PRISCILA THAÍS RODRIGUES DE ABREU

EFEITOS DA FOTOBIOMODULAÇÃO SOBRE QUERATINÓCITOS CULTIVADOS *IN VITRO*: *UMA REVISÃO SISTEMÁTICA DA LITERATURA*

Faculdade de Odontologia
Universidade Federal de Minas Gerais
Belo Horizonte
2018

Priscila Thaís Rodrigues de Abreu

EFEITOS DA FOTOBIOMODULAÇÃO SOBRE QUERATINÓCITOS CULTIVADOS *IN VITRO*: *UMA REVISÃO SISTEMÁTICA DA LITERATURA*

Dissertação apresentada ao Colegiado 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 Estomatologia

Orientadora: Profª. Drª. Tarcília Aparecida da Silva
Coorientadora: Profª. Drª. Ivana Márcia Alves Diniz

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PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

Efeitos da fotobiomodulação sobre queratinócitos cultivados in vitro: uma revisão sistemática da literatura

PRISCILA THAÍS RODRIGUES DE ABREU

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

Aprovada em 21 de dezembro de 2018, pela banca constituída pelos membros:

Prof(a). Ivana Marcia Alves Diniz - Coorientadora
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Belo Horizonte, 21 de dezembro de 2018.

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ATA DA DEFESA DE DISSERTAÇÃO DA ALUNA
PRISCILA THAÍS RODRIGUES DE ABREU

Aos 21 dias de dezembro de 2018, às 09:00 horas, na sala 3418 da Faculdade de Odontologia da Universidade Federal de Minas Gerais, reuniu-se a Comissão Examinadora composta pelos professores Ivana Marcia Alves Diniz (Coorientadora) – FO/UFMG, Giovanna Ribeiro Souto – PUC Minas e Carolina de Castro Martins – FO/UFMG, para julgamento da dissertação de Mestrado, área de concentração em Estomatologia, intitulada: **Efeitos da fotobiomodulação sobre queratinócitos cultivados in vitro: uma revisão sistemática da literatura.** A Presidente da Banca, abriu os trabalhos e apresentou a Comissão Examinadora. Após a exposição oral do trabalho pela aluna e arguição pelos membros da banca, a Comissão Examinadora considerou a dissertação:

(X) Aprovada

( ) Reprovada

Finalizados os trabalhos, lavrou-se a presente ata que, lida e aprovada, vai assinada por mim e pelos demais membros da Comissão. Belo Horizonte, 21 de dezembro de 2018.

Ivana marcia alves Diniz

Prof(a). Ivana Marcia Alves Diniz

Prof(a). Giovanna Ribeiro Souto

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Dedico este trabalho aos meus pais por todo apoio e encorajamento, ao meu irmão pelo contínuo incentivo e aos meus amigos que sempre torceram pelo meu bem.

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“É impossível para um homem aprender aquilo que ele acha que já sabe”

Epicteto

RESUMO

A terapia por fotobiomodulação (PBMT) consiste no uso da luz em baixas densidades de potência visando promover efeitos benéficos ao organismo. Tem sido amplamente aplicada na medicina, fisioterapia e na odontologia para promover analgesia, cicatrização de feridas e reparo tecidual. A literatura que visa avaliar os efeitos da PBMT *in vivo* e *in vitro* consta de vários estudos que buscam elucidar os mecanismos celulares e moleculares envolvidos nessa terapia. Embora a literatura pertinente seja vasta, tais mecanismos ainda não foram completamente elucidados. Ademais, há uma carência de revisões sistemáticas que sumarizem os principais resultados encontrados por esses estudos, de forma que sejam fornecidas evidências do que já poderia ser considerado consenso e dos aspectos que ainda apresentam lacunas do conhecimento. Sendo assim, este trabalho se propôs a realizar uma revisão sistemática da literatura sobre os efeitos da PBMT utilizando LASER/LED, operando no azul, vermelho ou infravermelho próximo, sobre a viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e NF-kB de queratinócitos cultivados *in vitro* sob diferentes densidades de energia. Uma busca sistemática foi realizada nas principais bases de dados eletrônicas – PubMed, Web of Science, Scopus e LILACS, sem restrição quanto ao idioma e ano de publicação. Foram resgatadas 3221 referências sobre o tema das quais, 1997 tiveram seus títulos e resumos lidos, 137 foram lidas na íntegra e 55 foram incluídas na revisão. Os dados extraídos incluíram autor e ano de publicação, parâmetros dos dispositivos de luz utilizados, tratamentos aplicados às células, ensaios de viabilidade, proliferação, migração e expressão de marcadores inflamatórios. Os estudos primários foram agrupados de acordo com o espectro da luz e com os resultados obtidos segundo as densidades de energia utilizadas, subdivididas em 0.1 a 5.0; 5.1 a 10.0; 10.1 a 15.0 e acima de 15.0 J/cm^2. Os resultados demonstraram que os efeitos fotobiomodulatórios foram alcançados na faixa de 0.1 a 5.0 J/cm^2, sobretudo quando o vermelho e o infravermelho foram utilizados. Doses superiores a 10.0 J/cm^2 tenderam a causar morte celular ou não gerar modificações nas células em cultivo. Entretanto, em todas as faixas avaliadas e independentemente do comprimento de onda utilizado, não houve diferença significativa entre as células irradiadas e não irradiadas quanto ao desfecho analisado. A maioria dos estudos não forneceu adequadamente os parâmetros dos dispositivos de luz e notou-se grande variabilidade quanto às doses de energia empregadas. Ademais, a heterogeneidade dos desenhos experimentais e dados impossibilitou a execução de uma meta-análise. A presente revisão pode auxiliar na condução de estudos laboratoriais com protocolos experimentais mais criteriosos, uso de doses de energia mais adequadas e na padronização de desenhos experimentais futuros.

Palavras-chave: Fototerapia. Queratinócitos. Dosimetria. Cicatrização

ABSTRACT

Photobiomodulation effects on keratinocytes cultured *in vitro*: a systematic review

Photobiomodulation therapy (PBMT) consists of the use of light at low power densities aiming to promote beneficial effects to the body. It has been widely used in medicine, physiotherapy and dentistry to promote relief of pain, wound healing and tissue repair. The literature that aims to evaluate the effects of PBMT *in vivo* and *in vitro* consists of several studies that seek to elucidate the cellular and molecular mechanisms involved in this therapy. Although the relevant literature is vast, such mechanisms have not yet been fully elucidated. In addition, there is a lack of systematic reviews summarizing the main results found by these studies, in order to provide evidence of what could already be considered as consensus and aspects that still have knowledge gaps. Thus, this work proposed a systematic review of the literature on the effects of PBMT, using LASER / LED operating in blue, red or near infrared light, on viability, proliferation, migration, expression of cytokines, growth factors and NF-kB of keratinocytes cultured *in vitro* according to the energy density. A systematic search was performed in the main electronic databases - PubMed, Web of Science, Scopus and LILACS, with no restriction on language and year of publication. 3221 references on the subject were rescued, 1997 had their titles and summaries read, 137 were read in full and 55 were included in the review. Data extracted included author and year of publication, parameters of the light devices used, treatments applied to cells, viability, proliferation, migration and expression of inflammatory markers assays. The primary studies were grouped according to the light spectrum and the results obtained according to the energy densities used, subdivided in 0.1 to 5.0; 5.1 to 10.0; 10.1 to 15.0 and above 15.0 J/cm^2. Studies that used more than one spectrum or more than one energy dose were allocated to more than one group. The results demonstrated that photobiomodulatory effects were achieved in the range of 0.1 to 5.0 J/cm^2, especially when red and near infrared were used. Doses above 10.0 J/cm^2 tended to cause cell death or did not generate changes in the cells in culture. However, in all the bands evaluated and independently of the wavelength used, there was no significant difference between the irradiated and non-irradiated cells regarding the analyzed outcome. Most of the studies did not adequately provide the parameters of the light devices and there was great variability in the energy doses employed. In addition, the heterogeneity of experimental designs and data made it impossible to perform a meta-analysis. The present review may help in the conduction of laboratory studies with more rigorous experimental protocols, use of more adequate energy doses and in the standardization of future experimental designs.

Keywords: Phototerapy. Keratinocyte. Dosimetry. Wound healing.

LISTA DE ABREVIATURAS E SIGLAS

ATP	Adenosine Triphosphate
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
ISSO	Internacional Society of Oral Oncology
LASER	Light Amplification by Stimulated Emission of Radiation
LED	Light Emitting Diode
LLLT	Low-Level Light/LASER Therapy
MACC	Multinacional Association of Supportive Care in Cancer
NF-kB	Nuclear Factor-Kappa b
OHAT	Office of Health Assessment and Translation
PBMT	Photobiomodulation Therapy
PDT	Photodynamic Therapy
PRISMA	Prefered Reporting Items for Systematic Review
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
TGF	Transforming Growth Factor
VEGF	Vascular Endhotelial Growth Factor
WALT	World Association for Laser Therapy

LISTA DE SÍMBOLOS

α	Alfa
β	Beta
γ	Gama
π	Pi
cm^2	Centímetros quadrados
J	Joules
mm^2	Milímetros quadradros
mW	Miliwatts
nm	Nanômetros
pH	Potencial hidrogeniônico
W	Watts

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1 CONSIDERAÇÕES INICIAIS

1.1 Histórico

O efeito da luz sobre os sistemas biológicos é conhecido desde as civilizações antigas. Egípcios e gregos relatavam as ações da luz do sol no tratamento de lesões de pele e Heródoto observou que a exposição à luz solar poderia fortalecer os ossos (GARCEZ, RIBEIRO e NÚÑEZ, 2012).

O termo LASER é o acrônimo de *Light Amplification by Stimulated Emission of Radiation* (Amplificação da Luz por Emissão Estimulada de Radiação) e surgiu em 1917 a partir de princípios físicos divulgados por Albert Einstein. Em 1960, Maiman publicou o primeiro trabalho de sucesso, baseado nas teorias de Einstein, relatando a emissão estimulada da luz dentro do espectro visível (694nm), a partir da excitação de um cristal de rubi (DA SILVA *et al.,* 2010). Desde então as ações da luz sobre os tecidos começaram a ser intensamente estudadas. A primeira evidência científica dos efeitos da irradiação com LASER em baixas intensidades decorreu do estudo realizado por Endre Mester na Hungria (1967) (MESTER, SZENDE e GARTNER, 1968). Os pesquisadores visavam repetir o estudo realizado previamente por McGuff, que consistia no tratamento por irradiação a LASER de tumores implantados no dorso de ratos. Entretanto, o LASER utilizado por Mester *et al.* possuía uma pequena fração da potência daquele utilizado por McGuff, de tal modo que não houve redução dos tumores. No entanto, Mester *et al.* observaram um rápido crescimento dos pêlos nas feridas dos animais irradiados, comparados aos seus controles (HAMBLIN, 2018). Evidenciou-se assim, que o LASER em baixas potências apresentaria efeitos estimulatórios sobre os tecidos biológicos.

Os efeitos estimulatórios do LASER eram atribuídos às suas características de coerência, monocromaticidade e colimação (KARU,1987). No entanto, à medida que os estudos avançaram outras fontes de luz que não dispunham de tais características, tal como LED (*Light Emitting Diode*), começaram a ser utilizadas. Verificou-se que estas fontes poderiam ser tão eficientes em promover efeitos fotobioestimulatórios quanto o LASER (KARU, 2010). O termo “Terapia com luz em baixas intensidades” (*Low-Level Light Therapy - LLLT*) passou então a ser amplamente utilizado, embora não houvesse muito bem um consenso do que seria a definição de

"baixaintensidade" (HAMBLIN, 2018). Dessa forma, atualmente o termo de melhor denominação empregado é "Terapia por Fotobiomodulação" (do inglês, Photobiomodulation *Therapy* – PBMT) (KHAN e ARANY, 2016).

1.2 Características e aplicações clínicas da PBMT

A PBMT é caracterizada pelo uso da luz visível e do infravermelho próximo com finalidades terapêuticas, em baixas densidades de potência, da ordem de miliwatts/cm^2 (mW/cm^2) e por seus efeitos térmicos serem quase imperceptíveis, o que a faz ser caracterizada como "não-térmica". Logo, diferem-na de outros tratamentos baseados na ação da luz, que podem ser ablativos (MARQUES *et al*., 2017). Portanto, os efeitos da PBMT sobre os tecidos são fotoquímicos, fotofísicos e fotobiológicos (ESTEVÃO, 2009).

Tais características, sobretudo a ausência de danos térmicos, fizeram da PBMT uma alternativa terapêutica potencial. Assim, os achados de Mester em 1967 foram extrapolados para a clínica, levando à aplicação da PBMT em humanos. Seu uso tem ocorrido ao longo de mais de 40 anos para a promoção de analgesia, redução da inflamação, cicatrização de feridas (ENGEL, KHAN e ARANY, 2016), assim como na reparação neural (SOLMAZ, ULGEN e GULSOY, 2017, TUCKER *et al*., 2018) e na estimulação do crescimento capilar em casos de alopecia (SHEEN *et al*., 2015).

Uma das principais aplicações atuais da PBMT, especificamente no campo da Odontologia é o tratamento de mucosites induzidas por quimio ou radioterapia. A *Multinacional Association of Supportive Care in Cancer* (MASCC) e a *International Society of Oral Oncology* (ISOO) estabelecem que a PBMT apresenta nível II de evidência científica para o tratamento de mucosite (Lalla *et al*., 2014). Antunes *et al*. (2017) demonstraram que pacientes em tratamento oncológico submetidos à PBMT experimentaram lesões de mucosite menos severas, redução da dor, maior capacidade de alimentar-se e hidratar-se, menor taxa de uso de opioides, bem como maior tolerância à quimioterapia, quando comparados aos seus controles. Diversos estudos corroboram os efeitos benéficos da PBMT neste sentido (CARVALHO *et al*., 2011; BESADOUN e NAIR, 2012; OTON-LEITE *et al*., 2015; SILVA *et al*., 2015; ELAD *et al*., 2018)

Os efeitos decorrentes da PBMT observados na clínica, primeiramente em animais e posteriormente em humanos, suscitaram

questionamentos sobre quais seriam os mecanismos celulares e moleculares envolvidos na PBMT que fossem capazes de explicar os seus resultados clínicos. Estes questionamentos levaram ao desenvolvimento de estudos laboratoriais, visando explicar os efeitos da radiação.

1.3 Estudos *in vitro* e os efeitos celulares e moleculares da PBMT

Historicamente, desde que os efeitos clínicos da PBMT puderam ser observados em 1967 com Mester *et al*., estudos laboratoriais vêm sendo desenvolvidos buscando elucidar os mecanismos celulares e moleculares que a radiação causa nos tecidos. Tiina Karu foi uma das pioneiras, desenvolvendo trabalhos na Rússia a partir da década de 1980. Karu elucidou que a absorção da luz se deve a fotorreceptores endógenos, denominados cromóforos, localizados na superfície mitocondrial e no interior das células (KARU, 1988). A citocromo c oxidase (cit c), uma enzima terminal da cadeia respiratória mitocondrial, seria a responsável pela absorção da luz na região do vermelho e do infravermelho próximo (600-1000 nm) em células eucarióticas (KARU, 1995). A pesquisadora demonstrou que a iluminação de mitocôndrias provenientes de pulmão de ratos resulta em aumento do potencial de membrana mitocondrial, do gradiente de prótons (pH), da produção de Adenosina Trifosfato (*Adenosine Triphosphate* – ATP), do consumo de oxigênio (O_2) e da síntese de RNA e proteínas (KARU, 1999). Deste modo, a pesquisadora observou que a irradiação com alguns comprimentos de onda poderia alterar algumas reações bioquímicas específicas ou o metabolismo celular inteiro (KARU, 2010).

Estes dados serviram como base para os estudos realizados posteriormente e ainda hoje são aceitas (HAMBLIN, 2017). Porém seus achados compreendem apenas uma fração do que ocorre no interior das células quando a luz é absorvida pelos fotorreceptores. Sabe-se, atualmente, que existem outros cromóforos relacionados à absorção dos comprimentos de onda dentro do espectro da luz visível. Proteínas do grupo heme e porfirinas participam do processo (GEORGE, HAMBLIN e ABRAHAMSE, 2018) e as opsinas e as flavoproteínas seriam as responsáveis pela absorção da luz na região do azul (400-450 nm) e do verde (aproximadamente 540 nm) (MAINSTER, 2006; HAMBLIN, 2017; WANG *et al*., 2018)

De fato há uma reação fotobiológica quando os fótons incidentes sobre as células são absorvidos. Inicialmente isto leva a mudanças diretamente relacionadas à configuração molecular do fotorreceptor, sendo estas alterações denominadas reações primárias. Posteriormente, este processo é acompanhado por alterações em uma série de sinais e funções celulares, as quais compõem as reações secundárias (HAMBLIN, 2017). Estas constituem cascatas moleculares, incluindo a sinalização mitocondrial retrógrada, que consiste na comunicação intracelular da mitocôndria com o núcleo, influenciando diversas atividades celulares (DE FREITAS e HAMBLIN, 2017).

Neste sentido, a absorção de luz pela cit c leva a um incremento na produção de ATP e cálcio intracelulares, além de espécies reativas de oxigênio (*Reactive oxygen species* - ROS), sobretudo o ânion superóxido ($O2^{-}$) e seu produto mais estável – peróxido de hidrogênio (H_2O_2) (GAO e XING; 2009, SPERANDIO *et al*., 2015; TATA e WAYNANT; 2011). São estas mudanças celulares e biomoleculares que levam aos efeitos microscópicos, tais como incremento na proliferação celular, diminuição da inflamação, estimulação à síntese de proteínas de reparo e cicatrização, dentre outros (DA SILVA *et al*., 2010). Estes efeitos têm potencial repercussão na redução da dor e menor tempo de cicatrização (ENGEL, KHAN e ARANY, 2016; TATA e WAYNANT; 2011).

Evidências indicam que a PBMT também exerce efeitos diretos na síntese e expressão de diversas citocinas, fatores de crescimento e fatores de transcrição, tais como o fator de transformação do crescimento beta (*Transforming growth factor betta* - TGF-β), o fator de crescimento epidérmico (*Epidermal growth fator* – EGF), o fator de crescimento do endotélio vascular (*Vascular endothelial growth fator* – VEGF), o fator de crescimento de fibroblastos (*Fibroblast growth fator* – FGF) e o fator Nuclear Kappa B (*Nuclear Factor – Kappa b*). Estes fatores exercem efeitos em fibroblastos, queratinócitos, células endoteliais e neutrófilos promovendo sua proliferação, migração e auxiliando no processo de reparo tecidual (CHEN *et al.*, 2011; FUSHIMI *et al.,* 2012; ARANY *et al*., 2014; YIN *et al*., 2017).

1.4 Justificativa

Embora diversos estudos *in vitro* tenham avaliado os efeitos da PBMT sobre diferentes tipos celulares, tais como células-tronco (MARQUES *et*

al., 2017, DE ANDRADE *et al*., 2018; DE SOUZA *et al*., 2018), queratinócitos (LEE *et al*., 2016; BUSCONE *et al*., 2017; RIZZI *et al.*, 2018) e fibroblastos (BASSO *et al*., 2016; MIGNON *et al*., 2017; HOURELD, AYUC e ABRAHAMSE, 2018), os mecanismos celulares e moleculares envolvidos neste processo ainda não foram completamente elucidados, o que demonstra a importância de se prosseguir na investigação a respeito do tema.

A PBMT apresenta importância especial para a cicatrização e a regeneração dos tecidos, bem como redução da dor e da inflamação (ENGEL, KHAN e ARANY, 2016). Assim, o desenvolvimento de terapias que otimizem as condições de sobrevivência e crescimento de células envolvidas nos processos de cicatrização e reparo, tais como células-tronco, queratinócitos e fibroblastos, é de extrema importância (MARQUES *et al.*, 2017). A PBMT pode induzir a proliferação e migração celulares, bem como a síntese de fatores de crescimento. A proliferação celular é um importante sinal fisiológico dos efeitos bioestimulatórios da PBMT e um dos mais importantes aspectos da cicatrização de feridas (SOLMAZ, ULGEN e GULSOY, 2017).

No entanto, apesar do número de estudos que avaliam os efeitos da PBMT utilizando o LASER e o LED *in vitro* ser vasto, há uma grande heterogeneidade em relação aos delineamentos experimentais, sobretudo quanto à densidade de energia aplicada às células e ao tempo de aplicação. Mais ainda, resultados contraditórios são frequentemente reportados. Ademais, vemos que poucas são as revisões da literatura existentes sobre o tema. Neste sentido, há a necessidade de se ampliar as revisões sistemáticas sobre o tema no intuito de analisar o estado-da-arte.

Sendo assim, o presente trabalho se propôs a realizar uma revisão sistemática da literatura sobre os estudos que analisam os efeitos da fotobiomodulação, utilizando comprimentos de onda nos espectros azul, vermelho e/ou infravermelho próximo, sobre queratinócitos cultivados *in vitro* por ser esta uma das principais células envolvidas no processo de cicatrização e reparo tecidual (KHAN e ARANY, 2016). Visamos avaliar os efeitos da PBMT sobre a viabilidade, proliferação e migração celulares, bem como sobre a expressão de marcadores inflamatórios, tais como citocinas, fatores de crescimento e outras moléculas como o NF-kB. Este é um fator de transcrição sensível a variações redox, tipicamente reportado em estudos que avaliam os

efeitos da PBMT *in vitro* (CHEN *et al*., 2011; HAMBLIN, 2018), o que justifica a sua inclusão no presente trabalho.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos da PBMT sobre queratinócitos cultivados *in vitro*, irradiados com LASER/LED nos comprimentos de onda dos espectros azul, vermelho e/ou infravermelho próximo, em relação à viabilidade, proliferação, migração e expressão de marcadores inflamatórios de acordo com densidade de energia (J/cm^2).

2.2 Objetivos específicos

a) Verificar quais são os principais parâmetros dos dispositivos de luz (LASER/LED) e especificações do tratamento utilizados nos estudos *in vitro*, tais como modo de operação, comprimentos de onda (dentro dos espectros azul, vermelho e infravermelho próximo), potência, densidade de potência, densidade de energia, energia total, área do spot, distância, tempo e frequência de irradiação e uso de fibra óptica, segundo recomendações da WALT.

b) Verificar os protocolos experimentais aplicados *in vitro*, tais como origem dos queratinócitos cultivados, ensaios realizados, tempos experimentais e os principais resultados produzidos pela PBMT na viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e NF-kB dos queratinócitos irradiados, quando comparados aos seus controles não irradiados.

c) Verificar a possibilidade de se estabelecer uma faixa terapêutica relativa à densidade de energia (J/cm^2) que apresente efeitos positivos sobre queratinócitos *in vitro*, visando fornecer dados consistentes que sirvam de base para estudos laboratoriais a serem realizados futuramente.

d) Realizar a análise de qualidade e risco de viés de cada estudo individualmente, por meio da ferramenta OHAT.

3 METODOLOGIA EXPANDIDA

3.1 Registro na base PROSPERO

A presente revisão sistemática foi submetida ao registro na base PROSPERO (*International prospective register of systematic reviews*), seguindo detalhadamente as especificações exigidas pela base. No entanto, por se tratar de uma base que aceita apenas o registro de revisões sistemáticas e meta-análises de estudos *in vivo,* não foi possível obter um número de registro.

3.2 Delineamento do estudo e pergunta da pesquisa

Esta revisão seguiu os critérios estabelecidos pela ferramenta *Preferred Reporting Items for Systematic Reviews and Meta-analyzes* (PRISMA), que determina os métodos para se conduzir revisões sistemáticas da literatura e meta-análises (Moher *et al.,* 2009).

A pergunta da pesquisa foi “qual a melhor faixa terapêutica em relação à densidade/dose de energia (J/cm^2) para se obter efeitos fotobiomoduladores na viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e outras moléculas como o NF-kB em queratinócitos cultivados *in vitro*?”. Os elementos que compõem a questão PICO (*Population, Intervention, Comparision, Outcomes*) são: 1) População: queratinócitos cultivados *in vitro*; 2) Intervenção: irradiação com LASER/LED em baixas potências, utilizando comprimentos de onda nos espectros azul, vermelho ou infravermelho próximo; 3) Comparação: queratinócitos cultivados *in vitro* não-irradiados considerados como controles; 4) Resultados: efeitos fotobiomoduladores sobre a viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e outras moléculas como o NF-kB.

3.3 Busca sistemática da literatura

As buscas foram realizadas nas principais bases de dados da grande área da saúde - PubMed (*U.S. National Library of Medicine* – Biblioteca Nacional de Medicina dos Estados Unidos), Web of Science (Thomson Reuters), Scopus (Elsevier) e LILACS (*Latin American and Caribbean Center on Health Sciences Information* - Literatura Latino-Americana e do Caribe em Ciências da Saúde). Visando englobar o máximo de publicações realizadas no

nível mundial e também a produção científica que vem sendo realizada sobre o tema em questão na América Latina. A primeira busca foi realizada em fevereiro de 2018 e a segunda busca em novembro do mesmo ano.

As estratégias de busca (chaves de busca) foram específicas para cada base de dados e compostas pelos descritores e seus acrônimos de acordo com o MeSH (*Medical Subject Headings*):

1) PubMed: photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR PBMT OR PBM OR LLLT OR laser OR “light emitting diode” OR “red laser therapy” OR “red laser therapies” OR “infra-red laser therapy” OR “infra-red laser therapies” OR “low intensity laser therapy” OR “low intensity laser therapies” OR “low-intensity laser therapy” OR “low-intensity laser therapies” OR “low level laser therapy” OR “low level laser therapies” OR “low-level laser therapy” OR “low-level laser therapies” OR “low level light therapy” OR “low level light therapies” OR “low-level light therapy” OR “low-level light therapies” OR “low power laser therapy” OR “low power laser therapies” OR “low-power laser therapy” OR “lowpower laser therapies” OR “low power laser irradiation” OR “low-power laser irradiation” OR “photobiomodulation therapy” OR “photobiomodulation therapies” OR “laser biostimulation” OR “laser phototherapy” OR “laser phototherapies” AND keratinocyte OR keratocyte;

2) Web of Science: photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR laser OR light emitting diode OR red laser therapy OR red laser therapies OR infra-red laser therapy OR infra-red laser therapies OR low intensity laser therapy OR low intensity laser therapies OR low-intensity laser therapy OR low-intensity laser therapies OR low level laser therapy OR low level laser therapies OR low-level laser therapy OR low-level laser therapies OR low level light therapy OR low level light therapies OR low-level light therapy OR low-level light therapies OR low power laser therapy OR low power laser therapies OR low-power laser therapy OR low-power laser therapies OR low power laser irradiation OR lowpower laser irradiation OR photobiomodulation therapy OR photobiomodulation therapies OR laser bioestimulation OR laser phototherapy OR laser phototherapies AND keratinocyte OR keratocyte;

3) Scopus: photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR laser OR “light emitting diode” OR “red laser” OR

“low intensity laser” OR “low level laser” OR “low level light” OR “low power laser” OR biostimulation OR phototherapy AND keratinocyte OR keratocyte;

4) LILACS: laser AND keratinocyte OR keratocyte;

3.4 Critérios de inclusão

Não houve restrição quanto ao idioma e ano de publicação e foram considerados elegíveis os estudos que comtemplavam os seguintes critérios:

- Estudos que utilizassem laser/LED em baixa potência;
- Comprimentos de onda dentro do espectro azul, vermelho ou infravermelho próximo;
- Estudos que avaliassem os efeitos da PBMT na viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e outras moléculas, como o NF-kB;
- Estudos que utilizassem queratinócitos normais cultivados *in vitro*, primários ou de linhagem, originados de mamíferos;
- Estudos de terapia fotodinâmica (*Photodynamic therapy* – PDT) que incluíssem em seus protocolos experimentais controles positivos de queratinócitos (células não expostas ao fotossensibilizador estudado, porém expostas à irradiação);
- Estudos in vivo e/ou *ex vivo*, desde que apresentassem em seu protocolo experimental avaliações *in vitro* realizadas em células cultivadas.

3.5 Critérios de exclusão

Foram excluídos os estudos que não contemplavam os critérios de elegibilidade e aqueles que apresentavam as seguintes características:

- Estudos unicamente *in vivo* e/ou *ex vivo*;
- Estudos que utilizaram LASER/LED com comprimentos de onda unicamente dentro dos espectros ultravioleta (UV) A e/ou B, violeta, verde, amarelo e alaranjado;
- Estudos que utilizaram LASER/LED cirúrgicos (em alta potência) não ajustados para operarem em baixa potência;

- Estudos de PDT que não apresentaram controles positivos de queratinócitos, ou seja, todos os grupos experimentais foram expostos ao fotossensibilizador;
- Estudos realizados unicamente com células normais ou alteradas que não eram queratinócitos;
- Estudos que apresentavam os queratinócitos apenas em co-cultivo com outros tipos celulares (ausência de monocultura de queratinócitos);
- Estudos que contemplavam apenas microscopia confocal a laser, citrometria de fluxo, microdissecção a laser e dispersão de luz laser;
- Referências para as quais apenas o resumo estava disponível.
- Estudos nos quais a densidade de energia utilizada não foi informada e não pôde ser calculada devido à carência de informações fornecidas.

3.6 Remoção de duplicatas e seleção dos estudos

Uma vez realizada a busca em cada base de dados as referências recuperadas foram importadas para o EndNote (Thompson Reuters, New York, NY, USA) e em seguida para o Reference Manager & Citation Generator (Mendeley, London, UK). Tais ferramentas permitiram a organização das referências por ordem alfabética, facilitando a leitura ordenada e a remoção de duplicatas. Na primeira etapa foram selecionadas as referências que claramente continham, em seus títulos e resumos, o assunto de interesse de acordo e também aquelas que geraram dúvidas. A seleção foi realizada por dois avaliadores de forma independente. Inicialmente ambos leram 10% do total da busca de forma independente e confrontaram as seleções feitas para que as discordâncias fossem ajustadas. Este processo foi realizado duas vezes até que se atingiu o índice de concordância Kappa de 0.9. Todas as dúvidas foram sanadas por um terceiro examinador, doutor em Odontologia e com experiência na área de fotobiomodulação.

Todas as referências selecionadas na primeira etapa foram tabuladas em ordem alfabética para que os artigos fossem recuperados e pudessem ser lidos na íntegra. Na segunda etapa os artigos selecionados previamente foram lidos pelos avaliadores, também de forma independente, e

apenas aqueles que contemplavam todos os critérios de elegibilidade foram selecionados para compor a revisão sistemática.

3.7 Extração e agrupamento dos dados

Uma vez que os estudos foram selecionados a partir da leitura na íntegra, procedeu-se à terceira etapa. Nesta etapa foram extraídos os seguintes dados de interesse: país e idioma de publicação; tipo de estudo (*in vitro*, *in vivo* e *ex vivo*); tipo de queratinócito utilizado (humano ou animal, de pele ou mucosa, primário ou de linhagem); características e parâmetros dos dispositivos de luz [fabricante, modelo e local, comprimento de onda, modo de operação, potência (W ou mW), densidade de potência/irradiância/intensidade/taxa de fluência (W/cm^2 ou mW/cm^2), densidade de energia/dose de radiação/fluência (J/cm^2), área do spot/área de irradiação (mm^2 ou cm^2), energia total (J) e uso ou não de fibra óptica]; especificações dos tratamentos realizados nas células em cultivo [irradiação em contato ou à distância, número de irradiações, frequência das aplicações, tempo de irradiação em segundos, superexposição evitada, dispositivo para checagem da potência (*powermeter*)]; tipos de ensaios realizados e tempos experimentais para avaliar a viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e outras moléculas como o NF-kB e os principais resultados encontrados. Extraímos também os dados relativos às metodologias empregadas, tais como número de réplicas (n), média e desvio-padrão de cada grupo experimental, visando a realização de uma meta-análise. Os dados foram registrados em um formulário desenvolvido para a extração de dados deste estudo, contendo as especificações citadas acima (Apêndice A).

A escolha dos parâmetros dos dispositivos de luz supracitados e as especificações dos tratamentos se deu com base nas orientações da *World Association for Laser Therapy* (WALT, 2006). A associação recomenda que os estudos realizados no campo da PBMT especifiquem detalhadamente todos os parâmetros dos dispositivos de luz utilizados nos estudos e como os tratamentos foram realizados, a fim de que haja confiabilidade dos resultados. Recentemente, no 12º Congresso Internacional da WALT sobre PBMT (12th International Congress for WALT – Photobiomodulation), ocorrido em outro de 2018 em Nice (França), a WALT recomendou que os pesquisadores informassem também sobre o uso ou não de fibra ótica, ademais de todos os

parâmetros dos dispositivos de luz supracitados. Infelizmente, por se tratar de um evento recente, não há publicações da associação citando especificamente a fibra ótica, deixando-nos impossibilitados de referenciá-la. Todavia, o uso ou não de fibra óptica foi considerado na fase de extração de dados desta revisão, seguindo já as novas diretrizes estabelecidas pela associação.

Quando os dados de interesse não foram fornecidos nos artigos originais, os autores foram contatados via *e-mail*. Dessa forma, cada autor em questão foi contatado duas vezes. Aguardamos até a etapa final de conclusão do artigo científico pela resposta dos autores. Na ausência de resposta, discriminamos o dado em questão como "Não reportado" (Not reported). Quando a densidade de energia não foi fornecida pelos autores e os demais parâmetros haviam sido informados, realizamos o cálculo mediante a fórmula *Densidade de energia (J/cm^2) = P (W) x T (segundos) / área do spot (cm^2).* Quando a área também não foi fornecida realizamos seu cálculo com base na fórmula *Área = π (3,14) x r^2*.

Os dados foram tabulados de acordo com o espectro da luz visível (azul, vermelho e infravermelho próximo), com o tipo de queratinócito utilizado, com a densidade de energia e com os efeitos decorrentes da PBMT na viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e NF-kB. Considera-se atualmente que os efeitos fotobiomodulatórios sejam obtidos em doses de 1.0 a 10.0 J/cm^2, para estudos *in vitro* (TATA e WAYNANT, 2011), o que pode ser considerado uma faixa muito ampla. Deste modo, visando alcançar o objetivo de se encontrar uma faixa de densidade de energia mais estreita para se produzir efeitos fotobiomodulatórios, criamos subdivisões de doses de energia: grupo de 0.1 a 5.0 J/cm^2, grupo de 5.1 a 10.0 J/cm^2, grupo de 10.1 a 15.0 J/cm^2 e grupo acima de 15.0 J/cm^2. Os estudos foram alocados em cada grupo, sendo que aqueles que utilizaram mais de uma dose de energia foram alocados em mais de um grupo, quando pertinente.

3.8 Análise de qualidade e risco de viés de cada estudo individualmente

A análise de qualidade dos estudos foi realizada mediante a avaliação dos seguintes critérios: randomização dos tratamentos; condições experimentais da cultura; alocação adequada dos grupos de estudo; se houve remoção de algum grupo experimental das análises de forma não explicada;

confiabilidade na caracterização da exposição; confiabilidade nos resultados obtidos; menção dos resultados de todos os ensaios realizados; e algum outro potencial risco de viés interno. Tais critérios foram estabelecidos seguindo a ferramenta *Office of Health Assessment and Translation* (OHAT) adaptado para estudos *in vitro* pelo Programa Nacional de Toxicologia - Departamento de Saúde e Serviços Humanos dos EUA (*National Toxicology Program – US Department of Health and Human Services*). Esta ferramenta foi inicialmente desenvolvida para a condução de revisões sistemáticas que visavam avaliar riscos potenciais à saúde humana ou iniquidades em saúde (ROONEY *et al*., 2014). Porém foi adaptada para estudos *in vitro* apresentando os critérios que devem ser contemplados por este tipo de estudo, visando à redução dos vieses internos e maior confiabilidade nos resultados.

O OHAT estabelece um *score* para os estudos *in vivo*, sejam em animais ou humanos, mas não o faz para estudos *in vitro*. Sendo assim, para a análise de qualidade dos estudos que compuseram a presente revisão sistemática nos limitamos a verificar se atendiam ou não aos critérios estabelecidos pela OHAT. Consideramos “Sim” (Yes) ou “Não” (No) quando foi possível perceber se o critério foi ou não contemplado, e “Incerto” (Unclear) quando não podíamos responder com segurança quanto ao critério estabelecido pela ferramenta. Ademais, a ferramenta não determina exatamente quais seriam “outros riscos potenciais à validade interna”, deixando este critério aberto à interpretação, o que pode variar dependendo do tipo de estudo, do seu objetivo e do desfecho estudado.

Sabe-se que a densidade de energia é um dos principais fatores, mas não o único, a interferir na resposta das células/tecidos à irradiação (HUANG *et al*., 2011). Dessa forma, a falta de métodos para se evitar a sobreirradiação das culturas, tal como o uso de papel preto e/ou distanciamento entre os poços-testes irradiados, assim como a não verificação da potência do aparelho previamente aos experimentos e a falta de detalhamento adequado dos parâmetros dos dispositivos (segundo recomendações da WALT), foram considerados como potenciais riscos à validade interna. Quando estes dados não foram contemplados ou não foram reportados nos artigos, consideramos que não poderíamos estar completamente seguros na caracterização da exposição e na confiabilidade dos resultados obtidos (“Unclear”).

3.9 Análise dos dados

As análises foram realizadas sempre em relação a dois grupos de comparação dos estudos primários: 1) Grupo experimental: queratinócitos irradiados com LASER/LED em baixas potências, utilizando luz azul, vermelha ou infravermelho próximo; 2) Grupo controle: queratinócitos não-irradiados. Nos estudos de PDT não visávamos avaliar os efeitos do fotossensibilizante estudado, apenas os efeitos da irradiação. Sendo assim, nesses estudos consideramos os seguintes grupos: 1) Grupo experimental: queratinócitos irradiados com LASER/LED em baixas potências, utilizando luz azul, vermelha ou infravermelho próximo sem fotossensibilizante (controle positivo dos estudos); 2) Grupo controle: queratinócitos não-irradiados e não expostos ao fotossensibilizante (controle negativo dos estudos). Com base nos grupos de densidades de energia criados, verificamos se a dose aplicada aumentou (“increased”), diminuiu (“decreased”) ou não alterou (“unmodified”) a viabilidade, proliferação, migração, expressão de citocinas, fatores de crescimento e NF-kB. Resultados que no estudo primário tenderam ao aumento ou à diminuição sem que houvesse diferença estatística em relação ao grupo controle foram considerados “unmodified”.

Se os estudos incluídos apresentassem homogeneidade metodológica, uma meta-análise seria conduzida, sendo verificada pela estatística I^2 (Higgins, Thompson, Deeks, & Altman, 2003). Se os estudos apresentassem heterogeneidade metodológica uma análise qualitativa seria realizada, apresentando os resultados em forma de tabelas.

4 RESULTADOS, DISCUSSÃO E CONCLUSÃO

Os resultados, a discussão e a conclusão do presente trabalho serão apresentados sob a forma de artigo científico, submetido ao periódico *PLoS ONE,* fator de impacto 3.057 e classificação A1 segundo a qualificação Qualis CAPES (triênio 2013-2016).

5 ARTIGO CIENTÍFICO

Photobiomodulation effects on keratinocytes cultured *in vitro*: a systematic review

Short running title: photobiomodulation and keratinocytes

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ABSTRACT

Photobiomodulation therapy (PBMT) has been widely used in the promotion of tissue repair. Regardless of the therapeutic benefits, there are instances where PBMT appears unsuccessful, and the strongest plea is due to inadequate light dosimetry arising from the use of unsuitable wavelengths. This systematic review aimed to verify the effects of PBMT on the cell function as well as on the release of molecules on cultured keratinocytes using blue, red or near infrared lights categorized in arbitrary ranges of energy densities (0.1–5.0; 5.1–10.0; 10.1–15.0; and over 15.0 J/cm^2). The electronic searches were conducted in PubMed, Web of Science, Scopus and LILACS databases, and included LASER or LED devices. A total of 55 articles evaluating the effects of PBMT on cell viability, proliferation, migration, cytokine and growth factors expression were included. Overall, the studies failed to provide detailed information about light dosimetry and detailed experimental conditions. However, it was possible to identify that the red and near infrared lights presented more stimulatory effects when compared to the blue light. In addition, for all analyzed parameters, favorable outcomes were mostly obtained in the range of 0.1–5.0 J/cm^2. The less explored energy densities range was the 10.1–15.0 J/cm^2; and high energy doses, above 15.0 J/cm^2, were ineffective or tend to cause more cell death. The vast majority of the energy densities tested produced unmodified results, regardless the wavelength applied.

Keywords: photobiomodulation therapy, keratinocytes, wound healing, dosimetry.

INTRODUCTION

Photobiomodulation therapy (PBMT), characterized by the use of light at low power, has been widely used for therapeutic purposes in order to produce analgesia, wound healing (Engel, Khan, & Arany, 2016) and tissue regeneration/repair (Diniz et al. 2018; Elad et al., 2018;). Mechanistically, it is achieved by the generation of energy and reactive oxygen species (ROS) in cells and tissues, which then biomodulate cell signalling and metabolism in an injured milieu. As such, pathways sensitive to redox signalling molecules, like the nuclear factor-κB (NF-κB) transcription factor and mitogen-activated protein kinases (MAPKs), or even growth factors (like transforming growth factor-β1) are mostly activated by PBMT (Arany et al. 2014; George, Hamblin, & Abrahamse, 2018).

Several studies have been carried out aiming to investigate the cellular and molecular events that explain the light action on the organic systems (Kim, Woo, Sohn, Jeong, & Kang, 2017; Yin, Zhu, Wang, & Zhao, 2017; Ferreira et al., 2018). The participation of photoreceptors located at the mitochondrial surface was already demonstrated in this process (Karu, 1995). The terminal enzyme of the respiratory chain, the cytochrome *c* oxidase (CcO), would be responsible for the absorption of wavelengths in the region of red (630 nm) to near infrared (around 1000 nm), and possibly, also in the region of blue lights (Karu, 2010; Hamblin et al. 2018). On the other hand, opsins and flavoproteins would be responsible for the absorption of wavelengths in the region of blue (around 420 nm) and green lights (around 540 nm) (Mainster, 2006; Hamblin, 2017; Wang, Huang, Wang, Liu, & Hamblin, 2017). The activation of retinal opsins by blue light, for example, can generate ROS, which is partly responsible for ocular phototoxicity caused by violet and blue light (Mainster, 2006). As such, PBMT would trigger direct or short-term responses represented, above all, by the stimulation of the ATP (Adenosine Triphosphate) synthesis concomitantly with a transient ROS production (Karu, 2010). This mechanism, in turn, would be directly linked to the activation of intracellular cascades that would culminate in indirect or long-term responses, such as DNA and RNA synthesis and further regulation of cell functions (Da Silva, da Silva, Almeida, Junior, & Matos, 2010).

A huge number of *in vitro* studies have demonstrated the benefits of PBMT on cells in culture (Houreld, Ayuc, & Abrahemse, 2014; Basso et al.,

2016; Rizzi et al., 2016; Solmaz, Ulgen, & Gulsoy, 2017; Diniz et al., 2018). Biostimulatory responses are generally considered to be in the range of 1.0 to 10.0 J/cm^2 and the inhibitory effects above 25.0 J/cm^2 (Tata & Waynant, 2011). Device settings such as power density and exposure time; and other experimental conditions such as the cell type are also important to set a PBMT as biostimulatory (Peplow, Chung, & Baxter, 2010; AlGhamdi, Kumar, & Moussa, 2012; Marques et al., 2017; Hamblin, 2018). Accordingly, the present study carries out a systematic review of the literature about the effects of PBMT using wavelengths in the blue, red and/or near infrared spectra applied exclusively on keratinocytes cultured *in vitro*, aiming to narrow down the most relevant energy densities described in the literature able to bio-modulate epithelial cells.

MATERIALS AND METHODS

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) Statement (Moher et al., 2009).

Eligibility criteria

The question of the research was: what is the best energy density range suited to produce stimulatory effects on the proliferation, viability, migration and expression of cytokines, growth factors and NF-κB in keratinocytes cultured *in vitro* submitted to PBMT? The items for the PICO question were: (1) Population: normal keratinocytes cultured *in vitro*, (2) Intervention: exposure to PBMT at various energy densities, (3) Comparison: keratinocytes unexposed to PBMT, (4) Outcomes: changes in proliferation, viability, migration and expression of cytokines, growth factors and NF-κB.

Inclusion criteria

Studies in which the LASER/LED was investigated as primary intervention (independent variable), which evaluated viability, proliferation, migration and expression of cytokines and growth factors, and NF-κB, using mammalian keratinocytes cultured *in vitro*; LASER/LED operating in blue, red or near-infrared light and photodynamic therapy (PDT) that had positive control of

keratinocytes (cells exposed to radiation but not exposed to the photosensitizer) were included.

Exclusion criteria

Studies using only ultraviolet A (UVA) and B (UVB), violet, green, yellow and orange lights; studies involving confocal laser microscopy; laser scanning cytometry; laser capture microdissection; laser light scattering; laser assisted laser desorption/ionization matrix and mass spectrometry by flight time; PDT studies without positive control; studies in which keratinocytes were co-cultured with other cell types; and studies for which only the conference abstract was available were excluded.

Information sources

Electronic searches without publication date or language restriction were undertaken in February 2018 and later updated in November 2018 in the following databases: PubMed (National Library of Medicine), Web of Science (Thomson Reuters), Scopus (Elsevier) and LILACS (Latin American and Caribbean Center on Health Sciences Information).

Search strategy

The search keys were composed of terms indexed by the Medical Subject Headings (MeSH). The following search strategy was used for the databases:

1) PubMed: photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR PBMT OR PBM OR LLLT OR laser OR “light emitting diode” OR “red laser therapy” OR “red laser therapies” OR “infra-red laser therapy” OR “infra-red laser therapies” OR “low intensity laser therapy” OR “low intensity laser therapies” OR “low-intensity laser therapy” OR “low-intensity laser therapies” OR “low level laser therapy” OR “low level laser therapies” OR “low-level laser therapy” OR “low-level laser therapies” OR “low level light therapy” OR “low level light therapies” OR “low-level light therapy” OR “low-level light therapies” OR “low power laser therapy” OR “low power laser therapies” OR “low-power laser therapy” OR “low-power laser therapies” OR “low power laser irradiation” OR “low-power laser irradiation” OR “photobiomodulation

therapy" OR "photobiomodulation therapies" OR "laser biostimulation" OR "laser phototherapy" OR "laser phototherapies" AND keratinocyte OR keratocyte;

2) Web of Science: photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR laser OR light emitting diode OR red laser therapy OR red laser therapies OR infra-red laser therapy OR infra-red laser therapies OR low intensity laser therapy OR low intensity laser therapies OR low-intensity laser therapy OR low-intensity laser therapies OR low level laser therapy OR low level laser therapies OR low-level laser therapy OR low-level laser therapies OR low level light therapy OR low level light therapies OR low-level light therapy OR low-level light therapies OR low power laser therapy OR low power laser therapies OR low-power laser therapy OR low-power laser therapies OR low power laser irradiation OR low-power laser irradiation OR photobiomodulation therapy OR photobiomodulation therapies OR laser bioestimulation OR laser phototherapy OR laser phototherapies AND keratinocyte OR keratocyte;

3) Scopus: photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR laser OR "light emitting diode" OR "red laser" OR "low intensity laser" OR "low level laser" OR "low level light" OR "low power laser" OR biostimulation OR phototherapy AND keratinocyte OR keratocyte;

4) LILACS: laser AND keratinocyte OR keratocyte;

The retrieved references were exported to the EndNote software (Thompson Reuters, New York, NY, USA) and Reference Manager (Mendeley, London, UK). Duplicates were removed upon identification.

Study selection

All retrieved references titles and abstracts were read independently by two examiners (P.T.R.A. and J.A.A.A.). Inter-examiner kappa scores were calculated for the determination of the level of agreement regarding the pre-selection of articles based on titles and abstracts ($\kappa = 0.9$). Titles/abstracts that met the eligibility criteria were included in this systematic review. When the title/abstract did not contain enough information to decide on inclusion or exclusion, the full texts were retrieved and evaluated by the two reviewers who applied the same eligibility criteria. Those references that met the eligibility criteria were also included in this systematic review. In cases of a divergence of

opinion, a third examiner (I.M.A.D.) with expertise in PBMT area made the final decision.

Data extraction

The following data were extracted from the articles: author(s) and year of publication; keratinocyte source used; light device parameters [manufacturer, wavelength in nanometers (nm), operation mode (continuous or pulsed), power in Watts or milliWatts (W/mW), power density (W/cm^2 or mW/cm^2), energy density (J/cm^2), total energy in Joules (J) and spot area (cm^2)]; treatment specifications (irradiation time (s), irradiation frequency, irradiation performed in contact or at a distance mode); assays performed and replicates; and the main results obtained, including mean and standard deviation/standard error.

The parameters of the LASER/LED devices considered in the present review were established according to the recommendations made by the World Association for Laser Therapy (WALT) (WALT, 2006). Accurate report of light parameters set the study as reproducible and comparable. If necessary, author(s) were contacted to retrieve additional information. In cases where the author(s) did not inform us the energy density (ED), it was calculated according to the following formula: *ED (J/cm^2) = P (W) x T (seconds) / spot area (cm^2).* When the spot area was not informed by the author(s), calculation was performed using the formula: *Area = π (3,14) x r^2*.

Data were grouped according to the visible light spectrum (blue, red or near infrared) and according to the energy densities used. Thus, arbitrary subdivisions of energy densities were created to compare the studies: 0.1 to 5.0 J/cm^2; 5.1 to 10.0 J/cm^2; 10.1 to 15.0 J/cm^2; and above 15.0 J/cm^2. Studies that fit into more than one energy density subgroup, were allocated accordingly into more than one category.

Quality assessment

The quality analysis of the included studies was performed by evaluating eight criteria, according to the Office of Health Assessment and Translation (OHAT) tool adapted for *in vitro* studies by the National Toxicology Program - US Department of Health and Human Services (Rooney, Boyles, Wolf, Bucher, & Thayer, 2014): (1) randomization of treatments; (2) adequate allocation of study groups; (3) experimental conditions of the culture; (4) complete data

without any experimental group being excluded from the analysis; (5) reliability in the exposure characterization; (6) reliability of the results; (7) mention of the results of all the tests performed; and (8) whether there were no other potential risks to the internal validity.

For each criterion, the included article was rated as “yes” or “no”, or “unclear”, when we could not be completely sure about the exposure characterization and/or outcomes. For example, studies missing information about the use of a method to avoid cells over-irradiation and/or for device power checking prior to the experiments were rated as “unclear” and, consequently, with potential risk of internal validity.

Synthesis of results

If the included studies presented methodological heterogeneity, only a qualitative analysis would be done making the results available in tables. If the included studies presented methodological homogeneity, a meta-analysis would be conducted. In the case of meta-analysis, statistical heterogeneity would be assessed by the I^2 statistic (Higgins, Thompson, Deeks, & Altman, 2003).

RESULTS

Studies selection

A total of 3221 references were identified in all databases searched. After the removal of duplicates, 1997 references were examined for their titles and abstracts; 1860 were excluded because they did not meet the criteria and 137 studies were selected for reading in full. Of these, 55 met the eligibility criteria and were included in this review. The flowchart shown in Figure 1 depicts the search and the selection process.

Irradiation parameters

Tables 1, 2 and 3 show the division of the studies according to the visible light spectrum, source of keratinocytes, energy densities, assays performed, experimental times and results in relation to cell proliferation, viability, migration, expression of cytokines and growth factors. The results refer to the irradiated groups in relation to the control groups not irradiated.

Amongst the 55 studies analyzed, 14 (25.5%) used blue light (Table 1), 34 (61.8%) used red light (Table 2) and 17 (30.9%) used infrared light (Table 3).

Wavelengths ranged from 405 nm (blue) to 1064 nm (near infrared). In the blue spectrum, the variation was from 405 to 488 nm; in the red spectrum was from 622 to 672 nm; and in the infrared spectrum was from 780 to 1064 nm. The energy density (J/cm^2) was the most frequently reported parameter for all wavelengths, varying from 0.3 to 168.5 J/cm^2 in the blue; from 0.1 to 271.0 J/cm^2 in the red; and from 0.12 to 1,080 J/cm^2 in the infrared spectra. Two studies (Soukos et al., 1996; Walter, Pabst, & Ziebart, 2015) did not report the energy density used and in this case, we performed the calculation using the formula as described.

According to WALT recommendations (WALT, 2006), 50 (90.9%) studies did not report the use of optical fibers, 47 (85.4%) did not report the total energy (J) supplied to the cells and 32 (58.1%) did not report the spot area. Twenty-eight (50.9%) did not report the distance between the device tip and the irradiated cells, or the operation mode or the power used (Supplementary Table 1).

Effects on viability and proliferation

Of the 55 studies, 50 (90.9%) evaluated the effects of PBMT on cell viability and proliferation. Of these, 42 (84.0%) studies performed the evaluation of proliferation and viability in short experimental times (48 hours maximum after irradiation), seven (14.0%) evaluated the effects of PBMT in long experimental times (greater than 48 h) and five (10.0%) did not report the experimental time used. Of studies using short experimental times, 17 (40.5%) studies evaluated proliferation and viability at experimental times below 24 h; 31 (73.8%) evaluated the effects of PBMT 24 h after irradiation and six (14.3%) performed the evaluation 48 h after irradiation. Of studies using long experimental times, 6 (85.7%) evaluated the effects of PBMT 72 h after irradiation and only one (14.3%) study performed the evaluation at experimental times greater than 72 h, specifically at the fifth and the eighth day after irradiation. It is observed that several studies have evaluated the PBMT effects in more than one experimental time. The MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) and Trypan Blue tests were the most used assays for assessing cell viability/proliferation (Supplementary Tables 2, 3 and 4).

Cell viability/proliferation outcomes according to the wavelength and energy density ranges are summarized in Figure 2. Of the 50 studies that evaluated the effects of PBMT on cell viability and proliferation, 13 (26.0%) evaluated the effects of blue light. Of these, 3 studies found an increase in viability and proliferation in the range of 0.1–5.0 J/cm^2 (Wataha et al., 2004; Buscone et al., 2017; Castellano-Pellicena et al., 2018). No cell death was found at this range. However, 5 studies reported that this energy density range did not modify viability (Wataha et al., 2004; Niu et al., 2015; Lee et al., 2016; Avola, Graziano, Panuzzo, & Cardile, 2018) or proliferation (Ma et al., 2016). The range of 5.1–10.0 J/cm^2 produced unmodified results in cell viability and proliferation (Lee et al., 2016; Ma et al., 2016) and cell death (Avola et al., 2018), specifically at 10.0 J/cm^2. In the range of 10.0 to 15.0 J/cm^2 only one study reported decrease in cell viability, specifically at 15.0 J/cm^2 (Avola et al., 2018). When using doses above 15.0 J/cm^2, most studies (n=7) found no change in viability (Lim et al., 2012; Dai et al., 2013; Dai et al., 2013; Zhang et al., 2013; Becker et al., 2016; Castellano-Pellicena et al., 2018) and proliferation (Ma et al., 2016). However, 3 studies found cell death at 20.0 and 40.0 J/cm^2 (Lee et al., 2016); 3 times at 33.0 J/cm^2, 3 times at 66.0 J/cm^2 and 3 times at 100.0 J/cm^2 (Liebmann, Born, & Kolb-Bachofen, 2010); and 25.0, 45.0, 50.0 and 85.0 J/cm^2 energy densities (Avola et al., 2018). There was no increase in cell viability at this range (Supplementary Table 2).

Of the 50 studies, 31 (62.0%) evaluated the effects of red light in cell viability and proliferation. The stimulatory effects were observed in the range of 0.1 to 5.0 J/cm^2 (Sheen et al., 2015; Sperandio et al., 2015; Gagnon et al., 2016). Only one study found decrease in cell viability at this range, specifically at 3.6 J/cm^2 (Silva et al., 2018). Most studies (n=14) found unmodified results in viability and proliferation (Artuc et al., 1989; Haas, Isseroff, Wheeland, Rood, & Graves, 1990; Yu, Chang, Yu, Chen, & Chen, 1996; Takashi et al., 2003; Babilas 2006; Maduray et al., 2010; Maduray et al., 2011; Maduray et al., 2012; Barron et al., 2013; Pellicioli et al., 2014; Niu et al., 2015; Lee et al. al., 2016; Ma et al., 2016; Silva et al., 2018). When doses in the range of 5.1 to 10.0 J/cm^2 were used, only one article reported increased cell viability (Sperandio et al., 2015) at the dose of 6.0 J/cm^2, and only one reported decrease in cell proliferation (Gagnon et al., 2016), specifically at the dose of 10.0 J/cm^2. Another nine studies reported no changes in cell proliferation and viability at this

range (Haas et al., 1990; Boehncke, Rück, Naumann, Sterry, & Kaufmann, 1996; Takahashi et al., 2003; Babilas et al., 2006; Maduray et al., 2012; Niu et al., 2015; Lee et al., 2016; Ma et al., 2016; Xiao et al., 2018). In the range of 10.1 to 15.0 J/cm^2, four studies found no change in cell viability or proliferation (Takahashi et al., 2003; Babilas et al., 2006; Maduray et al., 2012; Huang, Qian, Sun, & Cheng, 2016). On the other hand, one study showed an increase in cell viability at 12.0 J/cm^2 (Sperandio et al., 2015). There was no cell death at this range. Similar results were found at doses greater than 15.0 J/cm^2, where the majority of the studies (n=10) found no change in viability or proliferation (Boehncke et al., 1996; Soukos et al., 1996; Babilas et al., 2006; Tapajós et al., 2008; Liebmann et al., 2010; Maisch et al., 2011; Lim et al., 2012; Pellicioli et al., 2014; Lee et al., 2016; Ma et al., 2016). On the other hand, two studies found increase in cell viability at the doses of 271.0 J/cm^2 (Walter et al., 2015) and 30.0 J/cm^2 (Castellano-Pellicena et al., 2018) and two found decreasing in cell viability at the doses of 30.0 J/cm^2 (Szeimies et al. al., 1995) and 112.5 J/cm^2, respectively (Migliario et al., 2013) (Supplementary Table 3).

Of the 50 studies, 16 (32.0%) evaluated the effects of near infrared on cell viability and proliferation. There was observed an increased in the range of 0.1 to 5.0 J/cm^2 (Grossman, Schneid, Reuveni, Halevy, & Lubart, 1998; Basso, Oliveira, Kurachi, Hebling, & de Souza Costa, 2013; Basso, Pansani, Soares, Hebling, & de Souza Costa, 2018). Eight studies did not find any modification (Pogrel, Chen, & Zhang, 1997; Basso et al., 2013; Lee et al., 2015; Lee et al., 2016; Baroni et al., 2017; Basso et al., 2018; De Filippis, Perfetto, Guerrera,l Oliviero, & Baroni, 2018; Qin et al., 2018). Two studies found decrease in cell viability and proliferation (Basso et al., 2013; Basso et al., 2018). There was also an increase in proliferation in the range of 5.1 to 10.0 J/cm^2 (Basso et al., 2013; Rizzi et al., 2018). Four other studies evaluated cell viability at doses within this range and none of them found any modifications (Basso et al., 2013; Lee et al., 2016; Baroni et al., 2017; De Filippis et al., 2018). Only two studies evaluated cell viability using energy doses at the range of 10.1 to 15.0 J/cm^2, and both cases, none of them have observed any modifications in relation to the control group (Fickweiler, 1997; Engel et al., 2016). Nine studies have evaluated the effects of PBMT on cell viability/proliferation using doses above 15.0 J/cm^2, where six did not find changes in the irradiated group in relation to the controls (Fickweiler et al., 1997; Abels et al., 2000; Liebmann et al., 2010;

Gluth et al., 2015; Lee et al., 2016; Castellano-Pellicena et al., 2018), one found a decrease in cell viability (Engel et al., 2016) and one found increased proliferation, specifically at the dose of 32.47 J/cm^2 (Rizzi et al., 2018) (Supplementary Table 4).

Effects on migration

Of the 55 studies, only 9 (16.3%) evaluated the effects of PBMT on cell migration. Among these studies, cell migration was performed at short experimental times (48 hours maximum after irradiation). Six (66.6%) studies have evaluated cell migration in time-intervals less than 24 hours, all nine (100%) studies within 24 hours, and two (22.2%) within 36 and 48 hours after irradiation. Some studies have evaluated migration in more than one experimental time. All studies have evaluated cell migration using the cell scratch assay. These data are reported in Supplementary Tables 2, 3 and 4.

Cell migration outcomes according to the wavelength and energy density ranges are summarized in Figure 2. Of the nine studies that evaluated the effects of PBMT on cell migration, two (22.2%) used blue light. In these studies, the dose of 0.3 J/cm^2 (Fushimi et al., 2012) and 2.0 J/cm^2 (Castellano-Pellicena et al., 2018) did not produce changes in relation to the control group. When 30.0 J/cm^2 was used there was reduction in migration (Castellano-Pellicena et al., 2018) (Supplementary Table 2).

Six studies (66.6%) evaluated the effects of PBMT on cell migration using red light. In this case, there was stimulatory effects at the 0.1 a 5.0 J/cm^2 range, particularly when the cells were irradiated 3 times at 0.8 J/cm^2 (Haas et al., 1990); 0.6 J/cm^2 (Fushimi et al., 2012); 4.0 J/cm^2 (Pellicioli et al., 2014); and 0.1, 0.2 e 1.2 J/cm^2 (Gagnon et al., 2016). At the range of 5.1 to 10.0 J/cm^2 two studies have evaluated cell migration. One article found increase in migration with 8.0 J/cm^2 (Xiao et al., 2018) and another reported the reduction of migration at 10.0 J/cm^2 (Gagnon et al., 2016). There was also an increase in cell migration at the range of 10.1 to 15.0 J/cm^2, using the dose of 12.0 J/cm^2 (Huang et al., 2016); and at the range >15.0 J/cm^2, when using 20.0 J/cm^2 (Pellicioli et al., 2014) (Supplementary Table 3).

Of the nine studies that evaluated the effects of PBMT on cell migration, only two (22.2%) used infrared light. One reported an increase in cell migration at 6.5, 16.23, 32.47 and 48.7 J/cm^2 (Rizzi et al., 2018) and another reported

unmodified results at 1.2 J/cm^2 in relation to the non-irradiated controls (Lee et al., 2015) (Supplementary Table 4).

Effects on the production of cytokines and growth factors

Of the 55 studies, 15 (27.2%) evaluated the effects of PBMT on the production of cytokines, growth factors and/or NF-kB. Of these, five (33.3%) studies have evaluated the expression of cytokines and growth factors in experimental times less than 24 h, 10 (66.6%) evaluated 24 h after irradiation, one (6.6%) evaluated 48 hours after irradiation and one (6.6%) evaluated 96 h after irradiation. Four (26.6%) studies did not report the experimental time used. Some studies have evaluated the PBMT effects in more than one experimental time. The main assays performed were the Enzyme-Linked Immunosorbent Assay (ELISA) and Real Time-Polymerase Chain Reaction (RT-PCR) and semi-quantitative RT-PCR (Supplementary Tables 2, 3 and 4).

The cytokines evaluated were Interleukin 1-alpha (IL-1α) (Yu et al., 1996; Gavish, Asher, Becker, & Kleinman, 2004; Kim et al., 2016; De Filippis et al., 2018), Interleukin 1-beta (IL-1β) (Gavish et al., 2004), Interleukin 6 (IL-6) (Fushimi et al., 2012; Gavish et al., 2004; Kim et al., 2016; De Filippis et al., 2018; Qin et al., 2018, Interleukin 8 (IL-8) (Yu et al., 1996; Liebmann et al., 2010; Lee et al., 2015; Baroni et al., 2017), Tumor Necrosis Factor–alpha (TNF-α) (Yu et al., 1996; Fushimi et al., 2012; Kim et al., 2016; Baroni et al., 2017; De Filippis et al., 2018; Qin et al., 2018), Transforming Growth Factor-alpha (TGF-α) (Fushimi et a., 2012), Transforming Growth Factor-beta (TGF-β) (Becker et al., 2016; Kim et al., 2016; Baroni et al., 2017, Qin et al., 2018), Interferon-gamma (IFN- γ) (Yu et al., 1996), the Stem Cell Factor (SCF) (Yu, Wu, Kao, Chiau, & Yu, 2003) and Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) (Yu et al., 1996). The growth factors studied were Heparin–Binding EGF – like Growth Factor (HB-EGF), Vascular Endothelial Growth Factor (VEGF) (Fushimi et al., 2012; Basso et al., 2013; Lee et al., 2015), Nerve Growth Factor (NGF), Hepatocyte Growth Factor (HGF) (Yu et al., 2003), basic Fibroblast Growth Factor (bFGF) (Yu et al., 2003; Basso et al., 2013), Keratinocyte Growth Factor (KGF) (Gavish et al., 2004; Basso et al., 2018) and Epidermal Growth Factor (EGF) (Basso et al., 2018). Three studies evaluated NF-κB transcription factor (Haas et al., 1998; Niu et al., 2015; Becker et al., 2016).

Expression of cytokines, growth factors and other molecules according to the wavelength and energy density used are summarized in Figure 2. Among the 15 studies that evaluated the effects of PBMT on production of cytokines, growth factors and NF-kB, three (20.0%) studies used blue light. The energy densities at the range of 0.1 to 5.0 J/cm^2 did not modify the expression of these molecules (Niu et al., 2015), similar to the range above 15.0 J/cm^2 (Liebmann et al., 2010). On the other hand, one study found downregulation of NF-kB, TGF-β and TNF signaling pathways with 41.4 J/cm^2 (Becker et al., 2016) (Supplementary Table 2).

Among the 15 studies that evaluated the effects of PBMT on production of cytokines, growth factors and NF-kB, six (40.0%) used red and seven (46.6%) used infrared light. The effects of red and infrared light on the expression of cytokines, growth factors and NF-κB can be seen in Supplementary Tables 3 and 4, respectively. There was an increase in the production of cytokines and growth factors in the range of 0.1 to 5.0 J/cm^2. In this range, an increase of IL-1α, IL-6, IL-8, bFGF, NGF, EGF, KGF, VEGF, TGF-α, TGF-β and TNF-α was reported (Yu et al*.,* 1996; Yu et al., 2003; Fushimi et al., 2012; Gavish et al., 2004; Basso et al., 2013; Lee et al*.*, 2015; Baroni et al., 2017; Basso et al., 2018; Qin et al., 2018). Haas et al. (1998) performed a wound on the cells' monolayer (cell scratch assay) and found that the NF-kB was activated in the irradiated cells localized in the edge of the wound. At the range of 5.1 to 10.0 J/cm^2 there was no change in the expression of IL-1α, IL-6, TNF-α and TGF-β with the use of red light (Kim et al., 2016) and bFGF with the use of infrared light (Basso et al., 2013). On the other hand, increased expression of VEGF (Basso et al., 2013), TGF-β (Baroni et al., 2017) and IL-1α, IL-6, TNF-α and TGF-β (De Filippis et al., 2018) were reported. At the range above 15.0 J/cm^2, there was no change in the NF-κB expression (Walter et al., 2015).

Quality assessment

Figure 3 shows the established criteria for the quality and risk of bias of each *in vitro* study, according to the OHAT tool. Overall, we have observed that all 55 studies met the criteria of randomization, adequate allocation of groups and experimental conditions identical for the study groups. On the other hand, nine studies (16.3%) failed to explain, in a statistical way, either through graphs

or tables, the values of some of the assays performed (Haas et al., 1990; Boehncke et al., 1996; Haas et al., 1998; Abels et al., 2000; Yu et al., 2003; Maduray et al., 2012; Barron et al., 2013; Kim et al., 2016; Lee et al., 2016).

All the studies included here were considered in potential risk of internal validity, since they have failed to accurately provide the LASER/LED light parameters; or to report a method for over-irradiation prevention; or to inform whether power was checked prior experiments (Supplementary 5). Only two (3.6%) studies reported all light parameters (Silva et al., 2018; Rizzi et al., 2018). Only eight (14.5%) studies reported the use of methods to avoid over-irradiation of experimental groups, such as empty wells adjacent to the test wells or the use of black paper (Grossman et al., 1998; Wataha et al., 2004; Becker et al., 2016; Engel et al., 2016; Gagnon et al., 2016; Buscone et al., 2017; Avola et al., 2018; Castellano-Pellicena et al., 2018). Twenty (36.3%) studies reported power checking of the apparatus prior to the experiments, such as the use of a power meter (Haas et al., 1990; Yu et al., 1996; Grossman et al., 1998; Yu et al., 2003; Gavish et al., 2004; Wataha et al., 2004; Babilas et al., 2006; Maduray 2010; Maduray et al., 2011; Maisch et al., 2011; Fushimi et al., 2012; Maduray et al., 2012; Basso et al., 2013; Dai et al., 2013; Dai et al., 2013; Zhang et a., 2013; Pellicioli et al., 2014; Engel et al., 2016; Lee et al., 2016; Buscone et al., 2017).

DISCUSSION

The epithelium is an important barrier that protects the body from external aggression. Therefore, restoring its integrity upon injuries is of paramount importance, helping the maintenance of the organism health (Rizzi et al., 2018). The development of tools that stimulate the viability/survival, proliferation, and migration of the epithelial constituent cells, such as keratinocytes, may have therapeutic benefits. This review showed that both cell viability and proliferation were stimulated by PBMT mainly at energy doses within the range of 0.1 to 5.0 J/cm^2 in any of the three analyzed light spectra (blue, red and infrared). Cell migration and an increased expression of cytokines were also observed, but particularly after red and infrared light irradiations. Large heterogeneity was found among studies, thus limiting the reliability of the results.

The energy range set of 1.0 to 10.0 J/cm^2 reported in the study of Tata & Waynant (2011) is the classical therapeutic window for PBMT by promoting biomodulatory effects. Accordingly, for epithelial cells, the present review shows that the ideal range may be narrower than the 1.0 to 10.0 J/cm^2, i.e. around 0.1 to 5 J/cm^2. However, more than the energy density, the biological effects of PBMT may be dependent on the rate at which light is delivered, which is the power density of the light (W/cm^2) (Hamblin, 2018). This may explain why some studies using energy densities above 5.0 J/cm^2 have also obtained positive results. For instance, the increase in the proliferation and viability found in the range of 5.1 to 10.0 J/cm^2, when red or infrared lights were used, occurred at the doses of 6.0 J/cm^2 (Sperandio et al., 2015), 6.5 J/cm^2 (Rizzi et al*.,* 2018) e 7.0 J/cm^2 (Basso et al., 2013), which are immediately above to the range 0.1 to 5 J/cm^2. Otherwise, intriguing positive results were obtained at the >15.0 J/cm^2 range, with 271.0 J/cm^2 (Walter et al., 2015), 30.0 J/cm^2 (Castellano-Pellicena et al., 2018) and 32.47 J/cm^2 (Rizzi et al., 2018). Unfortunately, we were not able to analyze these and other included studies considering also the power density used due the failure in reporting important light parameters in many studies. Moreover, the great diversity of light setting protocols could turn data condensation highly speculative.

In fact, our results corroborate with those based on the Arndt-Schultz curve reported previously (Chaves et al., 2014; Hamblin et al. 2018), where a bimodal effect of PBMT is observed in relatively close energy densities. We have observed that when higher densities (over 10.0 J/cm^2) were tested, cell proliferation and viability tended to decrease (Szeimies et al., 1995; Liebmann et al., 2010; Migliario et al., 2015; Engel et al., 2016; Gagnon et al., 2016; Lee et al., 2016). One possible explanation may be related to the sensitive balance between ROS levels and ROS scavengers in cells and tissues before and after PBMT. Accordingly, a moderate increase in ROS can promote cell proliferation and differentiation, whereas excessive amounts of ROS can be toxic to the cells, causing oxidative damage to lipids, proteins and DNA (Trachootham, Alexandre, & Huang, 2009). In this light, Gagnon et al. (2016) found that the dose of 10.0 J/cm^2 produced the worst results regarding proliferation and migration, with results similar to those of the experimental group cultured with 1% fetal bovine serum, i.e., under oxidative stress. Lower doses (0.1 and 0.2 J/cm^2), though, yielded positive results similar to those of the non-irradiated

group cultured in 10% fetal bovine serum, which presented ideal conditions for cell culture growth. As such, the control of ROS generation in PBMT is apparently challenging to produce beneficial and detrimental outcomes (Hamblin et al. 2018).

Considering the effects of PBMT on the expression of cytokines, the analysis is hampered due to the dual behavior of these molecules, which may have a proinflammatory action or an anti-inflammatory action (Opal & Depalo, 2000). In addition to the inflammatory effects, cytokines IL-1α, IL-8, TGF- α and growth factor VEGF also play a role in the induction of keratinocyte proliferation and migration (Yu et al., 1996; Haas et al., 1998; Fushimi et al., 2012). It is interesting to note that in the study of Baroni et al. (2017), cytokine evaluation was performed on HaCaT infected with *Candida albicans* treated with or without irradiation. The fungus-infected and non-irradiated cells had high levels of the proinflammatory cytokines IL-8 and TNF-α, while presenting low levels of TGF-β. It was observed that in this context, IL-8 and TNF-α had a predominantly pro-inflammatory action since the cells were infected by the microorganism, whereas TGF-β had mainly an anti-inflammatory action. Irradiation with 2.0 and 4.0 J/cm^2 caused reduction of the proinflammatory cytokines and increase of the anti-inflammatory cytokine in the infected cells.

It is noteworthy that some cytokines released by the keratinocytes are mostly produced under inflammatory processes stimulus (Fellclan et al. 1996), and in the studies where it was not considered/simulated, some results may have been biased. Furthermore, a particular pattern of cytokine/ expression could be more related to cell culture conditions than reflect a physiological stimulus (Gröne, 2000). As such, particular attention to the *in vitro* experiments should be considered when analyzing those molecules once FBS is a natural source of growth factors, for example. In these cases, a blank control must be performed.

Most of the studies were inaccurate in providing all LASER/LED dosimetry parameters applied. This issue was previously raised in other reviews (Peplow et al., 2010; Tata & Waynant, 2011). Thus, for future research to be carried out in the PBMT field, we strongly suggest the reporting of the detailed description of light dosimetry, including not only the energy density used, but also power, power density, total energy supplied, the irradiation distance, the use or not of optical fiber, mode of action, as well as the irradiation time and the

treatment frequency (WALT, 2006). Care in providing full device setting guarantees reproducible studies and external validity.

It should be noticed that most of the energy densities and wavelengths tested in the studies, regardless of the assays performed, provided unmodified outcomes. This observation is particularly evident in the viability and proliferation assays, since they are more frequently used as a basic parameter of cytotoxicity in the *in vitro* studies. A common feature of these studies is the lack of reproduction of in *vitro* conditions that mimic an injured milieu before testing PBMT. Apparently, the redox signaling that occurs in PBMT can have opposite effects on healthy and stressed cells (Hamblin et al., 2018). As such, challenging cells chemically or by decreasing the fetal bovine serum concentration (mimicking oxidative stress), decreasing pH, simulating hypoxia, wounding, and other conditions, may represent a struggle for cell survival where PBMT may represent a benefit (Haas et al., 1998; Lim et al. 2012, Mignon et al. 2016, Tucker et al. 2018).

An important issue observed in data condensation were variations in treatment protocols and cell culture conditions, which may have resulted in different outcomes even when using the same light parameters (Mignon, Uzunbajakava, Raafs, Botchkareva, & Tobin, 2016). Moreover, cell viability, proliferation, migration and cytokines and growth factors expression patterns can be influenced in a dynamic time-varying manner, thus limiting data grouping and analysis. Indeed, the vast majority of the reviewed studies do not provide enough information either about the exposure protocols or about outcomes measuring and assessment. Otherwise, the OHAT tool was developed in 2014 to assess potential bias for *in vitro* and mechanistic studies, and so far was only barely used in the literature (Thayer et al., 2014). Accordingly, the OHAT is a checklist that may help researchers to be aware of the internal and external validity of their experimental design and/or data description. Thus, we strongly recommend that future experimental studies follow the OHAT guidelines.

Finally, missing mathematical data (such as replicates, mean and standard deviation) from the assays used to assess the treatments precluded a further meta-analysis. Hence, most of these data are presented, but numerically not explicit in graphs or tables. Additionally, some journals currently require the raw data from the assays performed by the researchers, particularly those from experimental studies. Encouraging data disclosure, particularly for systematic

review purposes, would be useful when performing future meta-analysis in basic research studies.

CONCLUSION

The present systematic review demonstrated the heterogeneity of the studies that evaluate the PBMT effects on keratinocytes cultured *in vitro*, particularly regarding the light parameters used. Meanwhile, investigations on potential mechanisms of PBMT using *in vitro* models are still reporting contradictory results. Overall, the results demonstrate that there is an increase in cell viability, proliferation, migration and expression of cytokines and growth factors when using doses in the range of 0.1 to 5.0 J/cm^2, both using red or near infrared lights. In addition, the vast majority of the tested energy densities produced unmodified results, regardless of the wavelength applied.

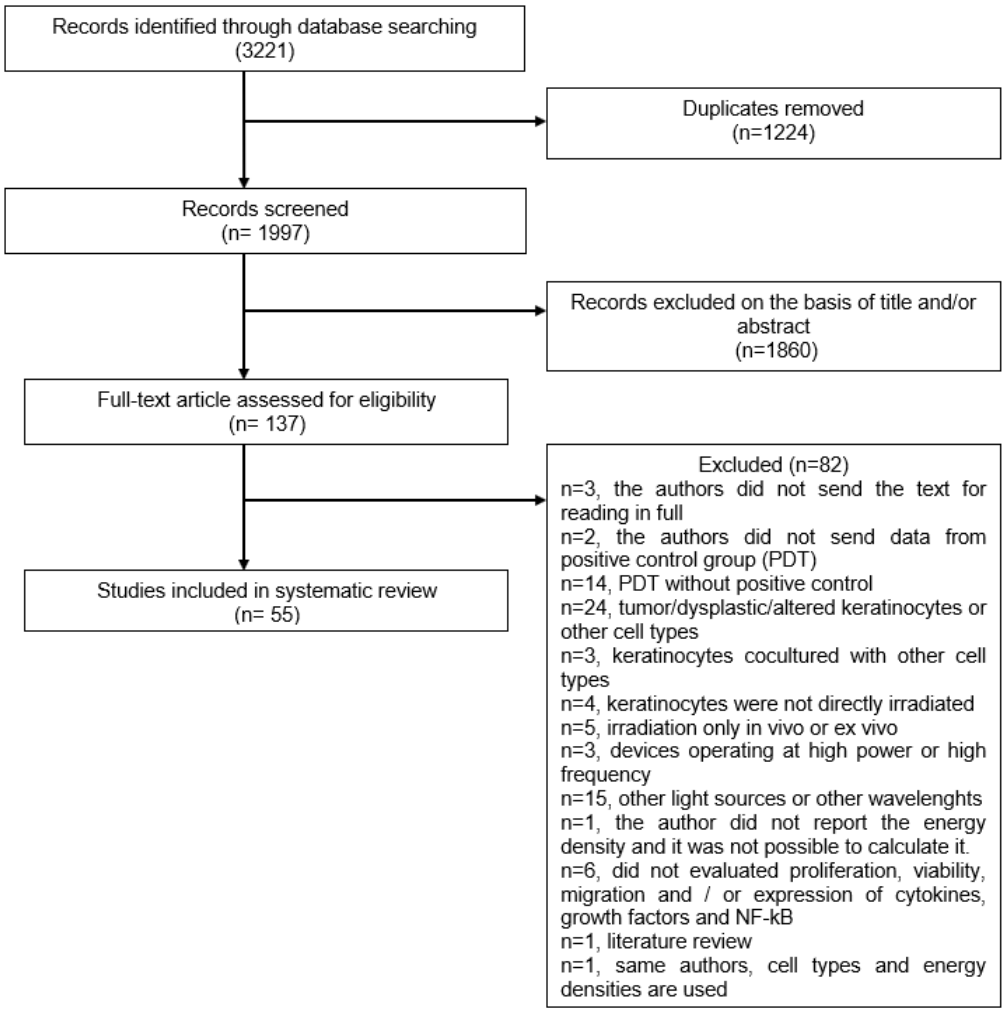

Figure 1 - Search flowchart according to the PRISMA Statement

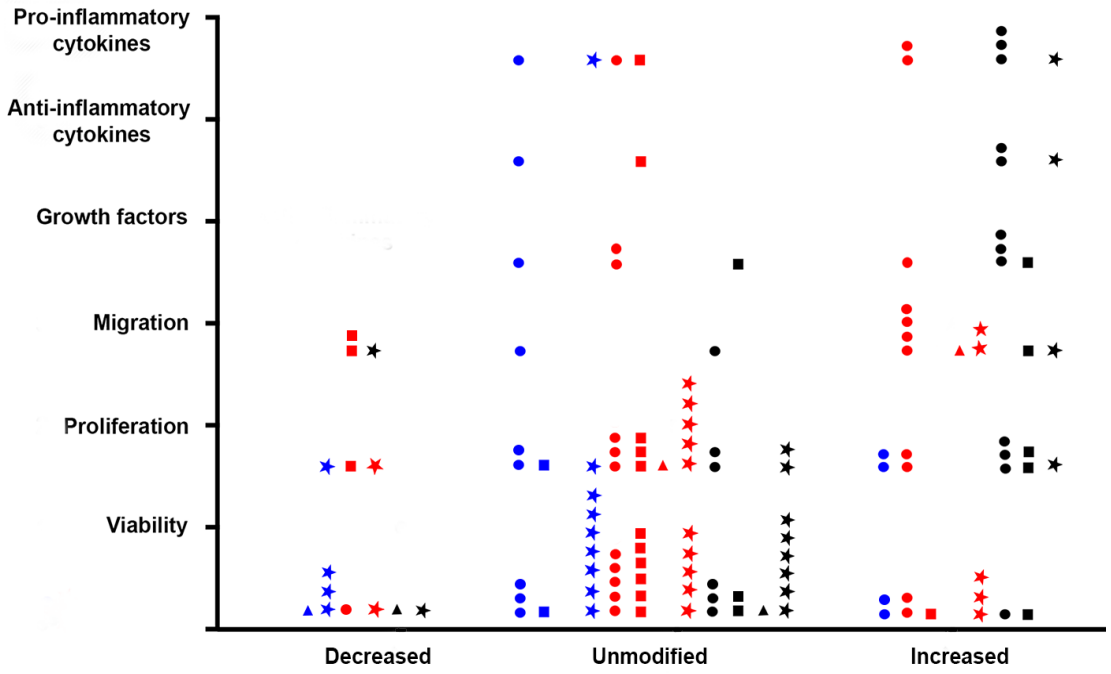

Figure 2 – Effects of PBMT on viability, proliferation, migration, growth factors, anti-inflammatory and pro-inflammatory cytokines according to energy density (J/cm^2). The symbols represent the groups of energy densities according the spectrum of visible light blue (blue), red (red) and black (near infrared): circle – 0.1 to 5.0 J/cm^2, square – 5.1 to 10.0 J/cm^2, triangle – 10.1 to 15.0 J/cm^2 and star – above 15.0 J/cm^2.

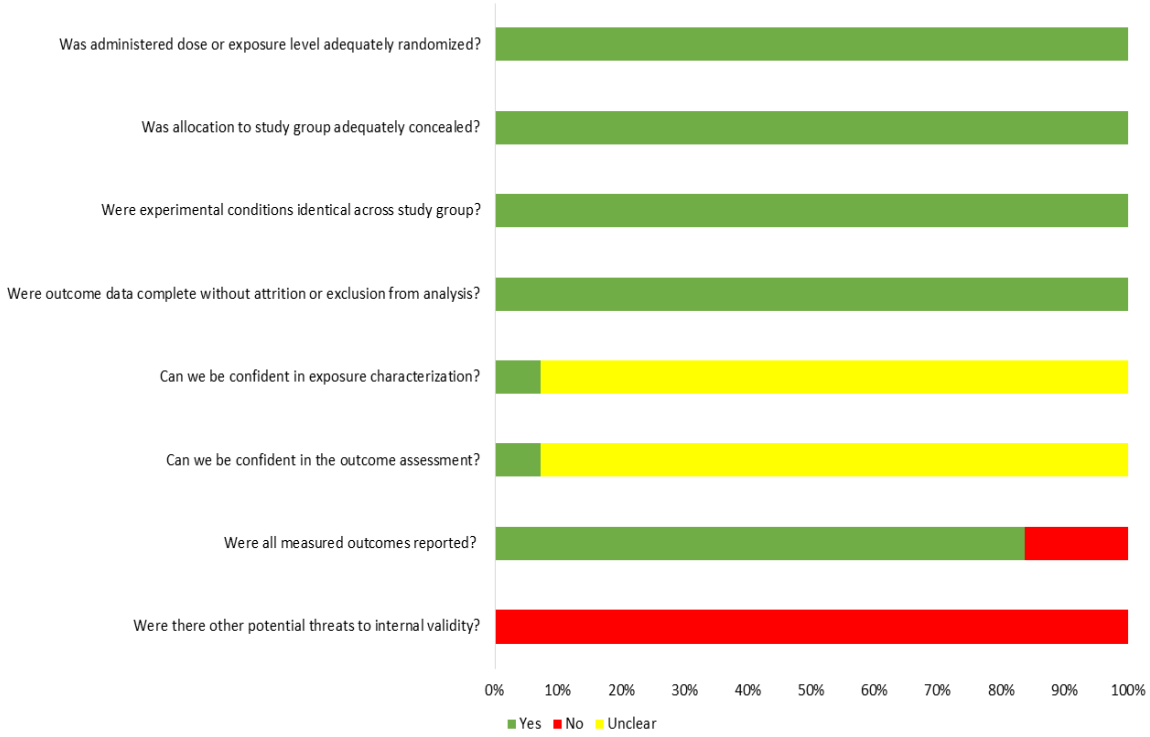

Figure 3 - Quality analysis and risk of bias of the primary studies

Supplementary 1: Device and treatment specifications of cells cultured *in vitro*, capable of interfering with photobiomodulatory effects

	Device specifications								Treatment specifications		
First author (year)	**Model**	**Wavelenght (nm)**	**Operation mode**	**Power**	**Power density**	**Spot size**	**Total energy**	**Optical fiber (diameter)**	**Distance of irradiation**	**Time of irradiation**	**Frequency of treatments**
Artuc (1989)	He-Ne laser	632 nm	Not reported	7.0 mW	Not reported	Not reported	5.0 J	Not reported	Not reported	720 sec (≈ 5 J)	Not reported
Haas (1990)	He-Ne laser (Spectraphysics Stabilite, Model 120, Mountain View, CA)	633 nm	Not reported	8.4 mW	Not reported	Not reported	Not reported	Not reported	Not reported	The maximal irradiation time was 1200 sec	Each group received one application. For KC's migration assay and KC's -fibroblasts coculture the cells were irradiated isolated or in combination three times, every 24h with 0.8 J/cm^2. This resulted in various combinations of irradiated and non-irradiated cells
Szeimies (1995)	Argon-pumped tunable dye laser (Models 2040 annd 375, Spectra Physics Lasers Inc., Mountain View, CA)	622 to 649 nm	Not reported	3.5 W	40 mW/cm^2	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Boehncke (1996)	Argon-ion laser	630, 662 nm	Not reported	Not reported	100 mW/cm^2	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Soukos (1996)	He-Ne laser (NEC Corp., Japan)	632.8 nm	Not reported	7.3 mW	Not reported	Not reported	438, 876 mJ	Not reported	Not reported	60, 120 sec	Not reported
Yu (1996)	He-Ne laser (Lasotronic Med 1000, Lasotronic Ag, Zugestr, Switzerland)	630 nm	Not reported	7.0 mW	7.0 mW/cm^2	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Fickweiler (1997)	Diode laser (Opto Power Corporation, California)	805 nm	Continuous	15.0 W	40.0 mW/cm^2	150.0 cm^2	Not reported	1.500 µm	Not reported	120, 240, 480 sec	Not reported
Pogrel (1997)	GaAlAs laser (Diolase 100, Berkeley, CA)	830 nm	Continuosly adjustable for 5- 100 mW	5, 10, 20, 30, 45, 60 mW and 0.1 W	Not reported	Not reported	Not reported	Not reported	1.1 cm	10, 20, 30, 45, 60, 120 sec	Each group received 1 irradiation. The powers were combined with the times to give a total of 24 combinations of time and power. Each assay was performed in duplicate or triplicate and incubated for 24 or 48 hours, resulting in 84 combinations of potency, irradiation and incubation time.
Grossman (1998)	Diode laser constructed at the Department of Physics, Bar-Ilan University (Israel)	780 nm	Continuous	6.5 mW	Not reported	0.32 cm^2	Not reported	Not reported	2.0 cm	0 to 180 sec	In a few experiments, cells were exposed repeatedly to 0.35 J/cm^2 or to 0.6 J/cm^2, every 24 hr (totalizing 3 consecutive exposures), or every 48 hr (totalzing two consecutive exposures), with the first exposure occurring 48 hr

											following seeding.
Haas (1998)	He-Ne laser (Uniphase model 1135P, Uniphase, Manteca, CA 95335)	633 nm	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Abels (2000)	Diode laser (Opto Power Corporation, California)	805 nm	Continuous	15.0 W	80.0 mW/cm^2	Not reported	Not reported	1.5 mm	Not reported	300 sec 900 sec	Each group received one application
Takahashi (2003)	Diode laser (LD670-05, Hamamatsu Photonics, Hamamatsu, Japan)	670 nm	Continuous	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Yu (2003)	He-Ne laser (Lasotronic Med 1000, Lasotronic Ag, Zugestr, Switzerland)	630 nm	Not reported	7.0 mW	7.0 mW/cm^2	Not reported	Not reported	Not reported	17.0 cm	Not reported	Not reported
Gavish (2004)	Ti-Sa laser	780 nm	Not reported	0.2 W	Not reported	100.0 cm^2	Not reported	Not reported	Not reported	1080 sec	Each group received one application
Wataha (2004)	Argon Laser (Accure, Model 3000, Laser Med)	488 nm	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	7.5 mm	10 sec	Each group received one application
Babilas (2006)	LED Omnilux	633 nm	Not reported	Not reported	40.0 mW/cm^2	Not reported	Not reported	Not reported	Not reported	75,150, 300, 600 sec	Not reported
Tapajós (2008)	Diode laser (BWF light source - Tech in)	670 nm	Continuous	Not reported	40.0 mW/cm^2	Not reported	Not reported	Not reported	Not reported	300 sec	Each group received one application
Liebmann (2009)	LED (Philips Research Institute, Aachen, Germany)	412, 419, 426, 453-632, 648-850, 940 nm	Not reported	Not reported	87.0, 126.0, 68.0, 66.0-38.0, 71.0-50.0, 32.0 mW/cm^2	Not reported	Not reported	Not reported	50mm	Not reported	Each group received one application every 24h hours, during 3 consecutive days
Maduray (2010)	Diode laser (Coherent laser system, USA)	672 nm	Continuous	20.0 – 30.0 mW	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Maduray (2011)	Diode laser (Coherent laser system, USA)	672 nm	Continuous	20.0 – 30.0 mW	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Maisch (2011)	LED system (Photocure, Oslo, Norway)	634 nm	Not reported	Not reported	56.0 mW/cm^2	144.0 cm^2	Not reported	Not reported	Not reported	660.7 sec	Not reported
Fushimi (2012)	LED - Mignon Belle LT-1 Crystalline (Mignon Belle Co., Ltd, Osaka, Japan)	456-638 nm	Continuous	6.93-7.56 W	0.75-0.25 mW/cm^2	70.0 cm^2	Not reported	Not reported	10.0 cm	1200 sec	Each group received one application. For analysis of protein levels by ELISA, each group received one application per day, during 3 consecutive days For migration assay the cells were irradiated with blue, red or green light. For protein levels and cytokines analyses the cells were irradiated only with red or green light.
Lim (2012)	LED (U-JIN LED, Korea)	470-635 nm	Continuous	Not reported	5.0 mW/cm^2	63.6 cm^2	Not reported	Not reported	Not reported	3600 sec	Each group received one application
Maduray (2012)	Diode laser (Coherent laser system, USA)	672 nm	Continuous	20.0 – 30.0 mW	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Barron (2013)	Photocure 128 PDT LED (Photocus, Oslo, Norway)	633 nm	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	110.0 cm	Not reported	Not reported

Basso (2013)	InGaAsP diode laser prototype (LASERTable, Optics Group of the Optics and Photonics Research Center at the Physics Institute of São Carlos, University of São Paulo, Brazil)	780±3 nm	Continuous	40.0 mW	Not reported	2.0 cm^2	Not reported	Not reported	2.5 cm	40, 120, 240, 400, 560 sec	The KC's were irradiated every 24h totaling three applications during three consecutive days.
Dai (2013)	LED - Omnilux Clear-UTM (Photo Therapeutics, Inc., Carlsbad, CA)	415 nm	Continuous	Not reported	19.5 mW/cm^2	Not reported	Not reported	Not reported	Not reported	1440, 2880, 4320, 5760, 8640 sec	Each group received one application
Dai (2013)	LED - Omnilux Clear-UTM (Photo Therapeutics, Inc., Carlsbad, CA)	415 nm	Continuous	Not reported	19.5 mW/cm^2	Not reported	Not reported	Not reported	Not reported	720,1440, 2880, 4320, 5760 sec	Each group received one application
Migliario (2013)	MLT two in one laser system (Medical Laser Technology GmbH, Ingelheim, Germany)	635 nm	Not reported	0.1- 0.3 W	Not reported	Not reported	6.6-9.0 J	320 µm	Not reported	60-30 sec	Not reported
Gluth (2014)	Coherent NIR light source (DIOMED 25; Dimed Limited, Cambridge, U.K.)	810 nm	Continuous	Not reported	6.0 mW/cm^2	Not reported	Not reported	600 µmol/L	10.0 cm	180 sec	Each group received one application
Pellicioli (2014)	InGaAlP diode laser (MM Optics Ltda., São Carlos, Brazil)	660 nm	Continuous	40.0 mW	Not reported	0.04 cm^2	0.48, 2.4, 12.0 J	Not reported	In contact (for MTT assay the laser was applied in a single point. For scratch assay the laser was applied in a five points)	4, 20 sec	Each group received three sessions of irradiations with 6-h intervals
Zhang (2014)	LED - Omnilux Clear-UTM (Photo Therapeutics, Inc., Carlsbad, CA)	415 ± 20 nm	Not reported	Not reported	19.5 mW/cm^2	Not reported	Not reported	Not reported	Not reported	1440, 2880, 4320, 5760, 8640 sec	Not reported
Chabert (2015)	TRIWINGS LED (BioMetrical electronics, France Biophoton)	630 nm	Not reported	Not reported	50 - 55 mW/cm^2	Not reported	Not reported	Not reported	20.0 cm	Not reported	Exposures were repeated twice a week, with at least 48 h between two exposures.
Lee (2015)	GaAlAs laser (NDLux, Seoul, Korea)	808 nm	Continuous	80.0 mW	Not reported	1.0 cm^2	0.4, 1.2, 2.4 J	Not reported	5.0 cm	5, 15, 30 sec	Each group received one application. The doses 0.4, 1.2 and 2.4 J/cm2 (total energy = 0.4, 1.2 and 2.4 J respectively) were used only for MTT assay.
Niu (2015)	LED (Chinese Semiconductor, Research Institute)	405-630,660 nm	Not reported	Not reported	161-300, 545 µW/cm^2	Not reported	Not reported	Not reported	25.0 mm	600 sec	Not reported
Sheen (2015)	LED (Lustrous Technology Ltd., Taipei, Taiwan)	630 nm	Not reported	Not reported	3.0 mW/cm^2	Not reported	Not reported	Not reported	Not reported	Not reported	In the energy densitty experiments, the cells were irradiated daily for 3 days. In the cell

											number experiments, the cells were irradiated daily for 5 days. To determine the serial effect of red light in proliferation, the cells were irradiated at 2J/cm2 every 24hours for 5 successive days
Sperandio (2015)	Diode laser (Photon Lase III; DMC Equipment, São Paulo, Brazil)	660 nm	Not reported	0.1 W	3.57 mW/cm^2	0.028 cm^2	84.0, 168, 336 mJ	Not reported	In contact	0.84, 1.68, 3.36 sec	Not reported
Walter (2015)	Diode laser (Periowave Ondine Biopharma Corp., Toronto, Canada)	670 nm	Not reported	28.0 mW	Not reported	Not reported	Not reported	Not reported	In contact	60 sec	Each group received one application
Becker (2016)	LED - Lumileds LUXEON Rebel LXML-PR01-0275 (Koninklijke Philips N.V., Eindhoven/Netherlands)	453 nm	Continuous	Not reported	23.0 mW/cm^2	Not reported	Not reported	Not reported	55.0 mm	1800 sec	Not reported
Engel (2016)	GaAlAs diode laser (Picasso, AMD, USA)	808 nm	Continuous	0.6, 0.7, 0.8, 0.9 W	0.0377, 0.0440, 0.0503, 0.0566 W/cm^2	Not reported	Not reported	Not reported	16.0 cm	300 sec	Each group received one application
Gagnon (2016)	He-Ne laser (Class IV laser system)	650 nm	Continuous	0.5, 1.0, 3.0, 12.0 W	Not reported	9.62 cm^2	Not reported	Not reported	5.0 cm	2, 2, 4, 8 sec	Each group received one application
Huang (2016)	LED - prototype device (Carnation-10; Lifotronic Technology Co., Ltd.)	640 nm	Not reported	15.0 W	30.0 mw/cm^2	Not reported	Not reported	Not reported	25.0 cm	400, 800 sec	Each group received one application.
Kim (2016)	LED (Omnilux revive, 633 nm, Phototherapeutics, Montgomeryville, PA, USA)	633 nm	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Lee (2016)	Not reported	480-630-850, 940 nm	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	10.0 cm	Not reported	Not reported
Ma (2016)	LED (633±10 nm, LED-IA, Wuhan Yage Ltd., P.R. China) - LED (418nm, LED, Wuhan Yage Ltd., P.R, China)	418-633 nm	Not reported	Not reported	100 mW/cm^2	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Baroni (2017)	Q- switched Nd-YAG laser (Medlite C6 laser, Conbio, USA)	1.064 nm	Pulsing mode (pulse width 5 ns)	Not reported	Not reported	Not reported	Not reported	Not reported	2.5 cm	Not reported	Laser irradiation was carried out twice at an interval of 1 sec.
Buscone (2017)	LED (Philips, The Netherlands)	453 nm	Not reported	Not reported	16 mW/cm^2	Not reported	Not reported	Not reported	Not reported	200 sec	Each group received one application per day during 10 consecutive days, totalizing 10 applications
Basso (2018)	InGaAsP diode laser prototype (LASERTable, Optics Group of the Optics and Photonics Research Center at the Physics Institute of São Carlos, University of São Paulo, Brazil)	780±5 nm	Continuous	70.0 mW	12.5 mW/cm^2	2.0 cm^2	Not reported	Not reported	2.5 cm	40, 120, 240 sec	Cells were irradiated for three consecutive times every 24 h.

Avola (2018)	Rayonet photochemical reactor (Southern New England Ultraviolet Company, Brandfort, CT)	450 nm	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	900, 1800, 5400, 10800 sec	Not reported
Castellano-Pellicena (2018)	Proprietary LED device	447-655-850 nm	Not reported	Not reported	50.0- 40.0-40.0 mW/cm^2	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
De Filippis (2018)	Q-switched 1064 nm Nd:YAG laser (Medlite Conbio C6 Nd-YAG laser, Cynosure USA)	1064 nm	Pulsing mode (pulse width of 5 ns	Not reported	Not reported	6.0 mm	Not reported	Not reported	2.5 cm	Not reported	Not reported
Qin (2018)	Q-switched 1064nm Nd:YAG (Medlite C, Conbio, USA)	1064 nm	Pulsing mode (pulse width of 5 ns	Not reported	Not reported	3.0 mm	Not reported	Not reported	5.0 mm	Not reported	Irradiation was carried out twice successively. Two consecutive pulses were irradiated on the same site of one spot. Each group received one application every 24h during 2 consecutive days
Rizzi (2018)	DMT Giotto laser equipment (DMT srl, Lissone, Italy)	980 nm	Continuous	1.0 W	649.35 mW/cm^2	1.54 cm^2	10, 25, 50, 75 J	600 µm	9.7 cm	10, 25, 50, 75 sec	
Silva (2018)	Not reported	640 ± 12.5 nm	Not reported	Not repoted	2.6 mW/cm^2	Not reported	Not reported	Not reported	20.7 mm	120, 300, 420, 660, 1020, 1380 sec	Not reported
Xiao (2018))	He-Ne laser (Carnation-22; Lifotronic, Shenzhen,China)	640 nm	Continuous	10 mW	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported

He-Ne: Helium-Neon laser; Nd-YAG: neodymium-doped yttrium aluminium garnet; InGaAsP: Indium Gallium Arsenide Phosphide; GaAlAs: Gallium Aluminum Arsenide; Ti-Sa: Titanium-Sapphire; InGaAlP: Indium Gallium Aluminum Phosphide; nm: nanometer; W: watts; mW: miliwatts; J: joules.

Supplementary 2: Photobiomodulatory effects of blue light on keratinocytes cultured *in vitro* according to the energy density

Groups of energy density (J/cm^2)	First author (year)	Source of Keratinocytes	Energy density (J/cm^2)	Proliferation	Viability	Migration	Cytokines/Growth Factor/NF-kB
0.1-5.0	Wataha (2004)	Human foreskin‡	5.0	Experimental time not reported (Population doubling time and MTT estimated by a least-squares regression) Results: increased	72h after irradiation (MTT) Results: unmodified		
	Fushimi† (2012)	HaCaT§	0.3			24h after irradiation (Cell Scratch) Results: unmodified	0, 4, 8 and 24h after irradiation (semi-quantitative RT- PCR) and on 4th day after irradiation (ELISA) IL-6, TNF-α, HB-EGF, TGF- α, VEGF Results: unmodified
	Lee† (2016)	Human Epidermis‡	1.0 2.5 5.0		After irradiation (MTT) Results: unmodified		
	Ma† (2016)	Keratinocytes from acne lesions‡	5.0	24h after irradiation (CCK-8) Results: unmodified			
	Buscone (2017)	Human Outer Root Sheath‡	3.2	4 and 8h after irradiation (EdU) Results: increased	24h after irradiation (Alamar Blue) Results: increased		
	Avola† (2018)	Human keratinocytes‡	5.0		24h after irradiation (MTT) Results: unmodified		
	Catellano-Pellicena† (2018)	Human Epidermis‡	2.0		24h after irradiation (Alamar Blue) Results: increased	0, 12 and 24h after irradiation (Cell Scratch) Results: unmodified	
5.1-10.0	Lee† (2016)	Human Epidermis‡	10.0		After irradiation (MTT) Results: unmodified		
	Ma† (2016)	Keratinocytes from acne lesions‡	10.0	24h after irradiation (CCK-8) Results: unmodified			
10.1-15.0	Avola† (2018)	Human keratinocytes‡	10.0 15.0		24h after irradiation (MTT) Results: decreased		
>15.0	Liebmann† (2009)	Human Neonatal foreskin‡	33.0 66.0	24h after irradiation (Alamar Blue)	24h after irradiation (Apoptosis evaluated by morphology and Hoechst/Propidium iodide		Experimental time not reported (RT-PCR) IL-8

				staining)		
Lim† (2012)	HaCaT§	100.0 18.0	Results: decreased	Results: decreased		Results: unmodified
Dai (2013)	HaCaT§	28.0-168.3		Immediately after irradiation (Trypan Blue) Results: unmodified		
Dai (2013)	HaCaT§	14.0-109.9		Immediately after irradiation (Trypan Blue) Results: unmodified.		
Zhang (2014)	HaCaT§	28.0-168.5		Immediately after irradiation (Trypan Blue) Results: unmodified		
Niu† (2015)	HaCaT§	1.604		24h after irradiation (CCK-8 and LDH) Results: unmodified		Experimental time not reported (Western Blot) NF-kB Results: unmodified
Becker (2016)	HaCaT§	41.4		24h after irradiation (FACS) Results: Unmodified		
Lee† (2016)	Human Epidermis‡	20.0 40.0		After irradiation (MTT) Results: decreased		
Ma† (2016)	Keratinocytes from acne lesions‡	20.0	24h after irradiation (CCK-8) Results: unmodified			
Avola† (2018)	Human keratinocytes‡	25.0 45.0 50.0 85.0		24h after irradiation (MTT) Results: decreased		
Catellano-Pellicena† (2018)	Human Epidermis‡	30.0		24h after irradiation (Alamar Blue) Results: unmodified	0,12 and 24h after irradiation (Cell Scratch) Results: decreased	

† Some articles reported more than one subgroup simultaneously. The authors used more than one wavelength and/or different energy densities.
‡ Prymary culture, § Lineage culture
CCK-8: Cell Counting Kit-8; EdU: (5-ethynyl-2'-deoxyuridine) assay; ELISA: Enzyme-Linked Immunosorbent Assay; FACS: Fluorescence Activated Cell Sorting; HB-EGF: Heparin-Binding Epidermal Growth Factor-like growth factor; IL-6: Interleukin-6; IL-8: Interleukin- 8; LDH: Lactate Dehydrogenase; MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay; NF-κB: Nuclear Factor-Kappa B; RT-PCR: Reverse Transcriptase – Polymerase Chain Reaction; TGF-α: Transforming Growth Factor-alpha; TNF-α: Tumor Necrosis Factor-alpha; VEGF: Vascular Endothelial Growth Factor

Supplementary 3: Photobiomodulatory effects of red light on keratinocytes cultured *in vitro* according to the energy density

Groups of energy density (J/cm^2)	First author (year)	Source of keratinocytes	Energy density (J/cm^2)	Proliferation	Viability	Migration	Cytokines/Growth factors/NF-kB
0.1-5.0	Artuc (1989)	Human foreskin‡	1.0 2.5 5.0		24h after irradiation (Trypan Blue) Results: unmodified		
	Haas† (1990)	Human neonatal foreskin‡	0.4 0.8 3.0	72h after irradiation (Coulter counter) 0 to 6h after irradiation (BrdU) Results: unmodified		0 to 6h after irradiation (Cell Scratch) Results: increased (with the dose 3x 0.8 J/cm^2)	
	Soukos† (1996)	Normal human gingiva‡	3.36¶		Experimental time not reported (MTS) Results: unmodified		
	Yu (1996)	Human adult foreskin‡	0.5 1.0 1.5	Immediately after irradiation (H-thymidine and H-leucine) Results: unmodified			Experimental time not reported (ELISA) IL-1α, IL-8, TNF-α, IFN-γ, GM-CSF Results: IL-1α, IL-8 increased; GM-CSF, TNF-α, IFN-γ unmodified
	Haas (1998)	Human neonatal foreskin‡	1.5				(Cell Scratch was combined with irradiation to evaluate the expression of NF-κB, immunohistochemistry) Results: NF-κB was present in the nucleus and markedly activated in keratinocytes along the wound edge
	Takahash† (2003)	Human‡	5.0		Experimental time not reported (Crystal Violet) Results: unmodified		
	Yu (2003)	Human adult foreskin‡	0.5 1.0 1.5				Experimental time not reported ELISA) NGF, SCF, HGF, bFGF Results: bFGF, NGF, increased; SCF, HGF unmodified
	Babilas† (2006)	Human epidermis	3.0		24h after irradiation (MTT) Results: unmodified		
	Maduray (2010)	(origin and type of cells were not reported)	4.5		24h after irradiation (CellTiter Blue) Results: unmodified		
	Maduray (2011)	Epidermis (immortalized cells)§	4.5		24h after irradiation (CellTiter Blue) Results: increased		
	Fushimi† (2012)	HaCaT§	0.6			24h after irradiation (Cell Scratch) Results: increased	0, 4, 8 and 24h after irradiation (semi-quantitative RT- PCR) and on 4th day after irradiation (ELISA) IL-6, TNF-α, HB-EGF, TGF-α, VEGF Results: only TGF-α mRNA increased
	Maduray† (2012)	Epidermis (immortalized cells)§	2.5 4.5		24h after irradiation (CellTiter Blue) Results: unmodified		
	Barron	HaCaT§	1.5		24h after irradiation		

	(2013)				(Neutral red) Results: unmodified	
	Pellicioli† (2014)	Normal oral spontaneously immortalized cell§	4.0	24h after irradiation (MTS) Results: unmodified		0, 12, 24, 36 and 48h after irradiation (Cell Scratch) Results: increased
	Chabert† (2015)	Normal human skin‡	4.0		Experimental time not reported (MTT) Results: MTT data for red light were not shown	
	Sheen (2015)	Outher root sheath from Wistar rats‡	1.0 2.0 4.0	72h after irradiation (Count of live cells). Results: increased		
	Sperandio† (2015)	HaCaT§	3.0		12, 24, 48 and 72h after irradiation (MTS) Results: increased after 48h	
	Gagnon† (2016)	Canine epidermis§	0.1 0.2 1.2	0, 24 and 48h after irradiation (WST-1) Results: immediately after irradiation unmodified; 24 and 48h increased		0, 12, 24, 36 and 48h after irradiation (Cell Scratch) Results: increased
	Lee† (2016)	Human epidermis ‡	1.0 2.5 5.0		After irradiation (MTT) Results: unmodified	
	Ma† (2016)	Keratinocytes from acne lesions‡	5.0	24h after irradiation (CCK-8) Results: unmodified		
	Silva (2018)	HaCaT§	0.31 0.78 1.0 1.7 2.6 3.6		48h after irradiation (MTT) Results: decreased with 3.6 J/cm^2	
5.1-10.0	Haas† (1990)	Human neonatal foreskin‡	7.2	72h after irradiation (Coulter counter) 0 to 6h after irradiation (BrdU) Results: unmodified		
	Boehncke† (1996)	HaCaT§	6.5	12 and 24h after irradiation (H-thymidine) Results: unmodified		
	Soukos† (1996)	Normal human gingiva‡	6.73¶		Experimental time not reported (MTS) Results: unmodified	
	Takahashi† (2003)	Human‡	10.0		Experimental time not reported (Crystal Violet) Results: unmodified	
	Babilas† (2006)	Human epidermis§	6.0		24h after irradiation (MTT) Results: unmodified	
	Maduray† (2012)	Epidermis (immortalized cells)§	7.5		24h after irradiation (CellTiter Blue) Results: unmodified	
	Chabert† (2015)	Normal human skin‡	8.0		Experimental time not reported (MTT)	

	Sperandio† (2015)	HaCaT§	6.0		Results: MTT data for red light were not shown 12, 24, 48 and 72h after irradiation (MTS) Results: increased		
	Gagnon† (2016)	Canine epidermis§	10.0	0, 24 and 48h after irradiation (WST-1). Results: immediately after irradiation unmodified; 24h and 48h decreased		0, 12, 24, 36 and 48h after irradiation (Cell Scratch) Results: 12, 24 and 36h decreased	
	Kim (2016)	Primary‡ and HaCaT§	6.0		Experimental time not reported (MTT) Results: irradiated only group data were not shown		24h and 48h after irradiation (ELISA) IL-1α, IL-6, TNF-α, TGF-β Results: 24h after: unmodified
	Lee† (2016)	Human epidermis‡	10.0		After irradiation (MTT) Results: unmodified		
	Ma† (2016)	Keratinocytes from acne lesions‡	10.0	24h after irradiation (CCK-8) Results: unmodified			
	Xiao (2018)	HaCaT§	8.0		12h after irradiation (CCK-8) Results: unmodified	0, 12 and 24h after irradiation (Cell Scratch) Results: 12 and 24h decreased	
10.1-15.0	Chabert† (2015)	Normal human skin‡	12.0		Experimental time not reported (MTT) Results: MTT data for red light were not shown		
	Huang† (2016)	Human neonatal and adult foreskin‡	12.0	24h after irradiation (CCK-8) Results: unmodified		0, 12 and 24h after irradiation (Cell Scratch) Results: increased	
	Szeimies (1995)	Human oral keratinocytes§	30.0		24h after irradiation (MTT) Results: decreased with 635 nm, 637 nm, 640 nm and 643 nm		
	Boehncke† (1996)	HaCaT§	30.0	12 and 24 after irradiation (H-thymidine) Results: unmodified			
	Babilas† (2006)	Human epidermis§	24.0		24h after irradiation (MTT) Results: unmodified		
>15.0	Tapajós (2008)	Normal oral spontaneously immortalized cell§	25.0		Experimental time not reported (Trypan Blue and Fluorescence microcopy) Results: unmodified		
	Liebmann† (2009)	Neonatal foreskin‡	20.0 40.0 60.0 100.0	24h after irradiation (Alamar Blue) Results: unmodified			
	Maisch (2011)	Neonatal human epidermis§	37.0		24h after irradiation (MTT) Results: unmodified		
	Lim† (2012)	HaCaT§	18.0		24h after irradiation (MTT) Results: increased		
	Migliario	HaCaT§	82.5	72h after irradiation			

(2013)		112.5	(Tox 8) Results: decreased			
Pellicioli† (2014)	HaCaT§	20.0	24h after irradiation (MTS) Results: unmodified		0, 12, 24, 36 and 48h after irradiation (Cell Scratch) Results: increased	
Niu† (2015)	Normal oral spontaneously immortalized cell§	3049 6538		24h after irradiation (CCK-8 and LDH) Results: unmodified		Experimental time not reported (Western Blot) NF-κB Results: unmodified
Walter (2015)	Human oral Keratinocyte§	271.0¶		Experimental time not reported (MTT) Results. increased		
Huang† (2016)	Human neonatal and adult foreskin‡	24	24h after irradiation (CCK-8) Results: unmodified		0, 12 and 24h after irradiation (Cell Scratch) Results: increased	
Lee† (2016)	Human epidermis ‡	20.0 40.0		After irradiation (MTT) Results: unmodified		
Ma† (2016)	Keratinocytes from acne lesions‡	20.0	24h after irradiation (CCK-8) Results: unmodified			
Castellano-Pellicena† (2018)	Human Epidermis	30.0		24h after irradiation (Alamar Blue) Results: increased		

†Some articles reported more than one subgroup simultaneously. The authors used more than one wavelength and/or different energy densities.

‡Primary culture, §Lineage culture.

¶Energy densities were not provided by the author (calculated by us according the ED formula)

bFGF, basic Fibroblast Growth Factor; BrdU, (5-Bromo-2'-Deoxyuridine) assay; CCK-8, Cell Counting Kit-8; ELISA, Enzyme-Linked Immunosorbent Assay; GM-CSF: Granulocyte-macrophage Colony-Stimulating Factor; HB-EGF, Heparin-Binding Epidermal Growth Factor-like growth factor; HGF, Hepatocyte Growth Factor; IFN-γ, Interferon-gamma; IL-1α, Interleukin-1 alpha; IL-6, Interleukin-6; IL-8, Interleukin-8; LDH, Lactate Dehydrogenase; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay; MTT, (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay; NF-κB, Nuclear Factor-Kappa B; NGF, Nerve Growth Factor; RT-PCR, Reverse Transcriptase–Polymerase Chain Reaction; SCF, Stem Cell Factor; TGF-α, Transforming Growth Factor-alpha; TGF-β, Transforming Growth Factor-beta; TNF-α, Tumor Necrosis Factor–alpha; TOX-8, Resazurin-based TOX-8 assay; VEGF, Vascular Endothelial Growth Factor; WST-1, (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assay.

Supplementary 4: Photobiomodulatory effects of near-infrared light on keratinocytes cultured *in vitro* according to the energy density

Groups of energy density (J/cm^2)	First author (year)	Source of keratinocytes	Energy density (J/cm^2)	Proliferation	Viability	Migration	Cytokines/Growth Factors/NF-kB
0.1–5.0	Pogrel (1997)	Human foreskin‡	0.12-4.24	24 and 48 h after irradiation (Crystal Violet) Results: unmodified			
	Grossman (1998)	Human young foreskin‡	0.35-3.6	24 and 48h after irradiation (Counting chamber and H-thymidine) Results: increased			
	Gavish (2004)	HaCaT§	2.0				0, 2, 4, 6, 8 and 10h after irradiation (semi-quantitative RT-PCR) IL-α, IL-6, IL-1β, KGF Results: IL-α, KGF increased at 2h, IL-6 activated immediately, increased at 2h, repressed at 6h and returned to control levels at 10h; IL-1β -decreased, but reapeared at 10h.
	Basso† (2013)	HaCaT§	0.5 1.5 3.0 5.0	24h after irradiation (Trypan Blue) Results: increased	24h after irradiation (MTT and Total protein production) Results: MTT: increased with 0.5, 1.5, 3.0 J/cm^2; unmodified with 5.0 J/cm^2; Total protein production: increased with 0.5, 3.0, 5.0 J/cm^2; decreased with 1.5 J/cm^2		24h after irradiation (q-PCR) bFGF, VEGF Results: bFGF unmodified; VEGF increased
	Lee (2015)	HaCaT§	1.2	24h after irradiation (Hoechst staining and Western Blot for Caspases 3, 7 and 9) Results: unmodified		24h after irradiation (Cell Scratch) Results: Unmodified	Experimental time not reported (ELISA) IL-8, VEGF Results: increased
	Lee† (2016)	Human epidermis‡	1.0 2.5 5.0		After irradiation (MTT) Results: unmodified		
	Baroni† (2017)	HaCaT§	2.0 4.0		24, 48 and 72 h after irradiation (MTT) Results: unmodified		Experimental time not reported (RT-PCR) TNF-α, TGF-β, IL-8 Results: TNF-α, IL-8 decreased, TGF-β increased
	Basso (2018)	Human Oral keratinocytes‡	0.5 1.5 3.0	24h after irradiation (Alamar Blue) Results: increased only with 0.5 J/cm^2			24h after irradiation (qPCR) EGF, KGF Results: increased only with 0.5 J/cm^2
	De Filippis† (2018)	HaCaT§	2.0 4.0		6, 12 and 24h after irradiation (MTT) Results: unmodified		
	Qin (2018)	HaCaT§	0.7 0.8		24h after irradiation (CCK-8) Results: unmodified		24h after irradiation (PCR) IL-6, TNF-α, TGF-β Results: increased

	Basso† (2013)	HaCaT§	7.0	24h after irradiation (Trypan Blue) Results: increased	24h after irradiation (MTT and Total protein production) Results: both increased		24h after irradiation (q-PCR) bFGF, VEGF Results: bFGF unmodified; VEGF increased
5.1–10.0	Lee† (2016)	Human epidermis‡	10.0		After irradiation (MTT) Results: unmodified		
	Rizzi† (2018)	HaCaT§	6.5	24h after irradiation (MTT) Results: increased		24h after irradiation (Cell Scratch) Results: increased	
	De Filippis† (2018)	HaCaT§	6.0 8.0		6, 12 and 24h after irradiation (MTT) Results: unmodified		
10.1–15.0	Fickweiler† (1997)	HaCaT§	12.0		24h after irradiation (MTT) Results: unmodified		
	Engel† (2016)	Normal Oral Keratinocytes Spontaneously Immortalized§	11.3 13.2		Immediately after irradiation (Alamar Blue) Results: decreased		
>15.0	Fickweiler† (1997)	HaCaT§	12.0		24h after irradiation (MTT) Results: unmodified		
	Abels (2000)	HaCaT§	24.0 48.0	Immediately after irradiation and 1, 2, 5 and 8 days after (Haemocytometer and polpulation doubling time by linear regression) Results: unmodified	24h after irradiation (MTT) Results: unmodified		
	Liebmann† (2009)	Neonatal Foreskin‡	40.0 60.0 80.0 120.0	24h after irradiation (Alamar Blue) Results: unmodified			
	Gluth (2014)	Cholesteatoma derived‡	1.080		2h after irradiation (Trypan Blue) Results: unmodified		
	Engel† (2016)	Normal Oral Keratinocytes Spontaneously Immortalized §	15.1 17.0		Immediately after irradiation (Alamar Blue) Results: decreased		
	Lee† (2016)	Human Epidermis‡	20.0 40.0		After irradiation (MTT) Results: unmodified		
	Castellano-Pellicena† (2018)	Human Epidermis‡	60.0		24h after irradiation (Alamar Blue) Results: unmodified		
	Rizzi† (2018)	HaCaT§	16.23 32.47 48.7	24h after irradiation (MTT) Results: increased		24h after irradiation (Cell Scratch) Results: increased	

† Some articles reported more than one subgroup simultaneously. The authors used more than one wavelength and/or different energy densities.
‡ Prymary culture, § Lineage culture
CCK-8: Cell Couting Kit-8; ELISA: Enzyme-Linked Immunosorbent Assay; Interleukin-1 alpha; bFGF. Basic Fibroblast Growth Factor; Interleukin-6; IL-8: Interleukin-8; IL-1β: Interleukin-1 beta; KGF: Keratinocyte Growth Factor; MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay; q-PCR: quantitative- Polymerase Chain Reaction; RT-PCR: Reverse Transcriptase –Polymerase Chain Reaction; TNF-α: Transforming Growth Factor-alpha; TGF-β: Transforming Growth Factor-beta; VEGF: Vascular Endothelial Growth Factor

Supplementary 5: Quality and risk analysis of bias according to criterias based on OHAT tool.

First author (year)	Was administered dose or exposure level adequately randomized?	Was allocation to study groups adequately concealed?	Were experimental conditions identical across study groups?	Were outcome data complete without attrition or exclusion from analysis?	Can we be confident in exposure characterization?	Can we be confident in the outcome assessment?	Were all measured outcomes reported?	Were there other potential threats to internal validity?
Artuc (1989)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Haas (1990)	Yes	Yes	Yes	Yes	Unclear	Unclear	Proliferation graph and BrdU labelled datas were not shown	Yes[1,2]
Szeimies (1995)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Boehncke(1996)	Yes	Yes	Yes	Yes	Unclear	Unclear	Viability data for irradiation-only group were not shown	Yes[1,2,3]
Soukos (1996)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Yu (1996)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2]
Fickweiler(1997)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Pogrel (1997)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3,]
Grossman(1998)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[1]
Haas (1998)	Yes	Yes	Yes	Yes	Unclear	Unclear	NF-Kβ translocation data for irradiated unwounded cells were not shown.	Yes[1,2,3]
Abels (2000)	Yes	Yes	Yes	Yes	Unclear	Unclear	Some MTT data were not shown	Yes[1,2,3]
Takahashi(2003)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3,]
Yu (2003)	Yes	Yes	Yes	Yes	Unclear	Unclear	Some RT-PCR data were not shown	Yes[1,2]
Gavish (2004)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2]
Wataha (2004)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[1]
Babilas (2006)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2]
Tapajós (2008)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Liebmann(2009)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Maduray (2010)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Maduray (2011)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,]
Maisch (2011)	Yes	Yes	Yes	Yes	Unclear	Unclear	Cell viability graph did not include irradiation- only group	Yes[1,2]
Fushimi (2012)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,]
Lim (2012)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Maduray (2012)	Yes	Yes	Yes	Yes	Unclear	Unclear	Cell viability graph did not include irradiation-only group	Yes[1,2,]
Barron (2013)	Yes	Yes	Yes	Yes	Unclear	Unclear	Some data were not shown	Yes[1,2,3]
Basso (2013)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2]
Dai (2013)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,]
Dai (2013)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,]
Migliario (2013)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Gluth (2014)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Pellicioli (2014)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2]

Zhang (2014)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2]
Chabert (2015)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3,]
Lee (2015)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Niu (2015)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Sheen (2015)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Sperandio(2015)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Walter (2015)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Becker (2016)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,3]
Engel (2016)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[1]
Gagnon (2016)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,3]
Huang (2016)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Kim (2016)	Yes	Yes	Yes	Yes	Unclear	Unclear	Optimal dose of irradiation and some MTT data were not shown	Yes[1,2,3]
Lee (2016)	Yes	Yes	Yes	Yes	Unclear	Unclear	Cell viability graph were not shown	Yes[1,2]
Ma (2016)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Baroni (2017)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Buscone (2017)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes[1]
Basso (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Avola (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,3]
Castellano-Pellicena (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,3]
De Filippis (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Qin (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Rizzi (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[2,3]
Silva (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]
Xiao (2018)	Yes	Yes	Yes	Yes	Unclear	Unclear	Yes	Yes[1,2,3]

BrdU: (5-Bromo-2'-Deoxyuridine) assay; MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay ; NF-kB: Nuclear Factor-Kappa B; RT-PCR: Reverse Transcriptase – Polimerase Chain Reaction

‡[1] Incomplete device parameters and treatment specifications

‡[2] Methods of avoiding overirradiation were not reported

‡[3] Check device power prior experiments were not reported

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6 CONSIDERAÇÕES FINAIS

O presente trabalho demonstrou a grande heterogeneidade existente quanto aos estudos *in vitro* que visam avaliar os efeitos da PBMT, sobretudo quanto aos desenhos experimentais, condições de cultivo das células, protocolos de irradiação e dosimetria. A ausência do detalhamento de todos os parâmetros dos dispositivos de luz utilizados, segundo as recomendações da WALT, dificulta a análise criteriosa de tais estudos, bem como a sua reprodutibilidade, comparação e a confiabilidade nos resultados obtidos. Neste sentido, torna-se difícil saber se a densidade de energia informada é a que realmente foi recebida pelas células e se os resultados alcançados devem-se exclusivamente à irradiação. Sendo assim, é de suma importância que os estudos passem a detalhar de forma criteriosa todos os parâmetros bem como se preocupem em adotar métodos que evitem a sobreirradiação das culturas e verifiquem a potência do aparelho previamente ao experimento (sobretudo nos casos em que a irradiação será realizada à distância).

Sabe-se que respondem à PBMT as células que apresentam características típicas de situações de estresse, tais como hipóxia, baixo pH, escassez de nutrientes (baixas concentrações de soro fetal bovino), similar ao que ocorre no tecido injuriado. Células que apresentam condições ótimas de crescimento e cultivo tendem a não responder à PBMT. Cremos que esse seja um dos mais importantes fatores que contribuíram para que a maioria dos estudos não verificasse modificações na viabilidade, proliferação e migração. Assim é importante também que os estudos simulem condições de estresse (baixos níveis de oxigênio, pH e concentrações de nutrientes) para que os efeitos da radiação possam ser verificados.

A principal dificuldade enfrentada na condução deste trabalho foi encontrar estudos similares que fornecessem todos os dados necessários para a realização de uma meta-análise. Os ensaios realizados pelos estudos primários permitem a descrição dos resultados de diferentes formas, seja porcentagem ou média e desvio-padrão. Justamente por isso é importante que haja uma padronização na descrição dos resultados para que tenhamos dados comparáveis. Solicitamos aos autores o fornecimento das amostras (n), médias e desvios-padrão, porém a taxa de resposta foi extremamente baixa, de tal forma que não obtivemos dados suficientes para comparação. Assim, é

importante que estudos futuros se preocupem em informar detalhadamente dados metodológicos da PBMT que podem influenciar consideravelmente os desfechos testados.

Este trabalho permitiu uma visão ampla, porém inicial do estado da arte no que concerne à PBMT. Sendo assim, novos estudos são necessários para que análises cada vez mais detalhadas possam ser realizadas. Salientamos que os resultados encontrados nesta revisão referem-se exclusivamente às analises realizadas em queratinócitos. Desta forma, compilar os resultados da PBMT sobre outros tipos celulares, tais como fibroblastos e células mesenquimais é extremamente importante, considerando-se que estas também estão diretamente envolvidas no processo de cicatrização e reparo tecidual.

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APÊNDICE A – Formulário de extração de dados e análise de qualidade

Citation Evaluation Form

Effects of photobiomodulation on keratinocytes cultured *in vitro*: a systematic review

Citation and publication status: nº _____
Location of trial / Language of publication
Country:_____
Language:_____

I. Type of Study
1. _____ *In vitro* only
2. _____ *In vivo* and *in vitro*
3. _____ *Ex vivo* and *in vitro*
4. _____ *In vivo*, *ex vivo* and *in vitro*

II. Type of keratinocytes
1. _____ Human
2. _____ Animal

III. Origin of keratinocytes
1. _____ Skin
2. _____ Mucosa

IV. Type of culture
1. _____ Primary
2. _____ Lineage
Wich: _____

V. Experimental conditions of the culture
1. _____ Same conditions (media, solvent, washes, plates) (desirable)
2. _____ Different conditions (media, solvent, incubator, plates)

VI. Randomization of treatments (dose, exposure in homogeneous cell suspension?)
1. _____ Yes
2. _____ No

VII. Allocation to study groups adequately concealed (in homogeneous cell suspension?)
1. _____ Yes
2. _____ No

VIII. Blinding during study
1. _____ Robotic system
2. _____ Nonblind (non robotic system)

IX. Exposure
a) Parameters of light
1. _____ Laser
2. _____ LED

Device specifications

Type of light	Manufacturer, location and model	Wave length (nm)	Power (mW/W)	Power density (W/cm^2)	Energy density (J/cm^2)	Spot size/ area of irradiation (cm^2)	Total energy (Joules)	Optical fiber (diameter)	Mode
Red									Continuous
Near infrared									Pulsed
Blue									

Treatment specifications

Contact	Number of applications (total number)	Frequency of treatments	Time(seconds)
Punctual			
Non punctual (distal)			

b) Exposure characterization
Avoided overexposure?
1. _____ Yes
2. _____ No
Checked device power prior experiments (power meter)
1. _____ Yes
2. _____ No
Other

X. Types of assay
1. _____ Proliferation _____
2. _____ Viability _____
3. _____ Migration _____
4. _____ Adhesion _____
5. _____ Expression of cytokines
Which: _____
6. _____ Expression of grown factors
Which: _____
7. _____ Expression of receptors
Which:_____
8. _____Other molecules

XI. Types of outcomes
Brief description:

XII. Were all measured outcomes reported?
1. _____ Yes
2. _____ No
XIII. Incomplete data (non-irradiated group, evidence of well or plate loss without explanation,…)
1. _____ Yes
2. _____ No

XIV. Appropriate statistical methods performed?
1. _____ Yes
2. _____ No

XV. Are there no other potential threats to internal validity
1. _____ Yes
2. _____ No

Which_____

XVI. Author's conclusion
Description:

XVII. Can we be confident in the outcome assessment?
Validity of conclusions: _____ adequate _____ inadequate

XVIII. Notes _____