

UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Odontologia
Colegiado de Pós-Graduação em Odontologia

Rafaela Ferrer de Oliveira

**FOTOATIVAÇÃO DE UMA POPULAÇÃO HETEROGÊNEA DE
CÉLULAS CUTÂNEAS EM UM MODELO DIABÉTICO *IN VITRO***

Belo Horizonte
2023

Rafaela Ferrer de Oliveira

FOTOATIVAÇÃO DE UMA POPULAÇÃO HETEROGÊNEA DE CÉLULAS CUTÂNEAS EM UM MODELO DIABÉTICO *IN VITRO*

Dissertação apresentada ao Colegiado do Programa de Pós-graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Mestre em Odontologia – área de concentração em Clínica Odontológica

Orientadora: Profa. Dra. Ivana Marcia Alves Diniz

Coorientadora: Profa. Dra. Marina Gonçalves Diniz

Belo Horizonte
2023

Ficha Catalográfica

048f Oliveira, Rafaela Ferrer de.
2023 Fotoativação de uma população heterogênea de células
T cutâneas em um modelo diabético in vitro / Rafaela Ferrer de
Oliveira. -- 2023.

57 f. : il.

Orientadora: Ivana Marcia Alves Diniz.
Coorientadora: Marina Gonçalves Diniz.

Dissertação (Mestrado) -- Universidade Federal de Minas
Gerais, Faculdade de Odontologia.

1. Fototerapia. 2. Terapia com luz de baixa intensidade.
3. Diabetes mellitus. 4. Ferimentos e lesões. 5.
Cicatrização. I. Diniz, Ivana Marcia Alves. II. Diniz,
Marina Gonçalves. III. Universidade Federal de Minas Gerais.
Faculdade de Odontologia. IV. Título.

BLACK - D047

Elaborada por: Mateus Henrique Silva Trindade - CRB 6/3883.



UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE ODONTOLOGIA
COLEGIADO DO CURSO DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

FOTOATIVÇÃO DE UMA POPULAÇÃO HETEROGÊNEA DE CÉLULAS CUTÂNEAS EM UM MODELO DIABÉTICO EX VIVO

RAFAELA FERRER DE OLIVEIRA

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA, como requisito para obtenção do grau de Mestre em ODONTOLOGIA, área de concentração CLÍNICA ODONTOLÓGICA.

Aprovada em 29 de março de 2023, pela banca constituída pelos membros:

Profa. Ivana Márcia Alves Diniz - Orientadora
Faculdade de Odontologia da UFMG

Profa. Marina Gonçalves Diniz
Faculdade de Odontologia da UFMG

Profa. Sara Santos Bernardes
UFMG

Prof. Paulo Eduardo Alencar de Souza
PUC - MINAS

Belo Horizonte, 29 de março de 2023.



Documento assinado eletronicamente por **Ivana Marcia Alves Diniz, Professora do Magistério Superior**, em 29/03/2023, às 17:26, conforme horário oficial de Brasília, com fundamento no art. 5º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Sara Santos Bernardes, Professora do Magistério Superior**, em 29/03/2023, às 17:27, conforme horário oficial de Brasília, com fundamento no art. 5º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Paulo Eduardo Alencar de Souza, Usuário Externo**, em 29/03/2023, às 17:27, conforme horário oficial de Brasília, com fundamento no art. 5º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



Documento assinado eletronicamente por **Marina Goncalves Diniz, Professora do Magistério Superior**, em 29/03/2023, às 17:29, conforme horário oficial de Brasília, com fundamento no art. 5º do [Decreto nº 10.543, de 13 de novembro de 2020](#).



A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **2169086** e o código CRC **23009BF0**.

Dedico este trabalho a todas as mulheres -
pesquisadoras, professoras, mães - que me
inspiraram e me deram força para seguir
meu caminho profissional.

AGRADECIMENTOS

Primeiramente, agradeço aos meus pais, irmãos e meu marido pelo apoio incondicional e porto seguro nos momentos de dificuldade. Sem eles, com certeza, eu não teria chegado até aqui.

À minha orientadora, Ivana, por todos os ensinamentos, pelo companheirismo e pelo carinho. Serei eternamente grata pela maestria em que transmite seu conhecimento, pelo amor que demonstra pela pesquisa e por me guiar na minha carreira.

À minha coorientadora, Marina, pela delicadeza, paciência e pela valiosa contribuição no desenvolvimento deste estudo e do meu crescimento profissional.

Aos meus amigos da pós-graduação, obrigada pelas conversas, amizade e parceria em todos os momentos. O apoio de vocês foi imprescindível para que eu finalizasse este trabalho.

Ao Colegiado de Pós-Graduação em Odontologia, pelo apoio institucional. E à Coordenação de Pessoal de Nível Superior (CAPES) pelo apoio financeiro.

À Deus, pela vida, por iluminar meu caminho e nunca me deixar só.

RESUMO

Atualmente, o diabetes mellitus (DM) continua sendo um problema de saúde crescente relacionado ao atraso na cicatrização cutânea e altas taxas de amputação, o que leva a uma grande necessidade de novas terapias. Uma abordagem alternativa para o tratamento de feridas diabéticas é a terapia de fotobiomodulação (PBMT). No entanto, o mecanismo que a PBMT pode ativar para estimular o reparo de feridas em uma condição prejudicada, como diabetes, ainda não é totalmente compreendido. Identificar e analisar o papel da PBMT na modulação das células cutâneas em um modelo *in vitro* de diabetes tipo I. Populações heterogêneas de células cutâneas foram isoladas enzimaticamente de camundongos não diabéticos e diabéticos e tratadas ou não com laser de diodo (InGaP, 660nm, 20mW, 5J/cm², 0,71 W/cm², 7s). Funções celulares essenciais, como capacidade de formação de colônias, viabilidade e migração celular foram quantificadas. A capacidade de diferenciação neuro e adipogênica e marcadores importantes para reparo de feridas também foram analisados. A PBMT estimulou a migração celular cutânea intervalada – ou seja, dia sim, dia não – independentemente do tipo de célula, apesar de ter produzido limitada viabilidade e crescimento celulares. Após 14 dias de cultivo, a expressão negativa do transportador de glicose 1 (*GLUT1*) confirmou o estado diabético em células *in vitro*. A PBMT promoveu a neurodiferenciação de ambas as linhagens celulares, mas aumentou a adipogênese apenas em células não diabéticas. A expressão diminuída de receptor antagonista de interleucina 1 (*IL1ra*) e o aumento de interleucina-1 α (*IL-1\alpha*) mostraram aumento do estado inflamatório nas células diabéticas em comparação com as células não diabéticas, o que foi agravado pela PBMT. Por outro lado, a PBMT aumentou a expressão de marcadores de células-tronco (*Nestina* – *NES* e Antígeno Neuro-Glial 2 – *NG2*) em células não induzidas à diferenciação. A PBMT em 660nm apresentou efeitos ambivalentes em células cutâneas *in vitro*. Apesar da PBMT ter contribuído para aumentar o estado hiperinflamatório das células diabéticas, seus efeitos na migração e na estimulação de células multipotentes foram positivos. É possível que a PBMT contribua para o influxo de células indiferenciadas no leito da ferida acelerando o reparo tecidual em feridas crônicas.

Palavras-chave: fotobiomodulação; diabetes; reparo de feridas; cicatrização.

ABSTRACT

Photoactivation of heterogeneous population of cutaneous cells in an *in vitro* diabetic model

Currently, diabetes mellitus (DM) remains a soaring health problem related to delayed skin healing and high amputation rates, which leads to a need for new therapies. An alternative approach to treating diabetic wounds is photobiomodulation therapy (PBMT). However, the mechanism that PBMT might activate to stimulate wound repair in an impaired condition such as diabetes is still not fully understood. Objective: To identify and analyze the role of PBMT in the modulation of cutaneous cells in an *in vitro* model of type I diabetes. Heterogeneous populations of skin cells were enzymatically isolated from non-diabetic and diabetic mice and treated or not with a diode laser (InGaIP, 660nm, 20mW, 5J/cm², 0.71 W/cm, 7s). Essential cell functions such as colony formation capacity, cell viability, and migration were quantified. Neuro and adipogenic differentiation ability and markers for wound repair were also analyzed. PBMT stimulated interval cutaneous cell migration – that is, every other day – regardless of cell type, despite producing limited cell viability and growth. After 14 days of culture, negative glucose transporter 1 (GLUT1) expression confirmed the diabetic state in cells *in vitro*. PBMT promoted neurodifferentiation of both cell lines but increased adipogenesis only in non-diabetic cells. Decreased expression of receptor antagonist interleukin 1 (IL1ra) and increased interleukin-1 α (IL-1 α) showed enhanced inflammatory status in diabetic cells compared to non-diabetic cells, which was exacerbated by PBMT. On the other hand, PBMT increased the expression of stem cell markers (Nestin – NES and Neuro-Glial Antigen 2 – NG2) in cells not induced to differentiation. PBMT at 660nm showed ambivalent effects on cutaneous cells *in vitro*. Although PBMT contributed to an increase in the hyperinflammatory state of diabetic cells, its effects on migration and stimulation of multipotent cells were positive. Therefore, PBMT may contribute to the influx of undifferentiated cells into the wound site, accelerating tissue repair in chronic wounds.

Keywords: photobiomodulation therapy; diabetes; repair; wounds.

LISTA DE TABELAS

Tabela 1 — Sequências de primers de camundongo usadas para análise qPCR.....	17
--	----

LISTA DE ABREVIATURAS E SIGLAS

bFGF	Basic Fibroblast Growth Factor
CEUA	Comitê de Ética de Pesquisa em Animais
CFU	Colony Forming Unit
DM	Diabetes Mellitus
DM1	Diabetes Mellitus Tipo 1
DM2	Diabetes Mellitus Tipo 2
EGF	Epidermal Growth Factor
<i>GAPDH</i>	Gliceraldeído-3-Fosfato Desidrogenase
<i>GLUT1</i>	Transportador de Glicose 1
<i>IL1ra</i>	Interleukin-1 Receptor Antagonist
<i>IL1α</i>	Interleukin 1 α
InGalP	Indio-gálio-alumínio-fosfeto
mRNA	Messenger RNA
MTT	Brometo de [3-(4,5-dimetiltiazol-2yl)-2,5-difenil] Tetrazolium
NES	<i>NESTIN</i>
NF- κ B	Nuclear Factor Kappa B
NG2	Neural/glial Antigen 2
ORO	Oil Red O
PBMT	Photobiomodulation Therapy
PBS	Phosphate Buphered Saline
PFA	Paraformaldehyde
pH	Potential Hydrogen
<i>PPARγ</i>	Peroxisome Proliferator-Activated Receptor γ
qPCR	Quantitative Polymerase Chain Reaction
STZ	Streptozotocin
TGF- β 1	Transforming Growth Factor B1
UV	Ultravioleta

LISTA DE SÍMBOLOS

α	Alfa
β	Beta
cm^2	Centímetros Quadrados
dL	Decilitro
J	Joules
J/cm^2	Joule por Centímetro Quadrado
Kg	Quilograma
mg/ml	Miligramma por Mililitro
min	Minutos
mm^2	Milímetros Quadrados
mM	Milimolar
mW	Miliwatts
ng	Nanograma
nm	Nanômetros
μm	Micrômetro
μl	Microlitro
μM	Micromolar
W	Watts
W/cm^2	Watt por Centímetro Quadrado

SUMÁRIO

1	CONSIDERAÇÕES INICIAIS	11
2	OBJETIVOS	15
2.1.	Objetivo geral	15
2.2.	Objetivos específicos	15
3	METODOLOGIA EXPANDIDA	16
3.1	Indução do DM e procedimentos em animais	16
3.2	Isolamento das células cutâneas	16
3.3	Parâmetros de fotobiomodulação	16
3.4	Unidade formadora de colônia (CFU-F)	17
3.5	Viabilidade celular	17
3.6	<i>Cell Scratch</i>	17
3.7	Diferenciação neurogênica	18
3.8	Diferenciação adipogênica	18
3.9	Análise de expressão de mRNA	19
3.10	Análise estatística	20
4	RESULTADOS	21
4.1	Artigo	21
5	CONSIDERAÇÕES FINAIS	53
	REFERÊNCIAS	54
	ANEXO A – Aprovação da comissão de ética no uso de animais	57

1 CONSIDERAÇÕES INICIAIS

O diabetes mellitus (DM) é um distúrbio metabólico complexo associado ao aumento do risco de doenças microvasculares e macrovasculares. Cerca de 415 milhões de adultos em todo o mundo são afetados pelo DM, e estima-se que esse número aumente para 642 milhões até 2040 (INTERNATIONAL DIABETES FEDERATION, 2015).

A principal característica do DM é a hiperglicemia, sendo dividido em dois tipos principais: diabetes mellitus tipo I (DM1) e diabetes mellitus tipo II (DM2). No DM1, as células β do pâncreas são danificadas, levando à diminuição do suprimento de insulina para a circulação sanguínea e a uma dependência absoluta de insulina exógena para regular os níveis de glicose no sangue. Este tipo é responsável por 5% a 10% de todos os casos. Já o DM2 é observado na maioria dos pacientes diabéticos ($\pm 85\%$) e resulta em um aumento anormal da resistência à ação da insulina, onde o corpo não consegue produzir esse hormônio de forma suficiente para superar esse quadro (DESHPANDE *et al.*, 2008; FEDERAÇÃO INTERNACIONAL DE DIABETES, 2015).

O DM está associado a inúmeras comorbidades, incluindo problemas cardiovasculares, acidente vascular cerebral, insuficiência renal crônica, neuropatia periférica e feridas cutâneas ou ulcerações diabéticas. As ulcerações cutâneas diabéticas apresentam-se como feridas dolorosas com desintegração do tecido dérmico, incluindo epiderme, derme e, em muitos casos, tecido subcutâneo (OKONKWO *et al.*, 2017).

A pele é o maior órgão do corpo e uma barreira física na interface com o ambiente. De origem ectodérmica e mesodérmica, a pele compreende uma população heterogênea de células (PARK, 2022). A camada mais externa, a epiderme, consiste principalmente de células dendríticas, melanócitos e queratinócitos, o último sendo responsável por sintetizar queratina, uma proteína com papel protetor à temperatura, pH extremo, patógenos, luz UV e estresse mecânico (KANITAKIS, 2002; KOLARSICK *et al.*, 2011; PARK, 2022). A epiderme abriga muitas outras populações de células, mas os queratinócitos compreendem a maioria delas. A derme, camada intermediária da pele, é um sistema integrado de tecido conjuntivo fibroso (colágeno), filamentoso e amorfo que acomoda fibroblastos, macrófagos, mastócitos e recebe vários estímulos

oriundos de redes nervosas e vasculares. Outras células oriundas da rede vascular, incluindo linfócitos e leucócitos, também entram na derme em resposta a diferentes fatores (KOLARSICK *et al.*, 2011). A derme encontra-se acima do tecido subcutâneo, ou panículo, que contém pequenos lóbulos de células adiposas conhecidas como lipócitos. Considerado um órgão endócrino, o tecido subcutâneo funciona como um depósito de energia (KOLARSICK *et al.*, 2011).

Após uma lesão tecidual, em um processo normal de reparo, o sistema imunológico desempenha um papel central ao fagocitar os detritos, bactérias e tecidos danificados do local e promovendo a regeneração da ferida (CERVERÓ-FERRAGUT *et al.*, 2017). Em lesões diabéticas, no entanto, ocorre um desequilíbrio nesse processo, e o sistema imunológico começa a promover mais dano que reparo (DENDEKKER; GALLAGHER, 2020).

O reparo de feridas é um processo complexo dividido em quatro fases sobrepostas: homeostase, inflamação, proliferação e remodelação (CAÑEDO-DORANTES; CAÑEDO-AYALA, 2019; DENDEKKER; GALLAGHER, 2020). A hemostasia ocorre imediatamente após o dano tecidual e resulta na formação de um coágulo sanguíneo, que interrompe a hemorragia e desencadeia o recrutamento de diferentes células imunes - incluindo neutrófilos, macrófagos e linfócitos - para prevenir a infecção, remoção de debris celulares e ativar ainda mais a resposta inflamatória (REINKE; SORG, 2012). A fase de proliferação coordena a reepitelização epidérmica e o reparo dérmico. E por último, a fase de remodelação remove as células desnecessárias e induz a remodelação da matriz extracelular.

Durante o reparo de feridas, as células-tronco (SCs – do inglês, *stem cells*), também desempenham papel importante. O tecido cutâneo apresenta um enorme potencial regenerativo, pois diferentes tipos de células-tronco estão localizadas na pele e seus anexos. A diferenciação dessas SCs em células neurais e adipogênicas contribui no reparo e regeneração do tecido danificado (PARFEJEVS *et al.* 2018; PLONER *et al.*, 2021; YANG *et al.*, 2019).

Ademais, adipócitos adjacentes à lesão da pele são essenciais para liberação de lipídios que atuam modulando a resposta inflamatória de macrófagos. Esses adipócitos também possuem a capacidade de desdiferenciação, voltando ao estado de célula-tronco, podendo de diferenciar novamente em miofibroblastos após uma injúria, recuperando o tecido perdido (SHOOK *et al.*, 2020).

Células nervosas também participam ativamente no reparo dos tecidos. A ativação de células gliais possui a capacidade de aumentar a proliferação e migração de queratinócitos, aumentar a neovascularização e acelerar o fechamento de feridas na pele (PARFEJEVS *et al.*, 2018). Além disso, neurônios e células da glia podem desempenhar um papel crítico na modulação da atividade de plaquetas no processo de cicatrização de feridas, e essa interação pode ser um fator chave na regulação da resposta geral de cicatrização (BEURA *et al.*, 2022).

Embora o processo de reparo seja complexo, está claro que anormalidades em neutrófilos e macrófagos contribuem fortemente para o estado pró-inflamatório e a persistência das feridas no diabetes (MARUYAMA *et al.*, 2007; WILGUS *et al.*, 2013). Além disso, a comunicação entre as células cutâneas neste ambiente hiper-inflamatório e hiperglicêmico ainda não é totalmente compreendida (ZHANG *et al.*, 2021).

Mesmo que muitos métodos estejam disponíveis para induzir o reparo em feridas diabéticas, muitas persistem depois de todos os tratamentos. Uma abordagem alternativa para feridas diabéticas é a PBMT (do inglês, *photobiomodulation therapy*). A PBMT, também conhecida como terapia a laser em baixa potência (até 100mW), é um tratamento não invasivo muito utilizado clinicamente para acelerar o reparo dos tecidos (MOSCA *et al.*, 2019).

Embora muito utilizada para cicatrização de feridas, a PBMT pode ser produzida por diferentes comprimentos de onda e potências, sem um protocolo de utilização bem estabelecido (KUFFLER, 2016; OLIVEIRA *et al.*, 2022). Na verdade, existe uma janela terapêutica para que os resultados positivos sejam alcançados após o tratamento com a fotobiomodulação (ABREU *et al.*, 2019). Apesar disso, os mecanismos de ação da PBMT ainda são pouco definidos ou até inconsistentes.

Desde o início da utilização da PBMT por Endre Mester, em meados dos anos 60, algumas teorias surgiram, incluindo a descoberta de alvos endógenos fotossensíveis, como: (1) complexos intracelulares (citocromo c oxidase nas mitocôndrias), (2) receptores na membrana celular (potencial receptor transitório vanilóide 1, opsinas), (3) fatores de transcrição sensíveis ao estado oxidativo (NF- κ B) e (4) complexos extracelulares (como fator de crescimento transformante- β 1/TGF- β 1) (ARANY, 2016; CHEN *et al.*, 2011; FREITAS; HAMBLIN, 2016).

No entanto, além da dicotomia nos resultados encontrados na literatura sobre o efeito benéfico do PBMT em modelos saudáveis, estudos em condições

diabéticas ainda são escassos. O mecanismo prevalente que a PBMT ativa para desencadear sua resposta de reparo em uma condição debilitada, como no diabetes, precisa ser explorado. Portanto, investigar os mecanismos moleculares e celulares da PBMT poderia fornecer a justificativa e a aplicação clínica aprimorada desse tratamento. Para isto, neste trabalho, utilizamos um modelo de diabetes tipo 1 *in vitro* para estudar o impacto da terapia com laser de baixa potência (PBMT) em uma população heterogênea de células cutâneas isoladas de camundongos diabéticos.

2 OBJETIVOS

2.1. Objetivo geral

Identificar e analisar o papel da PBMT na modulação de células cutâneas em um modelo *in vitro* de diabetes tipo I.

2.2. Objetivos específicos

a) Avaliar a viabilidade e proliferação celular por meio do teste MTT (brometo de [3-(4,5-dimetiltiazol-2yl)-2,5-difenil] tetrazolium) e CFU (do inglês, *colony-forming units*), entre grupos controle não expostos à PBMT e grupos expostos à irradiação, em células cutâneas de camundongos saudáveis e de camundongos diabéticos.

b) Avaliar a migração de células cutâneas de camundongos saudáveis e de camundongos diabéticos em um modelo de ferida *in vitro* (*cell scratch*) com ou sem a aplicação da PBMT.

c) Comparar a capacidade de diferenciação adipogênica de células cutâneas isoladas da pele de camundongos normais ou diabéticos após a irradiação através da avaliação da expressão gênica de *PPAR γ* (do inglês, *peroxisome proliferator-activated receptor gamma*) e coloração com ORO (do inglês, *oil red o*).

d) Comparar a capacidade de diferenciação neurogênica e a expressão de marcadores de indiferenciação de células cutâneas isoladas da pele de camundongos normais ou diabéticos após a irradiação através da avaliação da expressão gênica de *NESTIN* e Neuron/Glial antigen 2 (*NG2*).

e) Comparar a expressão gênica de *GLUT1*, *IL1 α* , *IL1ra* em células cutâneas de camundongos saudáveis e de camundongos diabéticos com ou sem a aplicação da PBMT pelo teste reação em cadeia de polimerase quantitativa (qPCR).

3 METODOLOGIA EXPANDIDA

3.1 Indução do DM e procedimentos em animais

O Comitê de Ética de Pesquisa em Animais (CEUA) da Universidade Federal de Minas Gerais aprovou o manejo e procedimentos deste trabalho (#62/2019). Todos os animais foram alojados em condições controladas de temperatura (24°C), sob um ciclo claro-escuro de 12:12h e alimentados *ad libitum*.

Camundongos C57Bl/6 de oito a dez semanas de idade (n = 6; 3 por grupo) foram usados para isolamento de células cutâneas. Os animais foram divididos em dois grupos, diabéticos e não diabéticos. O DM foi induzido quimicamente no grupo diabético conforme descrito anteriormente (KONRAD *et al.*, 2001). Durante cinco dias, 50 mg/kg de estreptozotocina (STZ) em tampão citrato de sódio 50 mM (FURMAN, 2015) foram injetados intraperitonealmente nos camundongos. O peso corporal e os níveis de glicose no sangue foram monitorados diariamente durante duas semanas após a aplicação, até que a condição diabética fosse confirmada. Camundongos diabéticos foram definidos como aqueles com mais de 300 mg/dL de glicose sanguínea.

3.2 Isolamento das células cutâneas

Os animais foram eutanasiados com sobredose anestésica (300 mg/kg de cetamina e 30 mg/kg de xilazina, intraperitoneal) conforme descrito anteriormente (GOMES *et al.*, 2022). O pêlo do dorso dos camundongos foi raspado, a pele coletada e as células isoladas (PARFEJEVS *et al.*, 2018). As células cutâneas foram expandidas em meio de cultivo Dulbecco's Modified Eagle Medium (ThermoFisher), suplementado com 10% de soro fetal bovino (Invitrogen) e 100 UI/mL de penicilina/estreptomicina (Gibco), até 90% de subconfluência. As células obtidas de camundongos diabéticos foram cultivadas em uma solução adicional de glicose 28 mM (ThermoFisher) para simular o ambiente diabético *in vitro* (LAN *et al.*, 2013; TARALLO *et al.*, 2012). Células em passagens iniciais (P1-P4) foram usadas para os experimentos. Para este estudo, o cultivo foi distribuído em quatro grupos: não diabéticos não irradiados, não diabéticos irradiados, diabéticos não irradiados e diabéticos irradiados.

3.3 Parâmetros de fotobiomodulação

A PBMT foi conduzida utilizando laser de diodo customizado de índio-gálio-alumínio-fosfeto (InGaAlP) em operação contínua em contato com o fundo das microplacas. Os parâmetros de dosimetria foram os seguintes: 660 nm, 20 mW, 0,028 cm² de área pontual, 0,71 W/cm², 7s, 5 J/cm², 0,14 J de energia total por ponto (OLIVEIRA *et al.*, 2022; VALLE *et al.*, 2020). Em todas as microplacas, a irradiação foi aplicada a uma distância de 1cm² entre cada ponto. A PBMT foi aplicada todos os dias durante uma semana. Os poços adjacentes aos grupos de tratamento foram mantidos vazios para evitar superexposição à luz, e papel preto foi usado para limitar a dosagem a um único poço. Antes de todos os experimentos, a potência do dispositivo foi verificada com um medidor de potência (LaserCheck; Coherent Inc., Santa Clara, CA, EUA).

3.4 Unidade formadora de colônia (CFU-f)

Para avaliar a proliferação celular por meio da eficiência de formação de colônias, 500 células/mL foram semeadas em triplicata utilizando microplaca de 6-poços, cultivadas por sete dias e fixadas em paraformaldeído à 4% por 30 minutos. A coloração foi realizada com azul de toluidina 0.1% em 1% paraformaldeído em PBS (Sigma-Aldrich) por 1 h, para contagem das unidades formadoras de colônia (QUEIROZ *et al.*, 2021). Imagens das placas de cultivo celular foram analisadas usando o software Image J (NIH, EUA). Apenas colônias com $n > 50$ células foram contabilizadas (DINIZ *et al.*, 2018).

3.5 Viabilidade celular

A medição da viabilidade celular foi realizada em sextuplicata através do teste com brometo de 3-(4,5-dimetiltiazol-2il)-2,5-difenil tetrazólio (MTT, ThermoFisher) em microplacas de 96-poços. As células foram semeadas na densidade de 10³ células/poço e analisadas nos tempos por 24, 48, 72h e 7 dias. Após cada tempo experimental, 5 mg/mL da solução de MTT foi adicionada as células e incubada à 37°C por 4 horas. Os cristais foram solubilizados com dimetil sulfoxido e a densidade óptica de cada poço foi medida em um comprimento de onda de absorção de 540nm em um espectrofotômetro (Varioskan Flash Multimode Reader, Thermo Scientific).

3.6 Cell Scratch

As células foram plaqueadas em microplacas de 48-poços à densidade de 2×10^4 células/poço. Após atingirem confluência, as células cutâneas foram submetidas ao ensaio “cell scratch” em quadruplicata (OLIVEIRA *et al.*, 2022). Utilizando uma ponteira p200, uma “ferida” foi simulada e a porcentagem da área durante o fechamento e migração das células foi calculada nos tempos 0, 12, 24 e 48h após a irradiação. Para esse fim, o software Image J (NIH, EUA) foi utilizado, tendo como referência a área total da imagem obtida com uma objetiva de 4x, de acordo o tempo experimental, sob um leitor multimodo de microplacas (Cytation 5 Cell Imaging, Winooski, VT, EUA).

3.7 Diferenciação neurogênica

As células foram plaqueadas em microplacas de 6-poços à densidade de 1×10^5 células/poço. Após atingirem confluência, as células cutâneas foram submetidas ao ensaio de diferenciação em quadruplicata. Para diferenciação neural, as células cutâneas foram cultivadas em meio neurobasal (Gibco) suplementado com suplemento 5% de soro fetal bovino, B27 1x (Gibco), 10.000 U/ml de penicilina-estreptomicina (Gibco), 20 ng/mL de fator de crescimento epidérmico (EGF, Invitrogen) e 40 ng/mL de fator básico de crescimento de fibroblastos (bFGF, Invitrogen) (FRESHNEY, 2015). As células foram incubadas por 14 dias e os meios trocados a cada 2-3 dias. Em seguida, foi realizada extração de mRNA e síntese de cDNA, e os genes Nestin e NG2 foram avaliados, como descrito abaixo. Células diabéticas e não-diabéticas cultivadas por 14 dias (não diferenciadas e não irradiadas) foram usadas como controle de referência.

3.8 Diferenciação adipogênica

As células foram plaqueadas em quadruplicata em microplacas de 6-poços à densidade de 1×10^5 células/poço. Células confluentes foram cultivadas por 14 dias em meio de indução ou regular, conforme relatado anteriormente (FRESHNEY, 2015). Para realizar a diferenciação adipogênica, as células foram cultivadas em meio α -MEM, suplementado com 10% de soro fetal bovino, 2 mM de L-glutamina, 100 μ M de ácido ascórbico, U/ml de penicilina-estreptomicina, 0,5 μ M de hidrocortisona, 10 μ g / mL de insulina, 3-isobutil-1-metilxantina 0,5 mM, indometacina 60 μ M. O meio de cultura foi trocado a cada 2-3 dias. A expressão gênica de *PPAR γ* foi avaliada por qPCR. Células de cada tipo cultivadas por 14 dias (não diferenciadas e não irradiadas)

foram usadas como controle de referência. O acúmulo de triglicerídeos foi fotografado e quantificado pela coloração Oil Red-O. Resumidamente, para preparar a solução estoque, ORO foi dissolvido em 0,5% de isopropanol e diluído com isopropanol e água (6:4). As células foram fixadas com paraformaldeído a 4% por 30 min, lavadas com PBS e coradas com ORO por 30 min à temperatura ambiente. As imagens das células coradas foram capturadas utilizando um leitor multimodo de microplacas (Cytation 5 Cell Imaging, Winooski, VT, EUA) com uma objetiva de 10x. A quantificação foi lipídica realizada pelo software Image J (NIH, EUA).

3.9 Análise de expressão de mRNA

Para análise de qPCR, cada grupo foi coletado para extração de RNA com RNeasy Mini Kit (Qiagen, Valencia, CA, EUA), de acordo com as instruções do fabricante. Alíquotas de 2.500ng de RNA total foram transcritas usando SuperScript VILO IV Master Mix (ThermoFisher). O cDNA sintetizado foi utilizado como molde para as reações utilizando o Power SYBR Green Master Mix (ThermoFisher). Os genes avaliados e as respectivas sequências de primers estão listados na Tabela 1. Os valores de Ct de medições duplicadas normalizadas para o controle endógeno Gliceraldeído-3-fosfato desidrogenase (*GAPDH*) foram comparados com a amostra de referência (não diabético não irradiado) usando a fórmula $2^{-\Delta\Delta CT}$.

Tabela 1 — Sequências de primers de camundongo usadas para análise qPCR

Gene	Sequências	Tamanho (pares de base)
<i>GLUT1</i>	F: 5' GGATCCCAGCAGCAAGAAGGT 3' R: 5' CGAACTGCAGTGATCCGAGC 3'	77
<i>IL1α</i>	F: 5' ACGTCAAGCAACGGGAAGAT 3' R: 5' AAGGTGCTGATCTGGGTTGG 3'	124
<i>IL1ra</i>	F: 5'AACCAGTCATTGCTGGTACTTA 3' R: 5' GCCCAAGAACACACTATGAAGGTC 3'	96
<i>NES</i>	F: 5' TGGCTACATACAGGACTCTGC 3' R: 5' AAGGATGTTGGGCTGAGGAC 3'	172
<i>NG2</i>	F: 5' CTGTTCTCACACAGAGGAGCC 3'	150

	R: 5' TGGACAGACGGTCAACTTCC 3'	
<i>PPAR_γ</i>	F: 5' GTCACACTCTGACAGGAGCC 3'	122
	R: 5' CACCGCTTCTTTCAAATCTTGT 3'	
<i>GAPDH</i>	F: 5' ACGGCCGCGCATCTTCTTGTGCA 3'	71
	R: 5' CGCCAAATCCGTTACACCCGA 3'	

Tabela 1. Sequências de primer de camundongo usadas para análise qPCR. Abreviações: *GLUT1*, transportador de glicose 1; *IL1α*, Interleucina 1α; *IL1ra*, antagonista do receptor da Interleucina 1; NES, *NESTIN*; *NG2*, Neural-Glial Antigen 2; *PPAR_γ*, receptor gama ativado por proliferador de peroxissoma; *GAPDH*, Gliceraldeído-3-fosfato desidrogenase.

Fonte: Elaborado pela autora, 2023.

3.10 Análise estatística

O teste de Shapiro-Wilk foi realizado para avaliar se os dados possuíam distribuição paramétrica ou não-paramétrica. A significância estatística entre os grupos foi calculada com o teste de comparação múltipla de Dunnet, ou teste t de Student, utilizando o software GraphPad Prism (GraphPad Prism versão 9.5.0 para Mac, La Jolla, EUA). O nível de significância foi definido como $p < 0,05$.

4 RESULTADOS

Os resultados e discussão serão apresentados no formato de artigo científico.

4.1 Artigo

Artigo a ser submetido ao periódico *Journal of Diabetes Research* (Qualis A3), fator de impacto 4.011, após as considerações da banca.

Photoactivation of heterogeneous population of cutaneous cells in an *in vitro* diabetic model

Rafaela Ferrer de Oliveira^{1,5}, João Victor Reis Cruvinel^{1,5}, Natalia Aparecida Gomes^{1,5}, Beatriz Gomes Lucardians^{1,5}, Caroline de Carvalho Picoli², Alinne do Carmo Costa², Márcio Sobreira Araújo³, Alexander Birbrair², Marina Gonçalves Diniz⁴, Ivana Márcia Alves Diniz^{1,5}

¹Department of Restorative Dentistry, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

²Department of Pathology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

³Oswaldo Cruz Foundation, Rene Rachou Institute, Belo Horizonte, MG, Brazil.

⁴Department of Microbiology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

⁵LASER | Biotechnologies, School of Dentistry, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Corresponding author: Prof. Ivana M. A. Diniz.

Department of Restorative Dentistry, School of Dentistry, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, Brazil. Zip code: 31.270-901. Telephone/Fax: +55-31-34092440. E-mail: ivanadiniz@gmail.com

ABSTRACT

Background: Currently, diabetes mellitus (DM) remains a soaring health problem related to delayed skin healing and high amputation rates, which leads to a need for new therapies. An alternative approach to treating diabetic wounds is photobiomodulation therapy (PBMT). However, the mechanism that PBMT might activate to stimulate wound repair in an impaired condition such as diabetes is still not fully understood. **Objective:** To identify and analyze the role of PBMT in the modulation of cutaneous cells in an *in vitro* model of type I diabetes. **Materials and methods:** Heterogeneous populations of skin cells were enzymatically isolated from non-diabetic and diabetic mice and treated or not with a diode laser (InGaIP, 660nm, 20mW, 5J/cm², 0.71 W/cm, 7s). Essential cell functions such as colony formation capacity, cell viability, and migration were quantified. Neuro and adipogenic differentiation ability and markers for wound repair were also analyzed. **Results:** PBMT stimulated interval cutaneous cell migration – that is, every other day – regardless of cell type, despite producing limited cell viability and growth. After 14 days of culture, negative glucose transporter 1 (GLUT1) expression confirmed the diabetic state in cells *in vitro*. PBMT promoted neurodifferentiation of both cell lines but increased adipogenesis only in non-diabetic cells. Decreased expression of receptor antagonist interleukin 1 (IL1ra) and increased interleukin-1 α (IL-1 α) showed enhanced inflammatory status in diabetic cells compared to non-diabetic cells, which was exacerbated by PBMT. On the other hand, PBMT increased the expression of stem cell markers (Nestin – NES and Neuro-Glial Antigen 2 – NG2) in cells not induced to differentiation. **Conclusions:** PBMT at 660nm showed ambivalent effects on cutaneous cells *in vitro*. Although PBMT contributed to an increase in the hyperinflammatory state of diabetic cells, its effects on migration and stimulation of multipotent cells were positive. Therefore, PBMT may contribute to the influx of undifferentiated cells into the wound site, accelerating tissue repair in chronic wounds.

Keywords: photobiomodulation therapy, diabetes, repair, wounds.

INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder associated with increased microvascular and macrovascular disease risk. Around 415 million adults worldwide are affected by DM, and the count is estimated to soar as high as 642 million by 2040 (International Diabetes Federation, 2015).

DM is characterized by hyperglycemia, and the condition can be categorized into two main types: type I diabetes mellitus (T1DM) and type II diabetes mellitus (T2DM). In T1DM, β -cells of the pancreas are damaged, leading to a decreased insulin supply to the circulation and an absolute dependence on exogenous insulin to regulate blood glucose levels. This type accounts for 5% to 10% of all cases of diabetes. Contrarily, T2DM is observed in most diabetic patients ($\pm 85\%$) and results in an abnormal increased resistance to the action of insulin where the body cannot produce enough insulin to overcome the resistance (Deshpande *et al.*, 2008; International Diabetes Federation, 2015).

DM is associated with numerous comorbidities, including cardiovascular disease, stroke, chronic kidney failure, peripheral neuropathy, and diabetic skin wounds or chronic ulcers. Diabetic skin ulcers manifest as painful wounds with the disintegration of dermal tissue, including the epidermis, dermis, and, in many cases, subcutaneous tissue (Okonkwo *et al.*, 2017).

Upon injury, in a normal wound repair process, the immune system plays a central role by phagocytizing the detritus, bacteria, and damaged tissue from the wound site and promoting wound regeneration (Cerveró-Ferragut *et al.*, 2017). In diabetic wounds, however, the immune system becomes dysregulated and promotes damage rather than repair (DenDekker & Gallagher, 2020).

Wound repair is a complex process divided into four overlapping phases: hemostasis, inflammation, proliferation, and remodeling (Cañedo-Dorantes & Cañedo-Ayala, 2019; DenDekker & Gallagher, 2020). Hemostasis occurs immediately after tissue damage and results in the formation of a blood clot, which stops bleeding and triggers the recruitment of different immune cells - including neutrophils, macrophages, and lymphocytes - to prevent infection, remove cellular debris, and further activate the inflammatory response (Reinke & Sorg, 2012). The proliferation phase coordinates

epidermal re-epithelialization and dermal repair. Lastly, the remodeling phase removes unnecessary cells and induces remodeling of the extracellular matrix.

Stem cells (SCs) also play an important role during wound healing. Cutaneous tissue has enormous regenerative potential because different types of stem cells are located in the skin and its appendages. The differentiation of these SCs into neural and adipogenic cells contributes to the repair and regeneration of damaged tissue (Yang *et al.*, 2019; Ploner *et al.*, 2021; Parfejevs *et al.*, 2018). In addition, adipocytes adjacent to skin lesions are essential for the release of lipids that act by modulating the inflammatory response of macrophages. These adipocytes also have the ability to dedifferentiate, returning to the stem cell state, and can differentiate again into myofibroblasts after an injury, recovering lost tissue (Shook *et al.*, 2020).

Nerve cells also actively participate in tissue repair. The activation of glial cells can increase the proliferation and migration of keratinocytes, increase neovascularization, and accelerate wound closure in the skin (Parfejevs *et al.*, 2018). In addition, neurons and glial cells may play a critical role in modulating the activity of platelets in the wound healing process, and this interaction may be a key factor in regulating the overall healing response (Beura *et al.*, 2022).

Even though numerous methods are available to induce repair in diabetic wounds, many of them remain recalcitrant to all treatments. An alternative approach for diabetic wounds is photobiomodulation therapy (PBMT). PBMT, also known as low-level laser therapy, is a non-invasive treatment widely used in clinics to accelerate the repair of tissues (Mosca *et al.*, 2019). Although broadly used, wound healing is induced by many different wavelengths and powers, with no optimal set of parameters yet established (Kuffler, 2016; Oliveira *et al.*, 2022). In fact, there is a therapeutic window for positive results to be achieved after treatment with PBMT (de Abreu *et al.*, 2019). Despite this, the mechanisms of action of PBMT are still not well defined or even inconsistent.

Since Endre Mester started to use PBMT in the mid-1960s (Hamblin, 2016), several theories have emerged, including the discovery of photosensitive endogenous targets, such as: (1) intracellular complexes (cytochrome c oxidase in mitochondria), (2) cell membrane receptors (transient receptor potential vanilloid 1, opsins), (3) activation of redox-sensitive transcription factors (NF- κ B) and (4) extracellular complexes (such as transforming growth factor- β 1/TGF- β 1)(Arany, 2016; Chen *et al.*, 2011; de Freitas & Hamblin, 2016).

However, in addition to the dichotomy found in the literature about the efficacy of PBMT in healthy models, studies on diabetic conditions are still not fully elucidated. The mechanism that PBMT activates to trigger its response in an impaired condition such as diabetes needs to be understood. Therefore, investigations into the molecular and cellular mechanisms of PBMT will provide the rationale and improved clinical application of this innovative treatment. Here, we replicate the type-1 diabetic condition *in vitro* to study the impact of light stimulation (PBMT) on a heterogeneous population of diabetic cutaneous cells.

MATERIALS AND METHODS

DM induction and animal procedures

The Animal Care and Use Committee at Universidade Federal de Minas Gerais approved animal handling and procedures in this work (#62/2019). All animals were housed in controlled conditions for temperature (24 °C) and under a 12:12-h light-dark cycle and fed *ad libitum*.

Eight to ten-week-old male or female C57Bl/6 ($n = 6$; 3 per group) were used for cutaneous cell isolation. The animals were divided into two groups, diabetic and non-diabetic. DM was chemically induced in the diabetic group as previously described (Konrad *et al.*, 2001). For five days, mice were injected intraperitoneally with a single high dose of 50 mg/kg streptozotocin (STZ) in 50 mM sodium citrate buffer (Furman, 2015) or buffer (control group). After two weeks of STZ, body weight and blood glucose levels were monitored daily until a diabetic condition was confirmed. Diabetic mice were defined as those with more than 300 mg/dL glucose. Mice with a successful course following diabetes induction were then used for experimentation.

Cell isolation and culture

Animals were euthanized with anesthetic overdose (300mg/kg ketamine and 30 mg/kg xylazine, *i.p.*) as previously described (Gomes *et al.*, 2022). Mice were shaved, the skin from the back was collected, and the cells were isolated, according to Parfejevs *et al.* 2018. Cells were expanded in growth media, composed of Dulbecco's Modified Eagle Medium (ThermoFisher), supplemented with 10% fetal bovine serum (Invitrogen) and 100 IU/mL penicillin/streptomycin (Gibco, USA), until 90%

subconfluence. Cells obtained from diabetic mice were cultured in an additional 28mM Glucose Solution (ThermoFisher) to mimic the environment of diabetes *in vitro* (Lan *et al.*, 2013; Tarallo *et al.*, 2012). Cells at early passages (P1–P4) were used for the experiments.

Experimental groups

The study was distributed into four groups: non-diabetic non-irradiated, non-diabetic irradiated, diabetic non-irradiated, and diabetic irradiated.

Photobiomodulation parameters

PBMT was conducted using indium–gallium–aluminum–phosphide (InGaAlP) customized diode laser in continuous operation in contact with the bottom of the microplates. The dosimetry parameters were as follows: 660 nm, 20 mW, 0.028 cm² spot area, 0.71 W/cm², 7 s, 5 J/cm², 0.14 J total energy per point (do Valle *et al.*, 2020; Oliveira *et al.*, 2022). In all microplates, the irradiation was applied at 1cm² between each point. Cells were irradiated immediately after plating and every day for one week. Wells adjacent to the test groups were kept empty to avoid overexposure to light, or a thick black paper cover was used to limit the dosage to a single well. Before all experiments, the device's potency was checked with a power meter (LaserCheck; Coherent Inc., Santa Clara, CA, USA).

Colony forming unit–fibroblastic (CFU-f)

To assess the colony-forming efficiency, 500 cells/mL were seeded in a 6-well microplate, cultured for seven days, and fixed in 4% paraformaldehyde for 30 minutes. The stain was performed with 1% toluidine blue (Sigma-Aldrich) for 1 hour to count the colony-forming units (Queiroz *et al.*, 2021). Images of the cell culture plates were analyzed using the Image J software (NIH, USA). Colonies with $n > 50$ cells were considered in the count (Diniz *et al.*, 2018).

Cell viability

Measurement of cell viability was performed in sextuplicate using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Invitrogen, ThermoFisher). The cells were seeded in 96-well plates at a density of 10³ cells/well and analyzed after 24, 48, 72h, and 7 days. After each experimental time, 5 mg/mL of

MTT solution was added and incubated at 37°C for four hours. The crystals were solubilized with dimethyl sulfoxide, and the optical density was measured at an absorbance wavelength of 540 nm in a spectrophotometer (Varioskan Flash Multimode Reader, Thermo Scientific).

Cell Scratch

Cells were plated in 48-well microplates at a 2×10^4 cells/well density. After reaching confluence, the skin cells were subjected to the "cell scratch" test in quadruplicate (Oliveira *et al.*, 2022). Using a p200 pipette tip, a scratch was made in the cell monolayer in the middle of each well. The area percentage of the "wound" was calculated in the times 0, 12, 24, and 48h after irradiation with the Image J software (NIH, Bethesda, MD, USA), having as reference the total image area obtained using a 4x objective under a microplate multimode reader (Cytation 5 Cell Imaging, Winooski, VT, USA).

Neurogenic differentiation

To differentiate into neural cells, the cutaneous cells were cultured in neurobasal medium (Gibco) supplemented with 5% fetal bovine serum, B27 supplement 1x (Gibco), 10,000 U/ml penicillin-streptomycin (Gibco), 20 ng/mL epidermal growth factor (EGF, Invitrogen) and 40 ng/mL basic fibroblast growth factor (bFGF, Invitrogen) (Freshney, 2015). Cells were incubated for 14 days, and the media changed every 2-3 days. Then, mRNA extraction and cDNA synthesis were performed, and the genes *NESTIN* and *NG2* were evaluated as described below. Cells from each type cultivated for 14 days undifferentiated and not irradiated were used as reference controls.

Adipogenic differentiation

As previously reported, confluent cells were cultivated for 14 days in adipogenic induction media or regular media as a control (Freshney, 2015). To perform adipogenic differentiation, cells were cultured in media α -MEM, supplemented with 10% of FBS, 2 mM L-glutamine, 100 μ M ascorbic acid, U/ml penicillin-streptomycin, 0,5 μ M hydrocortisone, 10 μ g/mL insulin, 0,5 mM 3-isobutyl-1-methylxanthine, 60 μ M indomethacin. Cell culture media were changed every 2–3 days. mRNA extraction and cDNA synthesis were performed, and the gene PPAR was evaluated. The triglyceride

accumulation was photographed and quantified by Oil Red-O staining. Briefly, to prepare the stock solution, ORO was dissolved in 0.5% isopropanol and diluted with isopropanol and water (6:4). Cells were fixed with 4% paraformaldehyde for 30 min, washed with PBS, and stained with ORO for 30 min at room temperature. Images of stained cells were captured using a multimode microplate reader (Cytation 5 Cell Imaging, Winooski, VT, USA), and lipid quantification was performed using Image J software (NIH, USA).

mRNA expression analyses

For qPCR analysis, each group was collected for total RNA extraction using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. Aliquots of 2,500 ng of total RNA were reverse transcribed using the SuperScript VILO IV Master Mix (ThermoFisher). The synthesized cDNA was used as a template for the reactions using the Power SYBR Green Master Mix (ThermoFisher). The genes evaluated, and respective primer sequences are listed in Table 1. The Ct values from duplicate measurements normalized to the endogenous control Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were compared to the reference sample (undifferentiated and non-irradiated controls of diabetic and non-diabetic cells) using the formula $2^{-\Delta\Delta CT}$.

Statistical Analysis

The Shapiro-Wilk test was performed to evaluate if the data had parametric or non-parametric distribution. Statistical significance amongst groups was calculated by Dunnet's multiple comparison test, or Student t-test, using the GraphPad Prism 8.0 Software (GraphPad Prism Version 9.5.0 for Mac, La Jolla, CA, USA). The level of significance was defined as $p < 0.05$.

RESULTS

1. The number of cell colonies was similar between treatments

As shown in Figure 1, concerning the number of colonies, the non-diabetic group did not show significant increase in the number of colonies between control and irradiated

cells ($p = 0.0533$). Still, despite the numerical increase of colonies after PBMT in the diabetic group, this rise was not statistically significant after seven days ($p = 0.1972$).

2. *Cell viability increase due to PBMT was limited to the 48h time-interval, regardless of the cell type*

We next sought to analyze metabolically active cells after PBMT through MTT assay (Figure 2). Cell viability 24h-post-irradiation was decreased compared to the control in both non-diabetic and diabetic cells treated with PBMT ($p = 0.0005$ and $p = 0.0403$, respectively). However, 48h after irradiation, PBMT-treated groups showed greater viability compared to the respective non-irradiated group ($p = 0.0093$ and $p = 0.0135$). At 72h, cell viability was similar in any group of non-diabetic cells. In the meantime, diabetic cells showed decreased viability after irradiation ($p = 0.0093$). Seven days later, cell viability was stabilized without statistical difference between PBMT and controls, regardless of the cell type (diabetic or not).

3. *Diabetic and non-diabetic cells presented interspaced migration responses to PBMT*

Data from the cell scratch assay are shown in Figure 3. At 0h, all groups presented similar scratch irrespective to cell type. At 12h, PBMT showed increased cell migration in both non-diabetic and diabetic cutaneous cells ($p = 0.0012$ and $p = 0.0008$, respectively). At 24h, diabetic neither non-diabetic cutaneous cells showed significant differences regarding the tested groups. After 48h, non-diabetic irradiated cutaneous cells showed another increase in wound closure compared to the control. Conversely, diabetic cells had repopulated the wounds almost completely, and no differences were observed between the control and PBMT-treated groups.

4. *mRNA expression analyses*

a. *Glucose transporter 1 (GLUT1)*

To confirm the maintenance of the diabetic *status* of isolated cutaneous cells *in vitro*, the expression of *GLUT1* was measured after 14 days of cell culture. This molecule is

a ubiquitous facilitative glucose transporter and regulates basal glucose uptake in most tissues, including the skin, where its expression appears downregulated (Pragallapati & Manyam, 2019; Spravchikov *et al.*, 2001). In this study, the *GLUT1* expression in non-diabetic cells was significantly upregulated compared to the diabetic ones ($p = 0.0010$) (Figure 4A). Furthermore, upon PBMT treatment in non-diabetic cells, the irradiated group showed improved expression of *GLUT1* compared to its control ($p = 0.018$) (Figure 4B). Conversely, the diabetic PBMT-treated group showed a numerical decrease in *GLUT1* mRNA expression without statistical significance (Figure 4C) when compared to the control diabetic non-irradiated.

b. Interleukin 1 α (IL1 α)

IL-1 α is an important mediator of inflammation and tissue damage in multiple organs. In the presence of a excess amount of *IL1 α* , inflammatory and autoimmune diseases may develop (Arend, 2002). Herein, concerning *IL1 α* , the cutaneous diabetic cells expressed more of this pro-inflammatory mediator compared to non-diabetic ones ($p < 0.0001$)(Figure 5A). After PBMT, the non-diabetic cells presented downregulated *IL1 α* mRNA expression ($p = 0.0002$) (Figure 5B) when compared to its control, whereas in diabetic cells, this marker expression was upregulated compared to the diabetic non-irradiated cells ($p < 0.0001$)(Figure 5C).

c. Interleukin 1 receptor antagonist (IL1ra)

The IL-1ra plays a role in buffering and dampening the overall effect of IL-1, thus regulating the effects of this pleiotropic cytokine (Oliveira *et al.*, 2022). In this study, when comparing the expression of *IL1ra* between the cell types before PBMT, minor expression of this interleukin is observed in diabetic cells when compared to the non-diabetic ones ($p < 0.0001$)(Figure 6A). In non-diabetic cells, following irradiation, occurs an upregulation of *IL1ra* ($p = 0.014$)(Figure 6B). However, in diabetic cells, the PBMT downregulates the expression of this pro-resolutive mediator ($p = 0.010$)(Figure 6C).

d. NESTIN

As an important player in tissue remodeling and cell regulation - particularly stem cells - (Bernal & Arranz, 2018), we evaluated the expression of *NESTIN* in diabetic and non-diabetic cutaneous cells in an *in vitro* model. Before PBMT, the non-differentiated diabetic cells presented a downregulated expression of this progenitor marker ($p < 0.0001$)(Figure 7A) compared to non-diabetic cells. In PBMT-treated groups, only the diabetic cells showed an upregulation of *NESTIN* after irradiation ($p = 0.023$)(Figure 7C), with the non-diabetic cells showing no difference between the control and PBMT ($p > 0.05$)(Figure 7B). After neurogenic differentiation, *NESTIN* was increased compared to the undifferentiated control, regardless of diabetic status ($p < 0.0001$). Under neurogenic stimulus, PBMT downregulated the expression of *NESTIN* in non-diabetic (Figure 7D) and diabetic cells (Figure 7E). In Figure 7F, we observe a representative image of neurogenic differentiated cutaneous cells.

e. Neural-Glial Antigen 2 (NG2)

NG2 is an important marker for type-1 pericytes, a type of cell knowledge for multipotent properties and their role in response to injuries (Ahmed & El-Badri, 2018). In this study, the non-differentiated diabetic cutaneous cells presented a downregulated expression of this marker when compared to non-diabetics before PBMT ($p < 0.0001$)(Figure 8A). After irradiation, the non-diabetic group presented similar expression of *NG2*, with no significant differences between the control and PBMT (Figure 8B). Moreover, diabetic PBMT-treated cells showed upregulation of *NG2* compared to control non-irradiated ($p = 0.040$)(Figure 8C). After neurogenic differentiation, the non-diabetic (Figure 8D) and diabetic cells (Figure 8E) presented similar expression of *NG2* when comparing PBMT-treated groups and their respective control without irradiation ($p > 0.05$). A downregulation of *NG2* mRNA was observed under neurogenic stimulus in both cell types compared to the undifferentiated control ($p < 0.0001$).

f. Peroxisome proliferator-activated receptor gamma (PPAR γ)

PPAR γ plays a key role in lipid metabolism and is a marker for lipid aggregates. Moreover, its activation also affects the epidermis through stimulation of keratinocyte differentiation, improvement of barrier homeostasis, and anti-inflammatory mediation

(Mao-Qiang *et al.*, 2004). In the cutaneous non-differentiated cells cultivated in this study, no significant differences between the non-diabetic and diabetic cells were observed regarding the expression of this marker ($p > 0.05$) (Figure 9A).

When evaluating the undifferentiated non-diabetic cell type, PBMT seems to downregulate the expression of *PPAR γ* compared to the non-irradiated cells ($p = 0.0053$) (Figure 9B). Conversely, within 14 days of adipogenic differentiation, these cells presented pronounced upregulation of this gene after irradiation ($p = 0.0018$) (Figure 9D). At the same time, the diabetic undifferentiated cells (Figure 9C) did not present a significant difference in the expression of *PPAR γ* between the control and PBMT. Following adipogenic stimulation, the expression also continued similar between PBMT-treated and control groups (Figure 9E).

5. *PBMT increases lipid positivity in diabetic cells*

After 14 days of cell culture, diabetic cutaneous cells demonstrated an increase in lipid-positive cells after PBMT compared to the non-irradiated group ($p = 0.0393$). Meanwhile, the non-diabetic cells showed no significant difference regarding lipid droplets compared to the control.

DISCUSSION

During normal wound healing, the immune system plays a orchestrated interplay to reestablish balance and promote tissue regeneration. However, in diabetic wounds, this process is dysregulated, and the immune system starts to promote more damage than repair (DenDekker & Gallagher, 2020). Considering the properties of PBMT, we hypothesized that this treatment could improve the biological responses of cells and tissues to overcome this impairment derived from diabetes mellitus. Thus, this study was designed to evaluate the impact of PBMT on cutaneous cells derived from type-1 diabetic mice.

In the conditions of this study, our preliminary data confirmed that PBMT improves the capacities of cutaneous cells from diabetic mice, depending on the moment of evaluation. After irradiation, the number of cell colonies was similar between the control and PBMT, although we observed a interspaced – or every other

day – increase in cell viability and migration. Indeed, some studies have shown the ability of PBMT to stimulate the proliferation and migration of different types of cells from diabetic mice, but these effects also depend on the parameters utilized and the moment of evaluation (Ayuk *et al.*, 2018; Jere *et al.*, 2018; Mokoena *et al.*, 2020; Oyeboode *et al.*, 2021).

Glucose represents a major source of metabolic energy in eukaryotic cells. Glucose transporters (1–14 GLUTs) are the family of integral membrane glycoproteins that play a key role in facilitating cellular uptake of this important nutrient (Thorens & Mueckler, 2010). In order to confirm the diabetic state after the culture expansion, we evaluated the expression of *GLUT1* in the diabetic cutaneous cells after 14 days of culture. This transporter is known to be downregulated in chronic hyperglycemia conditions (Pascual *et al.*), which, added to the greater expression of *IL1 α* by the diabetic group of this study, indicates that these cells are still demonstrating features of the diabetic condition.

In addition to that, previous studies also reported that keratinocytes exposed to high-glucose demonstrated impaired cell proliferation (Terashi *et al.*, 2005) and decreased *GLUT1* expression, a main glucose transporter in proliferating keratinocytes (Spravchikov *et al.*, 2001). Oppositely, we have observed greater cellular growth (colony forming units) in diabetic cutaneous cells compared to non-diabetic ones. Also, after PBMT, the *GLUT1* expression in non-diabetic cells was upregulated, in contrast with the diabetic group that not showed significant change regarding its respective control. Within the parameters of this study, PBMT seemed insufficient to promote a change in diabetic cutaneous cells to improve glucose uptake via *GLUT1*, suggesting that other mechanisms of energy source might have been playing a role in the growth of diabetic cells regardless of laser stimulus (Cibrian *et al.*, 2020)

As previously state, we saw greater expression of *IL1 α* by DM cells no treated with PBMT. The IL1 family plays a vital role in directing the inflammatory responses at skin in the beginning phases of wound healing (Johnston *et al.*, 2011). However, the persistence of pro-inflammatory cytokines in the wound microenvironment may contribute to wound persistence (Xiao *et al.*, 2020). Notwithstanding, the DM environment also impaired the expression of *IL1ra*, a naturally occurring cytokine that inhibits the IL-1 function preventing the release of inflammatory molecules by *IL1 α* activation (Volarevic *et al.*, 2010). In truth, the local application of *IL1ra* in diabetic wounds showed significant improvement in mice cutaneous wound healing (Perrault

et al., 2018; Tan *et al.*, 2021). This cytokine setup also corroborates with the inflammatory state of our diabetic cells *in vitro*.

Surprisingly, after PBMT, the DM cells kept the upregulated expression of IL1a and downregulation of *IL1ra*, worsening the proinflammatory scenario (Figures 5, 6). Nevertheless, we noticed that PBMT did not present the same magnitude of positive effects in diabetic wounds (unpublished data), confirming our current *in vitro* data. As such, PBMT limitations in diabetic wounds deserve further investigation and light protocol optimization.

NG2 is a marker expressed not only by glial cells but also by vascular mural cells, including pericytes, cells of mesenchymal lineages, and stem cells in the cutaneous tissue (Gomes *et al.*, 2022; Kadoya *et al.*, 2008). *NESTIN* is also a well-known and important marker of neural progenitor cells. Cells expressing *NESTIN* show characteristic features of stem cells, such as multipotency, high proliferation, limited self-renewal, and regeneration capacity (Gomes *et al.*, 2022; Wiese *et al.*, 2004).

A previous study from our group showed that PBMT has the ability to improve local microvasculature and increases pericytes and *NESTIN*-expressing progenitors towards skin regeneration (do Valle *et al.*, 2020). Herein, regarding *NESTIN* and NG2 expression, cutaneous cells from diabetic mice showed downregulation of these markers compared to non-diabetic ones, but PBMT was capable of recovering its expression, which can mean enhanced regeneration of tissues in diabetes by the modulation of stem cells. Therefore, this corroborates to our previous findings where PBMT has driven cutaneous or dental pulp regeneration through the stimulation of endogenous NG2⁺ and *NESTIN*⁺ stem cells (do Valle *et al.*, 2020; Gomes *et al.*, 2022).

After neurogenic stimulation, despite the overall increase of *NESTIN* mRNA compared to the undifferentiated control, this marker was significantly downregulated upon PBMT. This decrease is suggestive of a differentiation shift toward neurogenesis, once the cells lose this marker while differentiating into a more committed cell type, such as neurons (Gomes *et al.*, 2022; Mignone *et al.*, 2004). A study on the neurogenic potential of skin-derived precursors have shown that skin cells retain latent neurogenic properties, including the expression of *NESTIN*, after residing in a non-neural tissue. However, their differentiation into electrophysiologically active neurons requires additional measures (Fernandes *et al.*, 2006). Those findings align our current data. Further insights regarding PBMT role on neurogenic stimulation may help elucidate its

positive effects on neuron regrowth in a number of tissues and organs (Yazdani *et al.*, 2012)

PPAR γ is a nuclear receptor known to modulate lipid metabolism, but its activation also controls the expression of a network of genes involved in cell proliferation, differentiation, and inflammatory responses (Mao-Qiang *et al.*, 2004; Nicolas Di-Poi *et al.*, 2004). In this context, it has been proposed as a new target for the treatment of inflammatory wounds, such as occur in diabetes mellitus (Ramot *et al.*, 2015). Interestingly, in our study, the *PPAR γ* expression between diabetic and non-diabetic cells was similar, and after PBMT, it showed a pronounced expression only in non-diabetic cells.

Although PBMT was insufficient to significantly increase *PPAR γ* expression in DM cells, the Oil Red O assay showed increased lipid droplets in those cultures. Shook and collaborators have demonstrated the fundamental role of adipogenesis and fatty acids release - through adipocyte' lipolysis - as energy sources for myofibroblast differentiation in wound repair (Shook *et al.*, 2020). Also, another study demonstrated that proliferative stages of cutaneous wound healing require adipocytes to orientate fibroblast function (Schmidt & Horsley, 2013).

Some studies corroborate our findings showing a major presence of adipose cells after the light treatment (Mostafavinia *et al.*, 2021; Zaccara *et al.*, 2018). Whereas others have shown reduced expression of adipogenic markers and lipid droplets upon photobiomodulation (Bueno *et al.*, 2022). A different group also demonstrated that PBMT helps reduce plasma triglycerides and free fatty acid contents to ameliorate whole-body insulin resistance in diabetic mice (Gong *et al.*, 2020).

Finally, although adipocytes are primarily known for their role in the storage of triglycerides as an energy source (Schmidt & Horsley, 2013), they also function as endocrine cells that secrete growth factors and cytokines (Kilroy *et al.*, 2007; Ouchi *et al.*, 2011). Consequently, the stimulation of adipogenic differentiation by PBMT could result in a greater network between these cells involved in wound repair.

Several *in vitro* experimental models have been used to investigate the role of DM on cell biology. In this study, despite the evident differences in *GLUT1* mRNA expression between cell types, *in vitro* isolated cutaneous cells from diabetic mice showed viability, proliferation, and migration functions only slightly impaired. Limitations in the current model are possibly due to cell culture conditions provided by the glucose addition in the culture medium or even an eventual loss of the diabetic

phenotype over cell division *in vitro* (El-Osta *et al.*, 2008). Additionally, despite the similarity, a STZ model of diabetes in mice does not recapitulate all the aspects of the advanced human diabetic condition observed in the clinic (Liang *et al.*, 2018).

CONCLUSION

Our data suggest that PBMT at 660nm presented ambivalent effects on cells stressed by diabetic conditions, sometimes limiting cellular growth, and worsening the inflammatory scenario *in vitro*. Despite this, PBMT stimulated the expression of progenitor mediators in cutaneous diabetic cells. Altogether, our data suggest that protocol optimizations might be considered to balance its positive effects on skin regeneration in diabetic patients.

AUTHOR CONTRIBUTIONS

I. M. A. Diniz, R. F. Oliveira, M. G. Diniz, N.A. Gomes, contributed to the conception, design, data acquisition, interpretation, drafted and critically revised the manuscript. J. V. R. Cruvinel, B. G. Lucardians, C. C. Picoli, A. C. Costa contributed to data acquisition and interpretation. M. S. Araújo, A. Birbrair, performed statistical analyses, drafted and critically revised the manuscript.

ACKNOWLEDGMENTS

This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grants 438748/2018) and Coordenação de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES scholarships, Finance code 001). Those agencies have no involvement in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; or in the decision to submit the article for publication.

Conflict of interest: The authors have no conflict of interest to declare.

REFERENCES

- Ahmed, T. A., & El-Badri, N. (2018). Pericytes: The role of multipotent stem cells in vascular maintenance and regenerative medicine. *Advances in Experimental Medicine and Biology*, 69–86. https://doi.org/10.1007/5584_2017_138
- Arany, P. R. (2016). Craniofacial Wound Healing with Photobiomodulation Therapy: New Insights and Current Challenges. In *Journal of Dental Research* (Vol. 95, Issue 9, pp. 977–984). SAGE Publications Inc. <https://doi.org/10.1177/0022034516648939>
- Arend, W. P. (2002). The balance between IL-1 and IL-1Ra in disease. In *Cytokine & Growth Factor Reviews* (Vol. 13).
- Ayuk, S. M., Houreld, N. N., & Abrahamse, H. (2018). Effect of 660 nm visible red light on cell proliferation and viability in diabetic models in vitro under stressed conditions. *Lasers in Medical Science*, 33(5), 1085–1093. <https://doi.org/10.1007/s10103-017-2432-2>
- Bernal, A., & Arranz, L. (2018). *NESTIN*-expressing progenitor cells: function, identity and therapeutic implications. In *Cellular and Molecular Life Sciences* (Vol. 75, pp. 2177–2195). Birkhauser Verlag AG. <https://doi.org/10.1007/s00018-018-2794-z>
- Beura, S. K., Panigrahi, A. R., Yadav, P., Agrawal, S., & Singh, S. K. (2022). Role of Neurons and Glia Cells in Wound Healing as a Novel Perspective Considering Platelet as a Conventional Player. *Molecular neurobiology*, 59(1), 137–160. <https://doi.org/10.1007/s12035-021-02587-4>
- Bueno, N. P., Kfoury, C. C., Copete, I. N., de Oliveira, F. S., Arany, P., Marques, M. M., & Ferraz, E. P. (2022). Photobiomodulation treatments drive osteogenic versus adipocytic fate of bone marrow mesenchymal stem cells reversing the effects of hyperglycemia in diabetes. *Lasers in Medical Science*, 37(7), 2845–2854. <https://doi.org/10.1007/s10103-022-03553-9>
- Cañedo-Dorantes, L., & Cañedo-Ayala, M. (2019). Skin acute wound healing: a comprehensive review. *International journal of inflammation*, 2019.
- Cerveró-Ferragut, S., López-Riquelme, N., Martín-Tomás, E., Massa-Domínguez, B., Pomares-Vicente, J., Soler-Pérez, M., & Sánchez-Hernández, J. F. (2017). Quantitative analysis of blood cells and inflammatory factors in wounds. *Journal of Wound Care*, 26(3), 121–125.
- Chen, A. C. H., Arany, P. R., Huang, Y. Y., Tomkinson, E. M., Sharma, S. K., Kharkwal, G. B., Saleem, T., Mooney, D., Yull, F. E., Blackwell, T. S., & Hamblin, M. R. (2011). Low-Level laser therapy activates NF-κB via generation of reactive oxygen species in mouse embryonic fibroblasts. *PLoS ONE*, 6(7). <https://doi.org/10.1371/journal.pone.0022453>
- Cibrian, D., de la Fuente, H., & Sánchez-Madrid, F. (2020). Metabolic Pathways That Control Skin Homeostasis and Inflammation. In *Trends in Molecular Medicine* (Vol. 26, Issue 11, pp. 975–986). Elsevier Ltd. <https://doi.org/10.1016/j.molmed.2020.04.004>
- de Abreu, P. T. R., de Arruda, J. A. A., Mesquita, R. A., Abreu, L. G., Diniz, I. M. A., & Silva, T. A. (2019). Photobiomodulation effects on keratinocytes cultured in vitro: a critical review. In *Lasers in Medical Science* (Vol. 34, Issue 9, pp. 1725–1734). Springer London. <https://doi.org/10.1007/s10103-019-02813-5>
- de Freitas, L. F., & Hamblin, M. R. (2016). Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE Journal of Selected*

- Topics in Quantum Electronics*, 22(3), 348–364.
<https://doi.org/10.1109/JSTQE.2016.2561201>
- DenDekker, A. D., & Gallagher, K. A. (2020). Dysregulated inflammation in diabetic wounds. In *Wound Healing, Tissue Repair, and Regeneration in Diabetes* (pp. 81–95). Elsevier. <https://doi.org/10.1016/B978-0-12-816413-6.00005-8>
- Deshpande, A. D., Harris-Hayes, M., & Schootman, M. (2008). Epidemiology of Diabetes and Diabetes-Related Complications Diabetes Special Issue. *Physical Therapy*, 88, 11. www.ptjournal.org
- Diniz, I. M. A., Carreira, A. C. O., Sipert, C. R., Uehara, C. M., Moreira, M. S. N., Freire, L., Pelissari, C., Kossugue, P. M., de Araújo, D. R., Sogayar, M. C., & Marques, M. M. (2018). Photobiomodulation of mesenchymal stem cells encapsulated in an injectable rhBMP4-loaded hydrogel directs hard tissue bioengineering. *Journal of Cellular Physiology*, 233(6), 4907–4918.
<https://doi.org/10.1002/jcp.26309>
- do Valle, I. B., Prazeres, P. H. D. M., Mesquita, R. A., Silva, T. A., de Castro Oliveira, H. M., Castro, P. R., Freitas, I. D. P., Oliveira, S. R., Gomes, N. A., de Oliveira, R. F., Marquiere, L. F., Macari, S., do Amaral, F. A., Jácome-Santos, H., Barcelos, L. S., Menezes, G. B., Marques, M. M., Birbrair, A., & Diniz, I. M. A. (2020). Photobiomodulation drives pericyte mobilization towards skin regeneration. *Scientific Reports*, 10(1), 19257. <https://doi.org/10.1038/s41598-020-76243-7>
- El-Osta, A., Brasacchio, D., Yao, D., Pocai, A., Jones, P. L., Roeder, R. G., Cooper, M. E., & Brownlee, M. (2008). Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *Journal of Experimental Medicine*, 205(10), 2409–2417.
<https://doi.org/10.1084/jem.20081188>
- Fernandes, K. J. L., Kobayashi, N. R., Gallagher, C. J., Barnabé-Heider, F., Aumont, A., Kaplan, D. R., & Miller, F. D. (2006). Analysis of the neurogenic potential of multipotent skin-derived precursors. *Experimental Neurology*, 201(1), 32–48.
<https://doi.org/10.1016/j.expneurol.2006.03.018>
- Freshney, R. I. (2015). *Culture of animal cells: a manual of basic technique and specialized applications*.
- Furman, B. L. (2015). Streptozotocin-induced diabetic models in mice and rats. *Current Protocols in Pharmacology*, 70(1), 5.47.1-5.47.20.
<https://doi.org/10.1002/0471141755.ph0547s70>
- Gallagher, K. A. (2020). Dysregulated inflammation in diabetic wounds. In *Wound healing, tissue repair, and regeneration in diabetes* (pp. 81-95). Academic Press.
- Gomes, N. A., do Valle, I. B., Gleber-Netto, F. O., Silva, T. A., Oliveira, H. M. de C., de Oliveira, R. F., Ferreira, L. de A. Q., Castilho, L. S., Reis, P. H. R. G., Prazeres, P. H. D. M., Menezes, G. B., de Magalhães, C. S., Mesquita, R. A., Marques, M. M., Birbrair, A., & Diniz, I. M. A. (2022). *NESTIN* and *NG2* transgenes reveal two populations of perivascular cells stimulated by photobiomodulation. *Journal of Cellular Physiology*, 237(4), 2198–2210.
<https://doi.org/10.1002/jcp.30680>
- Gong, L., Zou, Z., Huang, L., Guo, S., & Xing, D. (2020). Photobiomodulation therapy decreases free fatty acid generation and release in adipocytes to ameliorate insulin resistance in type 2 diabetes. *Cellular Signalling*, 67.
<https://doi.org/10.1016/j.cellsig.2019.109491>

- Hamblin, M. R. (2016). Photobiomodulation or low-level laser therapy. In *Journal of Biophotonics* (Vol. 9, Issues 11–12, pp. 1122–1124). Wiley-VCH Verlag.
<https://doi.org/10.1002/jbio.201670113>
- International Diabetes Federation. (2015). *IDF diabetes atlas: Vol. 7th Edition*. International Diabetes Federation.
- Jere, S. W., Houreld, N. N., & Abrahamse, H. (2018). Photobiomodulation at 660 nm stimulates proliferation and migration of diabetic wounded cells via the expression of epidermal growth factor and the JAK/STAT pathway. *Journal of Photochemistry and Photobiology B: Biology*, 179, 74–83.
<https://doi.org/10.1016/j.jphotobiol.2017.12.026>
- Johnston, A., Gudjonsson, J. E., Aphale, A., Guzman, A. M., Stoll, S. W., & Elder, J. T. (2011). EGFR and IL-1 signaling synergistically promote keratinocyte antimicrobial defenses in a differentiation-dependent manner. *Journal of Investigative Dermatology*, 131(2), 329–337. <https://doi.org/10.1038/jid.2010.313>
- Kadoya, K., Fukushi, J. I., Matsumoto, Y., Yamaguchi, Y., & Stallcup, W. B. (2008). NG2 proteoglycan expression in mouse skin: Altered postnatal skin development in the NG2 null mouse. *Journal of Histochemistry and Cytochemistry*, 56(3), 295–303. <https://doi.org/10.1369/jhc.7A7349.2007>
- Kilroy, G. E., Foster, S. J., Wu, X., Ruiz, J., Sherwood, S., Heifetz, A., Ludlow, J. W., Stricker, D. M., Potiny, S., Green, P., Halvorsen, Y. D. C., Cheatham, B., Storms, R. W., & Gimble, J. M. (2007). Cytokine profile of human adipose-derived stem cells: Expression of angiogenic, hematopoietic, and pro-inflammatory factors. *Journal of Cellular Physiology*, 212(3), 702–709.
<https://doi.org/10.1002/jcp.21068>
- Konrad, R. J., Mikolaenko, I., Tolar, J. F., Liu, K., & Kudlow, J. E. (2001). The potential mechanism of the diabetogenic action of streptozotocin : inhibition of pancreatic β -cell O-GlcNAc-selective N-acetyl- β -D-glucosaminidase. *Biochemical Journal*, 356(1), 31–41.
- Kuffler, D. P. (2016). Photobiomodulation in promoting wound healing: a review. *Regenerative Medicine*, 11(1), 107–122. <https://doi.org/10.2217/rme.15.82>
- Lan, C. C. E., Wu, C. S., Huang, S. M., Wu, I. H., & Chen, G. S. (2013). High-Glucose environment enhanced oxidative stress and increased interleukin-8 secretion from keratinocytes. *Diabetes*, 62(7), 2530–2538.
<https://doi.org/10.2337/db12-1714>
- Liang, W. J., Zhou, S. N., Shan, M. R., Wang, X. Q., Zhang, M., Chen, Y., Zhang, Y., Wang, S. X., & Guo, T. (2018). AMPK α inactivation destabilizes atherosclerotic plaque in streptozotocin-induced diabetic mice through AP-2 α /miRNA-124 axis. *Journal of Molecular Medicine*, 96(5), 403–412. <https://doi.org/10.1007/s00109-018-1627-8>
- Mao-Qiang, M., Fowler, A. J., Schmuth, M., Lau, P., Chang, S., Brown, B. E., Moser, A. H., Michalik, L., Desvergne, B., Wahli, W., Li, M., Metzger, D., Chambon, P. H., Elias, P. M., & Feingold, K. R. (2004). Peroxisome-proliferator-activated receptor (PPAR)- α activation stimulates keratinocyte differentiation. *Journal of Investigative Dermatology*, 123(2), 305–312.
- Mignone, J. L., Kukekov, V., Chiang, A. S., Steindler, D., & Enikolopov, G. (2004). Neural Stem and Progenitor Cells in *NESTIN*-GFP Transgenic Mice. *Journal of Comparative Neurology*, 469(3), 311–324. <https://doi.org/10.1002/cne.10964>
- Mokoena, D. R., Houreld, N. N., Dhilip Kumar, S. S., & Abrahamse, H. (2020). Photobiomodulation at 660 nm Stimulates Fibroblast Differentiation. *Lasers in Surgery and Medicine*, 52(7), 671–681. <https://doi.org/10.1002/lsm.23204>

- Mosca, R. C., Ong, A. A., Albasha, O., Bass, K., & Arany, P. (2019). Photobiomodulation Therapy for Wound Care: A Potent, Noninvasive, Photoceutical Approach. *Advances in Skin and Wound Care*, 32(4), 157–167. <https://doi.org/10.1097/01.ASW.0000553600.97572.d2>
- Mostafavinia, A., Ahmadi, H., Amini, A., Roudafshani, Z., Hamblin, M. R., Chien, S., & Bayat, M. (2021). The effect of photobiomodulation therapy on antioxidants and oxidative stress profiles of adipose derived mesenchymal stem cells in diabetic rats. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 262. <https://doi.org/10.1016/j.saa.2021.120157>
- Nicolas Di-Poï, Liliane Michalik, Béatrice Desvergne, & Walter Wahli. (2004). Functions of Peroxisome Proliferator-Activated Receptors (PPAR) in Skin Homeostasis. *Lipids*, 39(11).
- Oliveira, R. F., Marquiere, L. F., Gomes, C. B. S., de Abreu, P. T. R., Ferreira, L. A. Q., Diniz, L. A., Gomes, N. A., Jácome-Santos, H., Moreno, A., Macari, S., Mesquita, R. A., Silva, T. A., Marques, M. M., & Diniz, I. M. A. (2022). Interplay between epithelial and mesenchymal cells unveils essential proinflammatory and pro-resolutive mediators modulated by photobiomodulation therapy at 660 nm. *Wound Repair and Regeneration*, 30(3), 345–356. <https://doi.org/10.1111/wrr.13010>
- Okonkwo, U. A., & DiPietro, L. A. (2017). Diabetes and wound angiogenesis. *International journal of molecular sciences*, 18(7), 1419.
- Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. In *Nature Reviews Immunology* (Vol. 11, Issue 2, pp. 85–97). <https://doi.org/10.1038/nri2921>
- Oyebode, O., Houreld, N. N., & Abrahamse, H. (2021). Photobiomodulation in diabetic wound healing: A review of red and near-infrared wavelength applications. In *Cell Biochemistry and Function* (Vol. 39, Issue 5, pp. 596–612). John Wiley and Sons Ltd. <https://doi.org/10.1002/cbf.3629>
- Parfejevs, V., Debbache, J., Shakhova, O., Schaefer, S. M., Glausch, M., Wegner, M., Suter, U., Riekstina, U., Werner, S., & Sommer, L. (2018). Injury-activated glial cells promote wound healing of the adult skin in mice. *Nature Communications*, 9(1), 236. <https://doi.org/10.1038/s41467-017-01488-2>
- Pascual, J. M., Wang, D., Lecumberri, B., Yang, H., Mao, X., Yang, R., & de Vivo, D. C. (n.d.). *GLUT1* deficiency and other glucose transporter diseases. In *European Journal of Endocrinology* (Vol. 150). www.eje.org
- Pecoraro, R. E., Reiber, G. E., & Burgess, E. M. (1990). Pathways to Diabetic Limb Amputation Basis for Prevention. *Diabetes Care*, 13(5), 513–521.
- Perrault, D. P., Bramos, A., Xu, X., Shi, S., & Wong, A. K. (2018). Local Administration of Interleukin-1 Receptor Antagonist Improves Diabetic Wound Healing. *Annals of Plastic Surgery*, 80(5S Suppl 5), S317–S321. <https://doi.org/10.1097/SAP.0000000000001417>
- Ploner, C., Rauchenwald, T., Connolly, C. E., Joehrer, K., Rainer, J., Seifarth, C., ... & Pierer, G. (2021). Oxidant therapy improves adipogenic differentiation of adipose-derived stem cells in human wound healing. *Stem Cell Research & Therapy*, 12(1), 1-16.
- Pragallapati, S., & Manyam, R. (2019). Glucose transporter 1 in health and disease. *Journal of Oral and Maxillofacial Pathology*, 23(3), 443–449. https://doi.org/10.4103/jomfp.JOMFP_22_18
- Queiroz, A., Wada, M. T., Rosin, F. C. P., Pelissari, C., & Trierveiler, M. (2021). Effects of serum-free culture media on human apical papilla cells properties.

- Archives of Oral Biology*, 121, 104962.
<https://doi.org/10.1016/j.archoralbio.2020.104962>
- Ramot, Y., Mastrofrancesco, A., Camera, E., Desreumaux, P., Paus, R., & Picardo, M. (2015). The role of *PPAR* γ -mediated signalling in skin biology and pathology: New targets and opportunities for clinical dermatology. In *Experimental Dermatology* (Vol. 24, Issue 4, pp. 245–251). <https://doi.org/10.1111/exd.12647>
- Schmidt, B. A., & Horsley, V. (2013). Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. *Development (Cambridge)*, 140(7), 1517–1527. <https://doi.org/10.1242/dev.087593>
- Shook, B. A., Wasko, R. R., Mano, O., Rutenberg-Schoenberg, M., Rudolph, M. C., Zirak, B., Rivera-Gonzalez, G. C., López-Giráldez, F., Zarini, S., Rezza, A., Clark, D. A., Rendl, M., Rosenblum, M. D., Gerstein, M. B., & Horsley, V. (2020). Dermal Adipocyte Lipolysis and Myofibroblast Conversion Are Required for Efficient Skin Repair. *Cell Stem Cell*, 26(6), 880-895.e6.
<https://doi.org/10.1016/j.stem.2020.03.013>
- Spravchikov, N., Sizyakov, G., Gartsbein, M., Accili, D., Tennenbaum, T., & Wertheimer, E. (2001). Glucose effects on skin keratinocytes implications for diabetes skin complications. *Diabetes*, 50(7), 1627–1635.
<http://diabetesjournals.org/diabetes/article-pdf/50/7/1627/367783/1627.pdf>
- Tan, J. L., Lash, B., Karami, R., Nayer, B., Lu, Y. Z., Piotto, C., Julier, Z., & Martino, M. M. (2021). Restoration of the healing microenvironment in diabetic wounds with matrix-binding IL-1 receptor antagonist. *Communications Biology*, 4(1).
<https://doi.org/10.1038/s42003-021-01913-9>
- Tarallo, S., Beltramo, E., Berrone, E., & Porta, M. (2012). Human pericyte-endothelial cell interactions in co-culture models mimicking the diabetic retinal microvascular environment. *Acta Diabetologica*, 49, 141–151. <https://doi.org/10.1007/s00592-012-0390-5>
- Terashi, H., Izumi, K., Deveci, M., Rhodes, L. M., & Marcelo, C. L. (2005). High glucose inhibits human epidermal keratinocyte proliferation for cellular studies on diabetes mellitus. *International Wound Journal*, 2(4), 298–304.
<https://doi.org/10.1111/j.1742-4801.2005.00148.x>
- Thorens, B., & Mueckler, M. (2010). Glucose transporters in the 21st Century. *Am J Physiol Endocrinol Metab*, 298, 141–145.
<https://doi.org/10.1152/ajpendo.00712.2009>.-The
- Volarevic, V., Al-Qahtani, A., Arsenijevic, N., Pajovic, S., & Lukic, M. L. (2010). Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. In *Autoimmunity* (Vol. 43, Issue 4, pp. 255–263). <https://doi.org/10.3109/08916930903305641>
- Wiese, C., Rolletschek, A., Kania, G., Blyszczuk, P., Tarasov, K. v., Tarasova, Y., Wersto, R. P., Boheler, K. R., & Wobus, A. M. (2004). *NESTIN* expression - A property of multi-lineage progenitor cells? In *Cellular and Molecular Life Sciences* (Vol. 61, Issues 19–20, pp. 2510–2522).
<https://doi.org/10.1007/s00018-004-4144-6>
- Xiao, T., Yan, Z., Xiao, S., & Xia, Y. (2020). Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. In *Stem Cell Research and Therapy* (Vol. 11, Issue 1). BioMed Central Ltd. <https://doi.org/10.1186/s13287-020-01755-y>
- Yang, R., Liu, F., Wang, J., Chen, X., Xie, J., & Xiong, K. (2019). Epidermal stem cells in wound healing and their clinical applications. *Stem cell research & therapy*, 10(1), 1-14.

- Yazdani, S. O., Golestaneh, A. F., Shafiee, A., Hafizi, M., Omrani, H. A. G., & Soleimani, M. (2012). Effects of low level laser therapy on proliferation and neurotrophic factor gene expression of human schwann cells in vitro. *Journal of Photochemistry and Photobiology B: Biology*, 107(1), 9–13.
<https://doi.org/10.1016/j.jphotobiol.2011.11.001>
- Zaccara, I. M., Mestieri, L. B., Moreira, M. S., Grecca, F. S., Martins, M. D., & Kopper, P. M. P. (2018). Photobiomodulation therapy improves multilineage differentiation of dental pulp stem cells in three-dimensional culture model. *Journal of Biomedical Optics*, 23(09), 1.
<https://doi.org/10.1117/1.jbo.23.9.095001>

TABLES

Gene	Sequences	Size
<i>GLUT1</i>	F: 5' GGATCCCAGCAGCAAGAAGGT 3' R: 5' CGAACTGCAGTGATCCGAGC 3'	77
<i>IL1α</i>	F: 5' ACGTCAAGCAACGGGAAGAT 3' R: 5' AAGGTGCTGATCTGGGTTGG 3'	124
<i>IL1ra</i>	F: 5'AACCAGCTCATTGCTGGTACTTA 3' R: 5' GCCCAAGAACACACTATGAAGGTC 3'	96
NES	F: 5' TGGCTACATACAGGACTCTGC 3' R: 5' AAGGATGTTGGGCTGAGGAC 3'	172
<i>NG2</i>	F: 5' CTGTTCTCACACAGAGGAGCC 3' R: 5' TGGACAGACGGTCAACTTCC 3'	150
<i>PPARγ</i>	F: 5' GTCACACTCTGACAGGAGCC 3' R: 5' CACCGCTTCTTTCAAATCTTGT 3'	122
<i>GAPDH</i>	F: 5' ACGGCCGCATCTTCTTGTGCA 3' R: 5' CGCCAAATCCGTTCACACCGA 3'	71

Table 1. Mouse primer sequences used for qPCR analysis. Abbreviations: *GLUT1*, Glucose transporter 1; *IL1 α* , Interleukin 1 α ; *IL1ra*, Interleukin 1 receptor antagonist; NES, *NESTIN*; *NG2*, Neural-Glial Antigen 2; *PPAR γ* , Peroxisome proliferator-activated receptor gamma; *GAPDH*, Glyceraldehyde- 3-phosphate dehydrogenase.

FIGURES

Figure 1. Colony Forming Units – Fibroblastic (CFU-F) assay of non-diabetic and diabetic cutaneous cells cultivated in physiological and high-glucose media, respectively, irradiated (PBMT) or not.

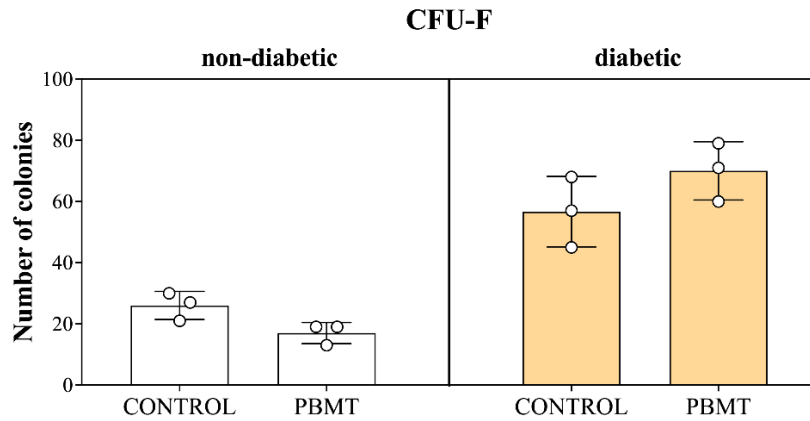


Figure 2. Cell viability of non-diabetic and diabetic cells stimulated by photobiomodulation therapy (PBMT). Bar graphs show cell viability upon irradiation after 24, 48, 72, and 7 days.

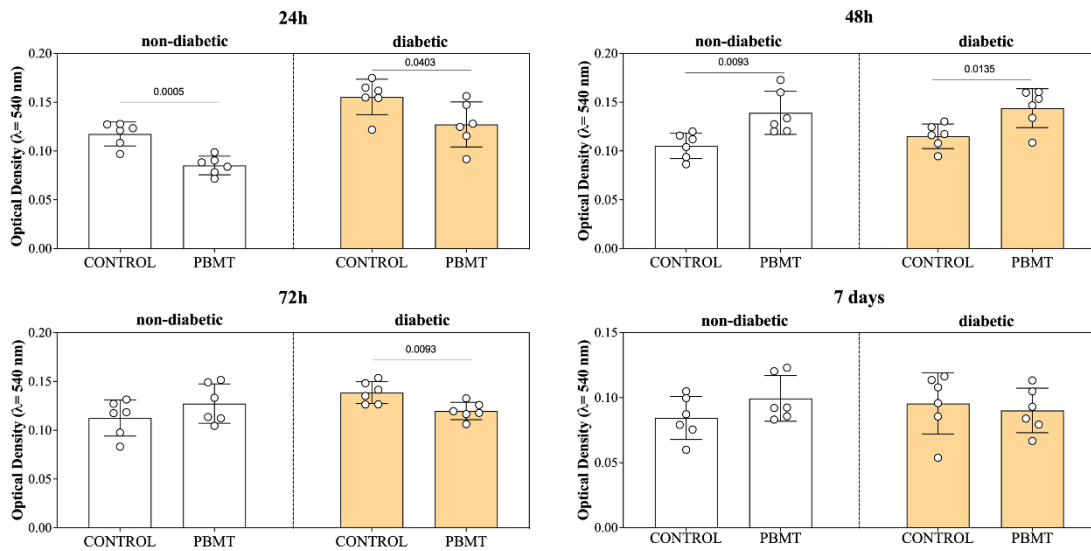


Figure 3. Cell scratch assay of non-diabetic and diabetic cutaneous cells cultivated in physiological and high glucose media, irradiated or not.

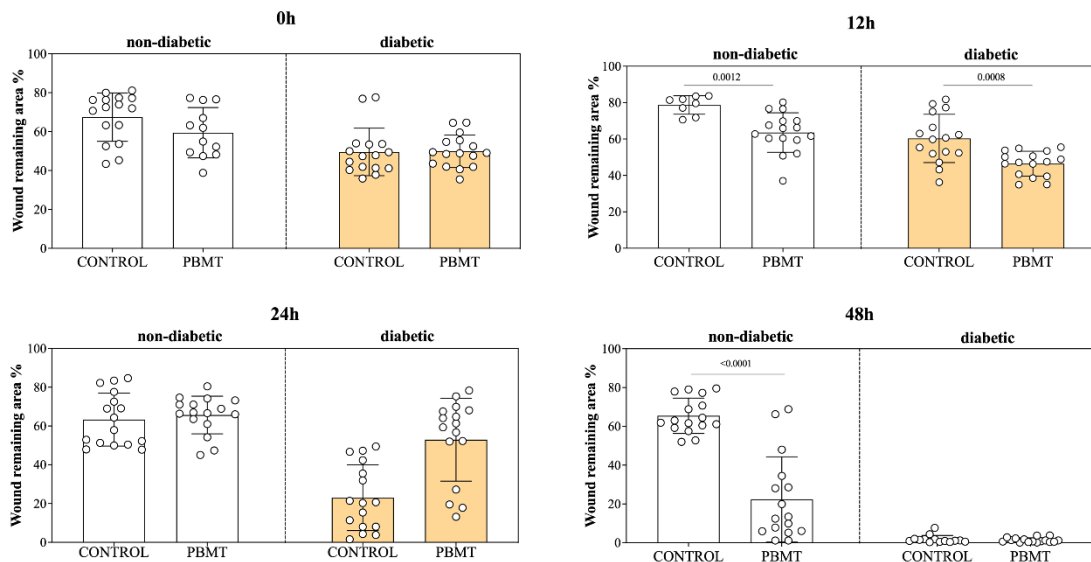


Figure 4. Relative expression of Glucose transporter 1 (*GLUT1*) in non-diabetic and diabetic cells upon photobiomodulation therapy (PBMT) after 14 days of cell culture.

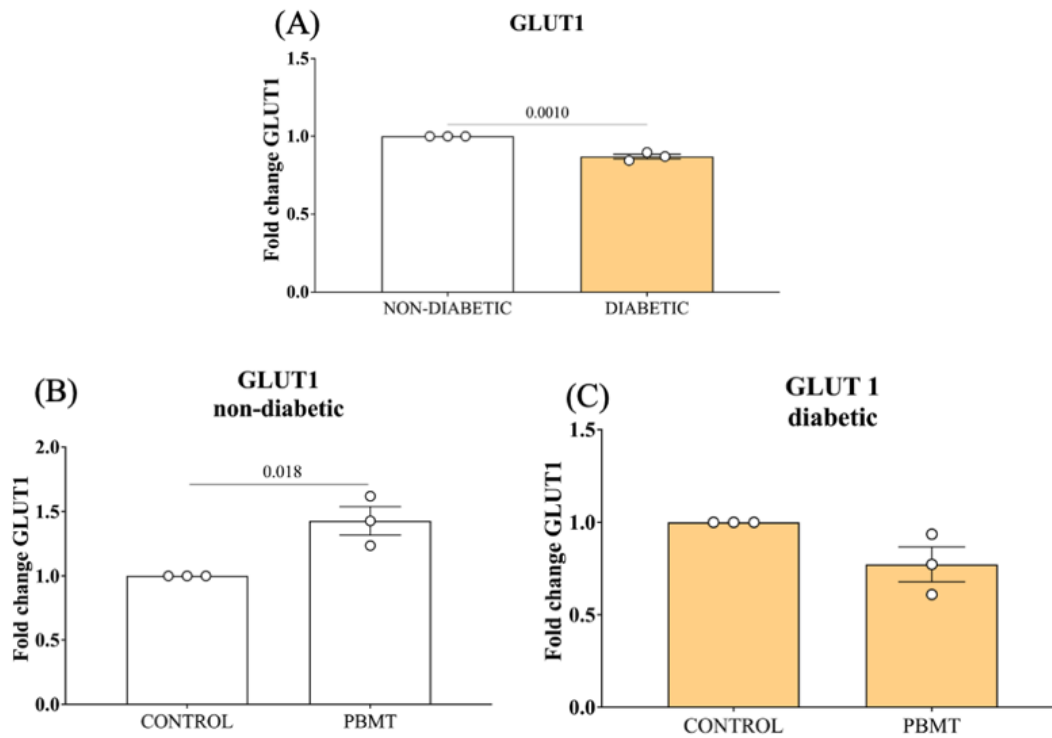


Figure 5. Relative expression of *IL1 α* in non-diabetic and diabetic cells upon PBMT or not after 14 days of cell culture.

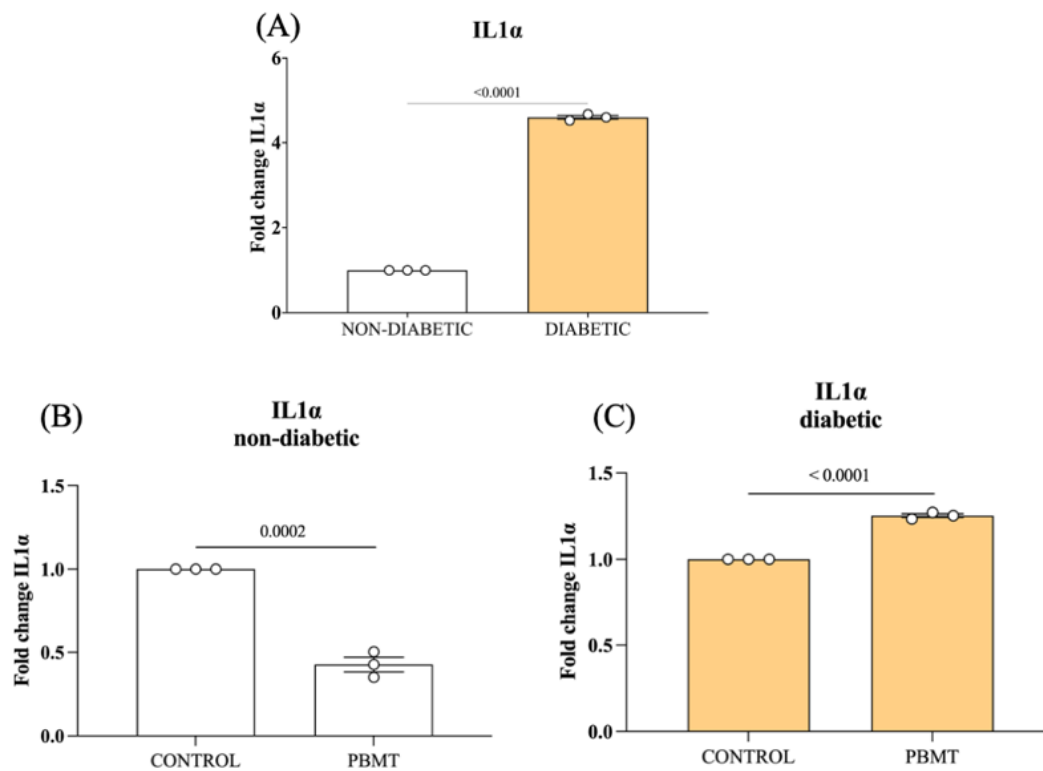


Figure 6. Relative expression of Interleukin 1 receptor antagonist (*IL1ra*) in non-diabetic and diabetic cells upon photobiomodulation therapy (PBMT) or not after 14 days of cell culture.

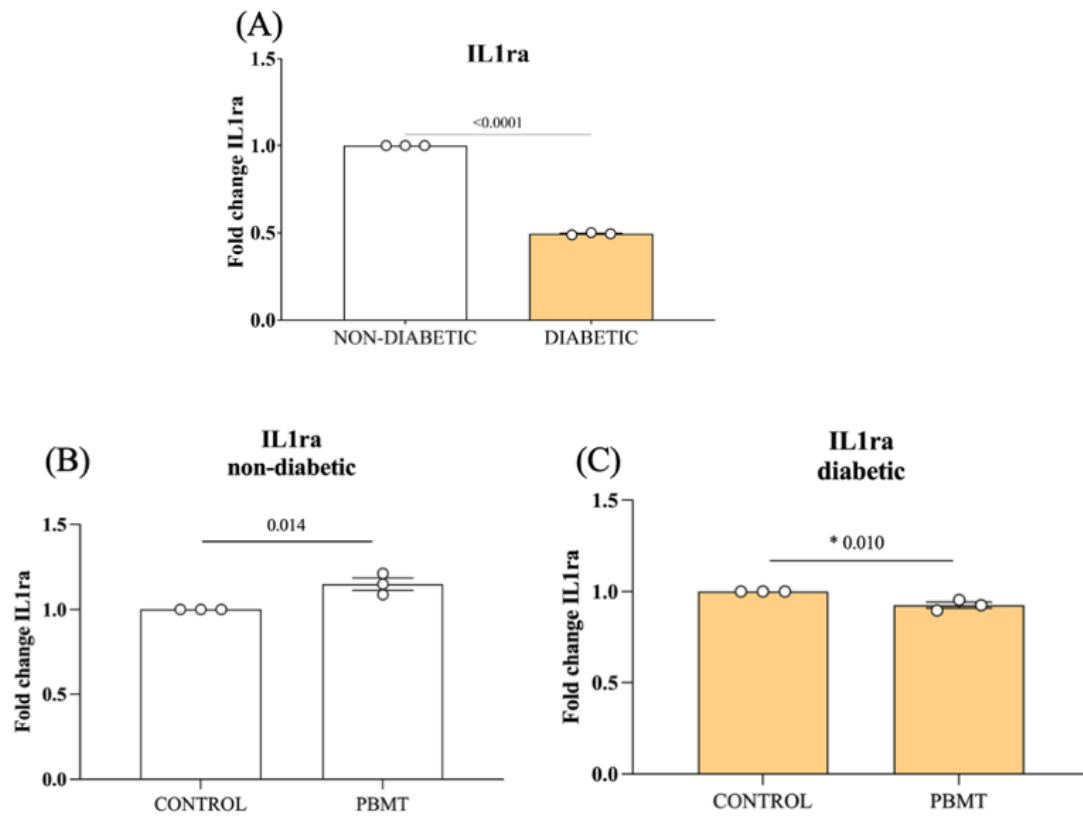


Figure 7. Relative expression of *NESTIN* regarding non-diabetic and diabetic cells upon photobiomodulation therapy (PBMT) or not after 14 days of cell culture in regular and neurogenic differentiation media.

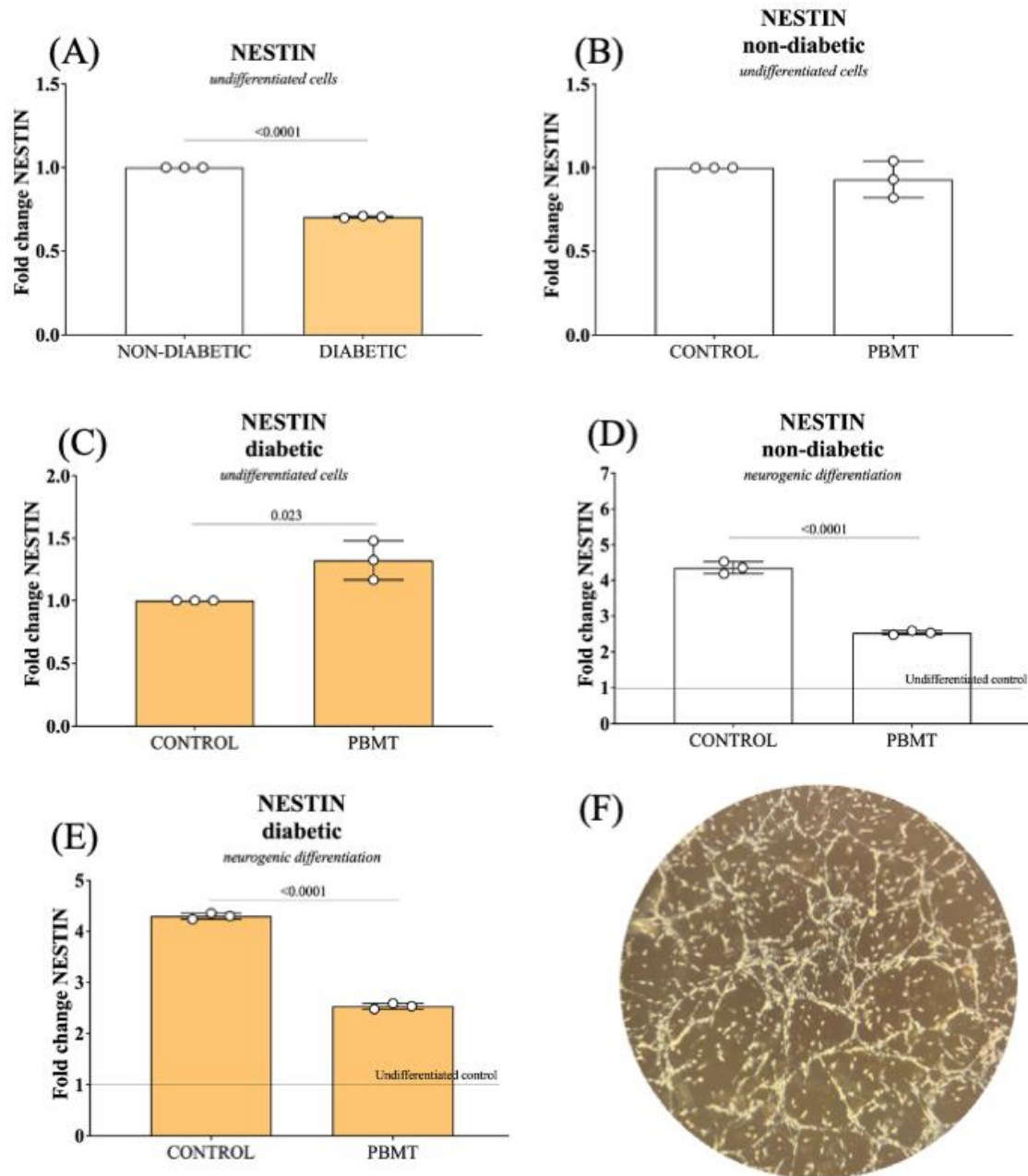


Figure 8. Relative expression of Neural/Glial Antigen 2 (*NG2*) regarding non-diabetic and diabetic cells upon PBMT or not after 14 days of cell culture in regular and neurogenic differentiation media.

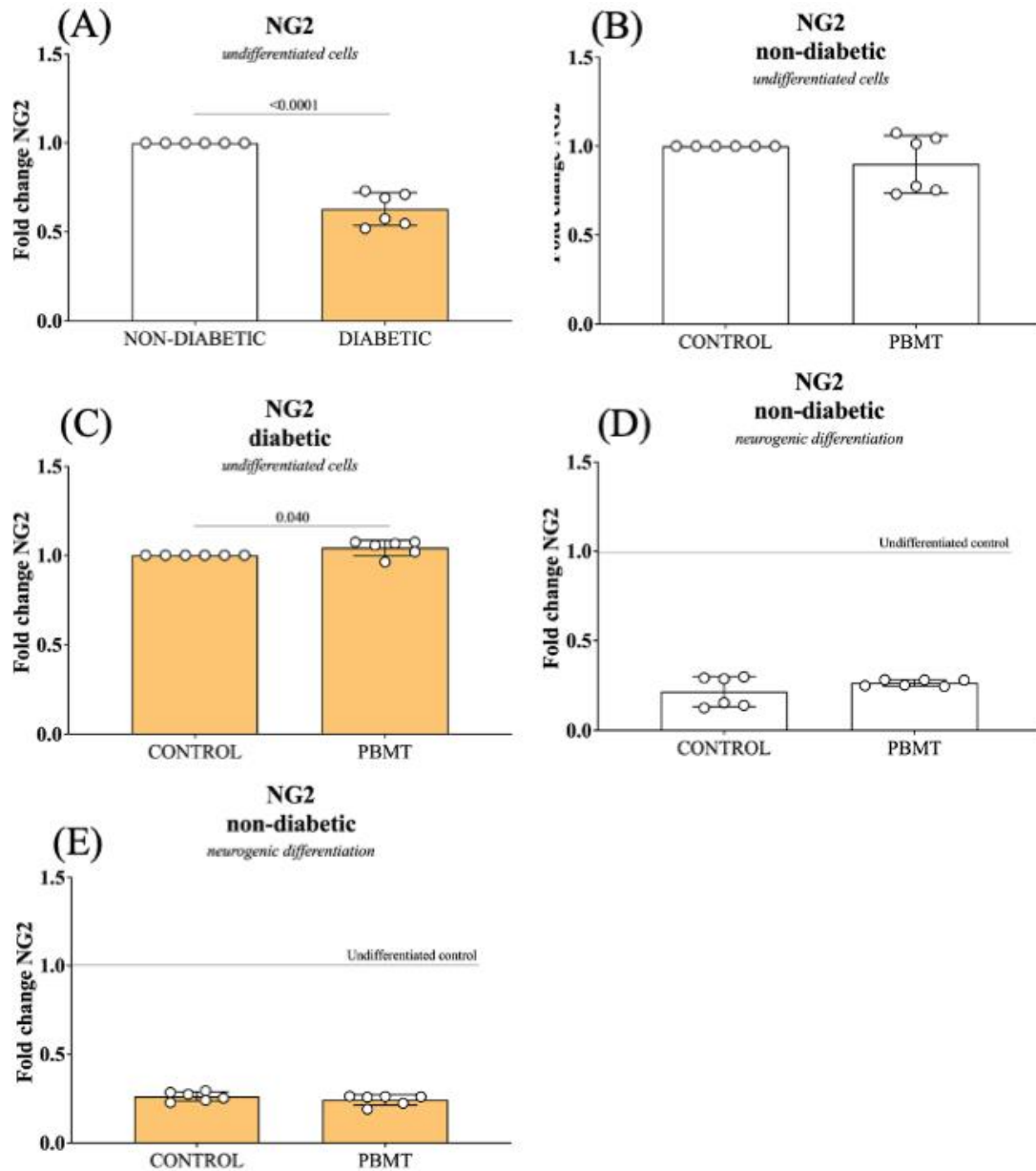


Figure 9. Relative expression of Peroxisome proliferator-activated receptor gamma (*PPAR* γ) regarding non-diabetic and diabetic cells upon PBMT after 14 days of cell culture in regular and adipogenic differentiation media.

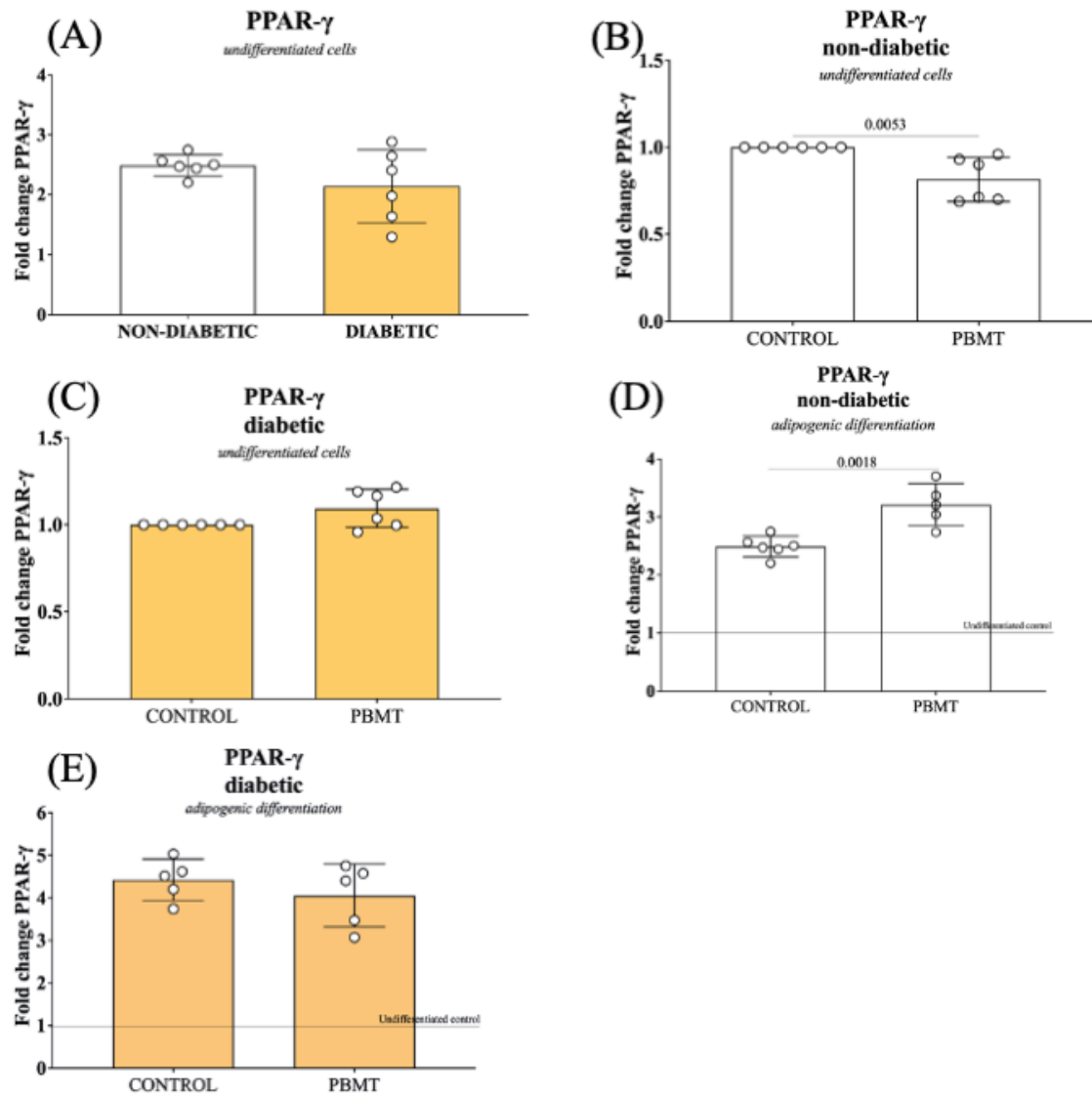
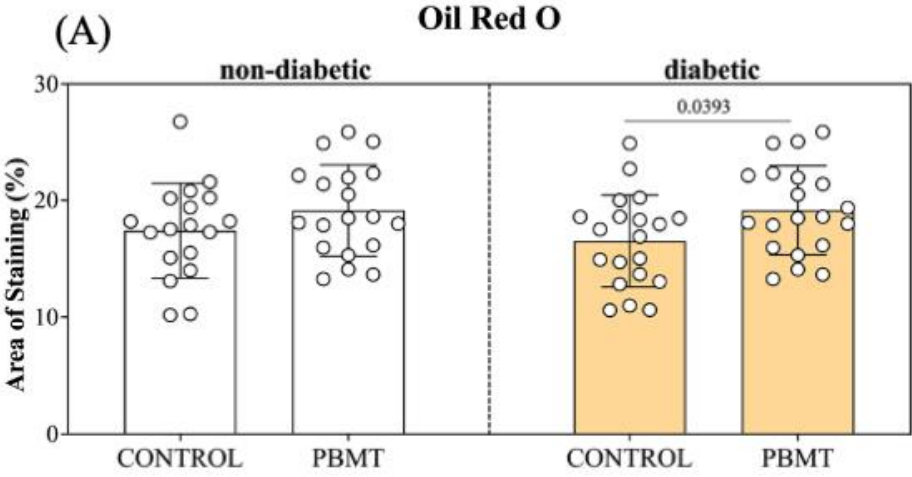
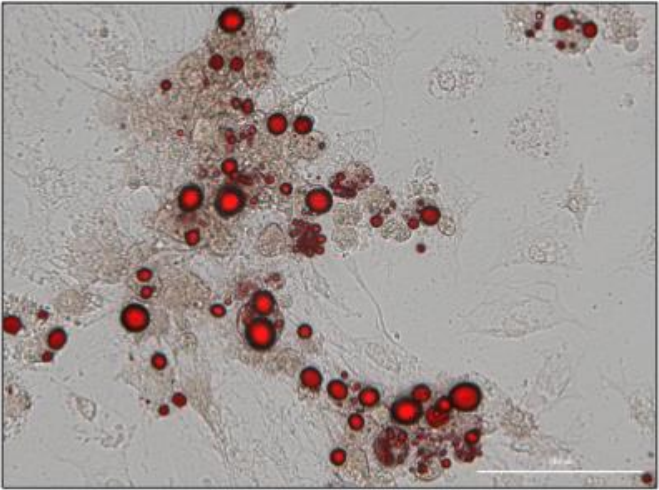


Figure 10. Quantification of lipid droplets by ORO analysis.



(B)



FIGURES LEGENDS

Figure 1. Colony Forming Units – Fibroblastic (CFU-F) assay of non-diabetic and diabetic cutaneous cells cultivated in physiological and high-glucose media, respectively, irradiated (PBMT) or not. Bar graph showing the efficiency of the control and PBMT-treated cutaneous cells in forming colonies. Data are shown as the mean $\pm$ SD (Student *t*-test; *n* = 3).

Figure 2. Cell viability of non-diabetic and diabetic cells stimulated by photobiomodulation therapy (PBMT). Non-diabetic and diabetic cutaneous cells were cultivated in physiological and high-glucose media, respectively, irradiated or not. Bar graphs show cell viability upon irradiation after 24, 48, 72, and 7 days. The values show the statistical differences between the control (non-irradiated) and PBMT-treated (irradiated) groups (Student *t*-test; *n* = 6). Data are shown as the mean $\pm$ SD.

Figure 3. Cell scratch assay of non-diabetic and diabetic cutaneous cells cultivated in physiological and high-glucose media, respectively, irradiated (PBMT) or not. The minor the area, the greater the closure. Data are shown as the mean $\pm$ SD. Student *t*-test or Mann-Whitney; *n* = 4, 16 fields.

Figure 4. Relative expression of Glucose transporter 1 (*GLUT1*) in non-diabetic and diabetic cells upon photobiomodulation therapy (PBMT) after 14 days of cell culture. (A) Bar graph showing the comparison between non-diabetic and diabetic cells without irradiation, (B) non-diabetic PBMT-treated group, (C) diabetic PBMT-treated group. Data are shown as the mean $\pm$ SD, *n* = 3 for each group (Student *t*-test).

Figure 5. Relative expression of *IL1 α* in non-diabetic and diabetic cells upon PBMT or not after 14 days of cell culture. (A) Bar graph showing the comparison between non-diabetic and diabetic cells without PBMT, (B) non-diabetic PBMT-treated group, (C) diabetic PBMT-treated group. Data are shown as the mean $\pm$ SD, *n* = 3 for each group (Student *t*-test).

Figure 6. Relative expression of Interleukin 1 receptor antagonist (*IL1ra*) in non-diabetic and diabetic cells upon photobiomodulation therapy (PBMT) or not after 14 days of cell culture (A) Bar graph showing the comparison between non-diabetic and diabetic cells without PBMT, (B) non-diabetic PBMT-treated group, (C) diabetic PBMT-treated group. Data are shown as the mean $\pm$ SD, *n* = 3 for each group (Student *t*-test).

Figure 7. Relative expression of *NESTIN* regarding non-diabetic and diabetic cells upon photobiomodulation therapy (PBMT) or not after 14 days of cell culture in regular and neurogenic differentiation media. (A) Bar graph showing the comparison between non-differentiated non-diabetic and diabetic cells without PBMT, (B) non-diabetic PBMT-treated, (C) diabetic PBMT-treated group, (D) non-diabetic cells PBM-treated

neurogenic differentiated and (E) diabetic cells PBMT-treated neurogenic differentiated. (F) Representative image of neurogenic stimulated cultures. Data are shown as the mean $\pm$ SD, $n = 3$ for each group (Student t -test).

Figure 8. Relative expression of Neural/Glial Antigen 2 (*NG2*) regarding non-diabetic and diabetic cells upon PBMT or not after 14 days of cell culture in regular and neurogenic differentiation media. A) Bar graph showing the comparison between non-differentiated non-diabetic and diabetic cells without PBMT, (B) non-diabetic PBMT-treated, (C) diabetic PBMT-treated group, (D) non-diabetic cells PBM-treated neurogenic differentiated, (E) diabetic cells PBMT-treated neurogenic differentiated.

Figure 9. Relative expression of peroxisome proliferator-activated receptor gamma (*PPAR γ*) regarding non-diabetic and diabetic cells upon PBMT after 14 days of cell culture in regular and adipogenic differentiation media. A) Bar graph showing the comparison between non-differentiated non-diabetic and diabetic cells without PBMT, (B) non-diabetic PBMT-treated, (C) diabetic PBMT-treated group, (D) non-diabetic cells PBM-treated neurogenic differentiated, (E) diabetic cells PBMT-treated neurogenic differentiated. Data are shown as the mean $\pm$ SD, $n = 6$ for each group (Student t -test).

Figure 10. Quantification of lipid droplets by Oil Red O (ORO) analysis of adipogenic stimulated cells. (A) Bar graph showing the comparison between non-diabetic and diabetic cells upon PBMT. Data are shown as the mean $\pm$ SD, $n = 2$, 20 fields for each group (Student t -test). The values show the statistical difference between the control (non-irradiated) and PBMT-treated (irradiated). (B) Representative image of Oil Red O staining after adipogenic differentiation. Scale bar = 100 μ m.

5 CONSIDERAÇÕES FINAIS

O objetivo deste estudo foi verificar a capacidade estimulatória da PBMT na modulação de células cutâneas em um modelo *in vitro* de diabetes tipo 1. Investigar os mecanismos moleculares e celulares da PBMT pode fornecer a justificativa e a aplicação clínica aprimorada desse tratamento para pacientes diabéticos.

Nas condições utilizadas neste trabalho, observamos que a PBMT em 660nm apresentou efeitos ambivalentes no modelo *in vitro* de células cutâneas de animais diabéticos quanto a viabilidade, migração e proliferação celulares. A avaliação de expressão gênica indicou que células cutâneas de animais diabéticos demonstraram um aumento do fator pro-inflamatório (*IL1 α*) e a diminuição do fator resolutivo investigado (*IL1ra*). Apesar disso, a fotobiomodulação foi capaz de aumentar a expressão de marcadores para células progenitoras – tronco. Ademais, a PBMT parece atuar nas células cutâneas através estimulação de PPAR, um marcador envolvido na comunicação celular para diferenciação e modulação da inflamação. Contudo, nossos resultados sugerem que otimizações no protocolo de aplicação da PBMT devam ser consideradas para um balanço positivo dos efeitos de regeneração tecidual em pacientes diabéticos.

REFERÊNCIAS

- ABREU, Priscila T. R. de *et al.* Photobiomodulation effects on keratinocytes cultured in vitro: a critical review. **Lasers in Medical Science**, v. 34, p. 1725-1734, 2019.
- ARANY, Praveen R. Craniofacial wound healing with photobiomodulation therapy: new insights and current challenges. **Journal of dental research**, v. 95, n. 9, p. 977-984, 2016.
- BEURA, Samir K. *et al.* Role of neurons and glia cells in wound healing as a novel perspective considering platelet as a conventional player. **Molecular Neurobiology**, p. 1-24, 2021.
- CAÑEDO-DORANTES, Luis; CAÑEDO-AYALA, Mara. Skin acute wound healing: a comprehensive review. **International journal of inflammation**, v. 2019, 2019.
- CERVERÓ-FERRAGUT, S. *et al.* Quantitative analysis of blood cells and inflammatory factors in wounds. **Journal of Wound Care**, v. 26, n. 3, p. 121-125, 2017.
- CHEN, Aaron CH *et al.* Low-level laser therapy activates NF-κB via generation of reactive oxygen species in mouse embryonic fibroblasts. **PloS one**, v. 6, n. 7, p. e22453, 2011.
- DESHPANDE, Anjali D.; HARRIS-HAYES, Marcie; SCHOOTMAN, Mario. Epidemiology of diabetes and diabetes-related complications. **Physical therapy**, v. 88, n. 11, p. 1254-1264, 2008.
- DINIZ, Ivana M. A. *et al.* Photobiomodulation of mesenchymal stem cells encapsulated in an injectable rhBMP4-loaded hydrogel directs hard tissue bioengineering. **Journal of Cellular Physiology**, v. 233, n. 6, p. 4907-4918, 2018.
- FRESHNEY, R. Ian. **Culture of animal cells: a manual of basic technique and specialized applications**. John Wiley & Sons, 2015.
- FURMAN, Brian L. Streptozotocin-induced diabetic models in mice and rats. **Current protocols in pharmacology**, v. 70, n. 1, p. 5.47. 1-5.47. 20, 2015.
- GALLAGHER, Katherine A. *et al.* Dysregulated inflammation in diabetic wounds. In: **Wound healing, tissue repair, and regeneration in diabetes**. Academic Press, 2020. p. 81-95.
- GOMES, Natália A. *et al.* *NESTIN* and *NG2* transgenes reveal two populations of perivascular cells stimulated by photobiomodulation. **Journal of Cellular Physiology**, v. 237, n. 4, p. 2198-2210, 2022.
- HAMBLIN, Michael R. Photobiomodulation or low-level laser therapy. **Journal of biophotonics**, v. 9, n. 11-12, p. 1122, 2016.
- INTERNACIONAL DIABETES FEDERATION, 33, 2015, Belgium. **IDF Diabetes Atlas**. Belgium: Internacional Diabetes Federation, 2015.

KANITAKIS, Jean. Anatomy, histology and immunohistochemistry of normal human skin. **European journal of dermatology**, v. 12, n. 4, p. 390-401, 2002.

KOLARSICK, Paul A. J.; KOLARSICK, Maria Ann; GOODWIN, Carolyn. Anatomy and physiology of the skin. **Journal of the Dermatology Nurses' Association**, v. 3, n. 4, p. 203-213, 2011.

KONRAD, Robert J. *et al.* The potential mechanism of the diabetogenic action of streptozotocin: inhibition of pancreatic β -cell O-GlcNAc-selective N-acetyl- β -D-glucosaminidase. **Biochemical Journal**, v. 356, n. 1, p. 31-41, 2001.

KUFFLER, Damien P. Photobiomodulation in promoting wound healing: a review. **Regenerative medicine**, v. 11, n. 1, p. 107-122, 2016.

LAN, Cheng-Che E. *et al.* High-glucose environment enhanced oxidative stress and increased interleukin-8 secretion from keratinocytes: new insights into impaired diabetic wound healing. **Diabetes**, v. 62, n. 7, p. 2530-2538, 2013.

MARUYAMA, Kazuichi *et al.* Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. **The American journal of pathology**, v. 170, n. 4, p. 1178-1191, 2007.

MOSCA, Rodrigo C. *et al.* Photobiomodulation therapy for wound care: a potent, noninvasive, photoceutical approach. **Advances in skin & wound care**, v. 32, n. 4, p. 157-167, 2019.

OLIVEIRA, Rafaela F. *et al.* Interplay between epithelial and mesenchymal cells unveils essential proinflammatory and pro-resolutive mediators modulated by photobiomodulation therapy at 660 nm. **Wound Repair and Regeneration**, v. 30, n. 3, p. 345-356, 2022.

OKONKWO, Uzoagu A.; DIPIETRO, Luisa A. Diabetes and wound angiogenesis. **International journal of molecular sciences**, v. 18, n. 7, p. 1419, 2017.

PARFEJEVS, Vadims *et al.* Injury-activated glial cells promote wound healing of the adult skin in mice. **Nature communications**, v. 9, n. 1, p. 236, 2018.

PARK, Sangbum. Building vs. Rebuilding epidermis: Comparison embryonic development and adult wound repair. **Frontiers in Cell and Developmental Biology**, p. 3969, 2022.

PECORARO, Roger E.; REIBER, Gayle E.; BURGESS, Ernest M. Pathways to diabetic limb amputation: basis for prevention. **Diabetes care**, v. 13, n. 5, p. 513-521, 1990.

PLONER, Christian *et al.* Oxidant therapy improves adipogenic differentiation of adipose-derived stem cells in human wound healing. **Stem Cell Research & Therapy**, v. 12, n. 1, p. 1-16, 2021.

QUEIROZ, Aline *et al.* Effects of serum-free culture media on human apical papilla cells properties. **Archives of Oral Biology**, v. 121, p. 104962, 2021.

REINKE, Julia M.; SORG, H. Wound repair and regeneration. **European surgical research**, v. 49, n. 1, p. 35-43, 2012.

SHOOK, Brett A. *et al.* Dermal adipocyte lipolysis and myofibroblast conversion are required for efficient skin repair. *Cell Stem Cell*, v. 26, n. 6, p. 880-895. e6, 2020.

TARALLO, Sonia *et al.* Human pericyte–endothelial cell interactions in co-culture models mimicking the diabetic retinal microvascular environment. **Acta diabetologica**, v. 49, p. 141-151, 2012.

VALLE, Isabella B. do *et al.* Photobiomodulation drives pericyte mobilization towards skin regeneration. **Scientific reports**, v. 10, n. 1, p. 19257, 2020.

YANG, Ronghua *et al.* Epidermal stem cells in wound healing and their clinical applications. **Stem cell research & therapy**, v. 10, n. 1, p. 1-14, 2019.

WILGUS, Traci A.; ROY, Sashwati; MCDANIEL, Jodi C. Neutrophils and wound repair: positive actions and negative reactions. **Advances in wound care**, v. 2, n. 7, p. 379-388, 2013.

ZHANG, Shan *et al.* High glucose causes distinct expression patterns of primary human skin cells by RNA sequencing. **Frontiers in Endocrinology**, v. 12, p. 603645, 2021.

ANEXO A – Aprovação da comissão de ética no uso de animais**UNIVERSIDADE FEDERAL DE MINAS GERAIS****CEUA**
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Prezado(a):

Esta é uma mensagem automática do sistema Solicite CEUA que indica mudança na situação de uma solicitação.

Protocolo CEUA: 62/2019**Título do projeto:** PAPEL DA FOTOATIVACÃO NA MODULAÇÃO DE PERICITOS EM MODELO DIABÉTICO DE FERIDAS**Finalidade:** Pesquisa**Pesquisador responsável:** Ivana Marcia Alves Diniz**Unidade:** Faculdade de Odontologia**Departamento:** Departamento de Odontologia Restauradora**Situação atual:** **Decisão Final - Aprovado**

Aprovado na reunião do dia 10/06/2019. Validade: 10/06/2019 à 09/06/2024
Belo Horizonte, 10/06/2019.

Atenciosamente,

Sistema Solicite CEUA UFMG
https://aplicativos.ufmg.br/solicite_ceua/

Universidade Federal de Minas Gerais
Avenida Antônio Carlos, 6627 – Campus Pampulha
Unidade Administrativa II – 2º Andar, Sala 2005
31270-901 – Belo Horizonte, MG – Brasil
Telefone: (31) 3409-4516
www.ufmg.br/bioetica/ceua - cetea@prpq.ufmg.br