroRNAs in diabetic complications-special emphasis on wound healing, Genes (Basel) 5 (2014) 926–956.

[45] E.J. Mulholland, N. Dunne, H.O. McCarthy, MicroRNA as therapeutic targets for chronic wound healing, Mol. Ther. Nucleic Acids 8 (2017) 46–55.

[46] A. Das, F. Reis, Y. Maejima, Z. Cai, J. Ren, mTOR signaling in cardiometabolic disease, Cancer, and aging, Oxid. Med. Cell. Longev. (2017) 6018675, https://doi.org/10.1155/2017/6018675.

[47] H. Huang, W. Cui, W. Qiu, M. Zhu, R. Zhao, D. Zeng, et al., Impaired wound healing results from the dysfunction of the Akt/mTOR pathway in diabetic rats, J. Dermatol. Sci. 79 (2015) 241–251.

[48] J.L. Jacot, D. Sherris, Potential therapeutic roles for inhibition of the PI3K/Akt/mTOR pathway in the pathophysiology of diabetic retinopathy, J. Ophthalmol. (2011) 589813, https://doi.org/10.1155/2011/589813.

[49] S. Bhan, R. Mitra, A.K. Arya, H.P. Pandey, K. Tripathi, A study on evaluation of apoptosis and expression of Bcl-2-Related marker in wound healing of streptozotocin-induced diabetic rats, ISRN Dermatol. (2013) 739054, https://doi.org/10.1155/2013/739054.

[50] L. Jiang, Y. Dai, F. Cui, Y. Pan, H. Zhang, J. Xiao, et al., Expression of cytokines, growth factors and apoptosis-related signal molecules in chronic pressure ulcer wounds healing, Spinal Cord 52 (2014) 145–151.

[51] W.X. Hong, M.S. Hu, M. Esquivel, G.Y. Liang, R.C. Rennert, A. McArdle, et al., The role of hypoxia-inducible factor in wound healing, Adv. Wound Care 3 (2014) 390–399.

[52] I.R. Botusan, V.G. Sunkari, O. Savu, A.I. Catrina, J. Grunler, S. Lindberg, et al., Stabilization of HIF-1 is critical to improve wound healing in diabetic mice, Proc. Natl. Acad. Sci. U.S.A. 105 (2009) 19426–19431.

[53] L. Jing, S. Li, Q. Li, Akt/hypoxia-inducible factor-1α signaling deficiency compromises skin wound healing in a type 1 diabetes mouse model, Exp. Ther. Med. 9 (2015) 2141–2146.

[54] X. Shu, S. Shu, S. Tang, L. Yang, D. Liu, K. Li, et al., Efficiency of stem cell based therapy in the treatment of diabetic foot ulcer: a meta-analysis, Endocr. J. 65 (2018) 403–413.

[55] J. Berlanga-Acosta, J. Fernández-Montequín, C. Valdés-Pérez, W. Savigne-Gutiérrez, Y. Mendoza-Marí, A. García-Ojalvo, et al., Diabetic foot ulcers and epidermal growth factor: revisiting the local delivery route for a successful outcome, Biomed Res. Int. (2017) 2923759, https://doi.org/10.1155/2017/2923759.

[56] L. Yazdanpanah, M. Nasiri, S. Adarvishi, Literature review on the management of diabetic foot ulcer, World J. Diabetes 6 (2015) 37–53.

[57] A. Yadav, A. Gupta, Noninvasive red and near-infrared wavelength-induced photobiomodulation: promoting impaired cutaneous wound healing, Photodermatol. Photoimmunol. Photomed. 33 (2017) 4–13.

[58] E. Zhang, B. Gao, L. Yang, X. Wu, Z. Wang, Notoginsenoside Ft1 promotes fibroblast proliferation via PI3K/Akt/mTOR signaling pathway and benefits wound healing in genetically diabetic mice, J. Pharmacol. Exp. Ther. 356 (2016) 324–332.

[59] M.R. Hamblin, Mechanisms and applications of the anti-inflammatory effects of photobiomodulation, AIMS Biophys. 4 (2017) 337–361.

[60] K.H. Beckmann, G. Meyer-Hamme, S. Schröder, Low level laser therapy for the treatment of diabetic foot ulcers: a critical survey, Evid. Complement. Alternat. Med. (2014) 626127, https://doi.org/10.1155/2014/626127.

[61] C.N. Tchanque-Fossuo, D. Ho, S.E. Dahle, E. Koo, C. Li, R.R. Isseroff, et al., A systematic review of low-level light therapy for treatment of diabetic foot ulcer, Wound Repair Regen. 24 (2016) 418–426.

[62] E. Vinesh, S.M. Jeyapriya, M.S. Kumar, M. Arunachalam, Photobiomodulation and oral wound healing, Indian J. Multidiscip. Dent. 7 (2017) 129–134.

[63] L.F. De Freitas, M.R. Hamblin, Proposed mechanisms of photobiomodulation or low-level light therapy, IEEE J. Sel. Top. Quantum Electron. 22 (2016) 7000417, https://doi.org/10.1109/JSTQE.2016.2561201.

[64] J.J. Anders, R.J. Lazalame, P.R. Arany, Low-level light/laser therapy versus photobiomodulation therapy, Photomed. Laser Surg. 33 (2015) 183–184.

[65] T. Obayashi, K. Funasaka, E. Ohno, R. Miyahara, Y. Hirooka, M. Hamaguchi, et al., Treatment with near-infrared radiation promotes apoptosis in pancreatic cancer cells, Oncol. Lett. 10 (2015) 1836–1840.

[66] S. Farivar, T. Malekshahabi, R. Shiari, Biological effects of low level laser therapy, J.

Laser Med sci. 5 (2014) 58–62.

[67] Y. Shingyochi, S. Kanazawa, S. Tajima, R. Tanaka, H. Mizuno, M. Tobita, A low-level carbon dioxide laser promotes fibroblast proliferation and migration through activation of akt, ERK, and JNK, PLoS One 12 (2017) e0168937, , https://doi.org/10.1371/journal.pone.0168937.

[68] T. Kiyosaki, N. Mitsui, N. Suzuki, N. Shimizu, Low-level laser therapy stimulates mineralization via increased Runx2 expression and ERK phosphorylation in osteoblasts, Photomed. Laser Surg. 1 (2010) 167–172.

[69] Y.D. Shaul, R. Seger, The MEK/ERK Cascade: From Signaling Specificity to Diverse Functions 1773, (2007), pp. 1213–1226.

[70] A.M. El-Makakey, R.M. El-Sharaby, M.H. Hassan, A. Balbaa, Comparative study of the efficacy of pulsed electromagnetic field and low level laser therapy on mitogen-activated protein kinases, Biochem. Biophys. Rep. 9 (2017) 316–321.

[71] L. Pang, Y. Zhang, Y. Yu and, S. Zhang, Resistin promotes the expression of vascular endothelial growth factor in ovary carcinoma cells, Int. J. Mol. Sci. 14 (2013) 9751–9766.

[72] K.E. Johnson, T.A. Wilgus, Vascular endothelial growth factor and angiogenesis in the regulation of cutaneous wound repair, Adv. Wound Care (New Rochelle) 3 (2014) 647–661.

[73] J. Feng, Y. Zhang, D. Xing, Low-power laser irradiation (LPLI) promotes VEGF expression and vascular endothelial cell proliferation through the activation of ERK/Sp1 pathway, Cell. Signal. 24 (2012) 1116–1125.

[74] Y. Kawano, Y. Utsunomiya-Kai, K. Kai, I. Miyakawa, T. Ohshiro, H. Narahara, The production of VEGF involving MAP kinase activation by low level laser therapy in human granulosa cells, Laser Ther. 21 (2012) 269–274.

[75] S.W. Jere, H. Abrahamse, N.N. Houreld, Photobiomodulation at 660 nm stimulates proliferation and migration of diabetic wounded cells via the expression of epidermal growth factor and the JAK/STAT pathway, J. Photochem. Photobiol 179 (2018) 74–83.

[76] Y. Dang, B. Liu, L. Liu, X. Ye, X. Bi, Y. Zhang, J. Gu, The 800nm diode laser irradiation induces skin Collagen synthesis by stimulating TGF-beta/smad signalling pathway, Lasers Med. Sci. 26 (2011) 837–843.

[77] I. Khan, P. Arany, Biophysical approaches for oral wound healing: emphasis on photobiomodulation, Adv. Wound Care (New Rochelle) 4 (2015) 724–737.

[78] J. Zhang, D. Xing, X. Gao, Low-power laser irradiation activates Src tyrosine kinase through reactive oxygen species-mediated signalling pathway, J. Cell. Physiol. 217 (2008) 518–528.

[79] S. Emanuele, A. D'Anneo, G. Calvaruso, C. Cernigliaro, M. Giuliano, M. Lauricella, The double-edged sword profile of redox signaling: oxidative events as molecular switches in the balance between cell physiology and cancer, Chem. Res. Toxicol. 31 (2018) 201–210.

[80] S. Song, F. Zhou, W.R. Chen, Low-level laser therapy regulates microglial function through Src-mediated signaling pathways: implications for neurodegenerative diseases, J. Neuroinflammation 9 (2012) 219, https://doi.org/10.1186/1742-2094-9-219.

[81] F.F. Sperandio, F.S. Giudice, L. Corrêa, S.P. Décio, M.R. Hamblin, et al., Low-level laser therapy can produce increased aggressiveness of dysplastic and oral cancer cell lines by modulation of Akt/mTOR signaling pathway, J. Biophotonics 6 (2013) 839–847 J.A..

[82] J.E. Zecha, R.G. Raber-Durlacher, J.B. Nair, S.T. Epstein, S.Elad Sonis, et al., Low level laser Therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations, Support. Care Cancer 24 (2016) 2781–2792. A.D. Liebert, B.T. Bicknell and.

[83] R.D. Adams, Protein conformational modulation by photons: a mechanism for laser treatment effects, Med. Hypotheses 82 (2014) 275–281.

[84] A.C.A. Pellicioli, M.D. Martins, C.S. Dillenburg, M.M. Marques, C.H. Squarize, et al., Laser phototherapy accelerates oral keratinocyte migration through the modulation of the mammalian target of rapamycin signaling pathway, J. Biomed. Opt. 19 (2014) 2, https://doi.org/10.1117/1.JBO.19.2.028002.

[85] L. Zhang, D. Xing, X. Gao, S. Wu, Low-power laser irradiation promotes cell proliferation by activating PI3K/Akt pathway, J. Cell. Physiol. 219 (2009) 553–562.

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