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# Evaluation de l'effet de la photobiomodulation sur la viabilité et la fonctionnalité des cellules bêta et îlots pancréatiques

Quentin Perrier

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THÈSE

Pour obtenir le grade de

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**Evaluation de l'effet de la photobiomodulation sur la viabilité et la fonctionnalité des cellules bêta et îlots pancréatiques**

**Impact of photobiomodulation on the survival and functionality of beta cells and pancreatic islets**

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<b>AA</b> : Antimycine A	<b>RCR</b> : <i>Respiratory control ratio</i>
<b>ADP</b> : Adénosine diphosphate	<b>ROS</b> : <i>Reactive oxygen species</i>
<b>AMP</b> : Adénosine monophosphate	<b>SDS</b> : <i>Substrate deprivation stress</i>
<b>ATP</b> : Adénosine triphosphate	<b>SIK</b> : <i>Simultaneous islet kidney transplantation</i>
<b>AUC</b> : <i>Area under the curve</i>	<b>SKP</b> : <i>Simultaneous kidney pancreas transplantation</i>
<b>CCCP</b> : <i>Cabonylcyanide m-chlorophenyl hydrazone</i>	<b>STZ</b> : <i>Streptozotocin</i>
<b>CCO</b> : Cytochrome C oxydase	<b>TGF-<math>\beta</math></b> : <i>Transforming growth factor</i>
<b>CI</b> : Contre-indication	<b>TMRM</b> : <i>Tetramethyl rhodamine methyl ester</i>
<b>COX</b> : Cyclo-oxygénase	<b>TNF<math>\alpha</math></b> : <i>Tumor necrosis factor</i>
<b>DFG</b> : Débit de filtration glomérulaire,	<b>TRP</b> : <i>Transient receptor potential</i>
<b>DT1</b> : Diabète de type 1	<b>VEGF</b> : <i>Vascular endothelial growth factor</i>
<b>ELISA</b> : <i>Enzyme-linked immunosorbent assay</i>	
<b>FBS</b> : <i>Foetal bovine serum</i>	
<b>GSIS</b> : <i>Glucose stimulated insulin secretion</i>	
<b>GTT</b> : <i>Glucose tolerance test</i>	
<b>HOMA-IR</b> : <i>Homeostatic model assessment of insulin resistance</i>	
<b>HUVEC</b> : Human Umbilical Vein Endothelial Cells	
<b>IAK</b> : <i>Islet after kidney transplantation</i>	
<b>IBMIR</b> : <i>Instant blood mediated inflammatory reaction</i>	
<b>IFN-<math>\gamma</math></b> : Interféron gamma	
<b>IL-1<math>\beta</math></b> : Interleukine 1 bêta	
<b>ITA</b> : <i>Islet transplantation alone</i>	
<b>ITT</b> : <i>Insulin tolerance test</i>	
<b>KRB</b> : <i>Kreb's ringer buffer</i>	
<b>LED</b> : <i>Light emitting diode</i>	
<b>LIF</b> : <i>Leukemia inhibitory factor</i>	
<b>LLLT</b> : <i>Low level laser therapy</i>	
<b>MEC</b> : Matrice extracellulaire	
<b>MIN6</b> : <i>Mouse insulinoma cells</i>	
<b>MMP2</b> : Métaloprotéinase 2	
<b>MTG</b> : <i>Mitotracker green</i>	
<b>NASA</b> : <i>National Aeronautics and Spatial Administration</i>	
<b>NF<math>\kappa</math>B</b> : Nuclear factor-kappa B	
<b>NO</b> : Oxyde nitrique	
<b>OMS</b> : Organisation mondiale de la santé	
<b>PAK</b> : <i>Pancreas after kidney transplantation</i>	
<b>PBM</b> : Photobiomodulation	
<b>PTA</b> : <i>Pancreas transplantation alone</i>	
<b>QoL</b> : <i>Quality of life</i>	
<b>RCT</b> : <i>Randomized controlled trial</i>	

## **Introduction**

### **1. Le diabète, entre recherche et innovations thérapeutiques**

#### **1.1 Le diabète**

##### **1.1.1 Définition**

Le diabète est caractérisé par une hyperglycémie chronique. Il est défini selon l'OMS par la présence d'au moins un des critères suivant<sup>1</sup> :

- Glycémie à jeun  $\geq 1,26$  g/L
- HbA1c  $\geq 6,5\%$
- Glycémie plasmatique aléatoire  $\geq 2$  g/L avec des signes cliniques de syndrome cardinal
- Glycémie  $\geq 2$ g/L 2h après un test d'hyperglycémie provoquée orale

Le diabète est une préoccupation majeure de santé publique, avec 537 millions d'adultes probablement atteints dans le monde en 2021 (prévalence de 10.5% en moyenne)<sup>2</sup> et 3,6 millions de personnes en France d'après Santé Publique France<sup>3</sup>.

##### **1.1.2 Le diabète de type 1**

Le diabète de type 1 (DT1) représente 10% des formes de diabète. Il s'agit d'une maladie auto-immune résultant de l'infiltration de macrophages et lymphocytes au sein des îlots pancréatiques, favorisant une inflammation et aboutissant ainsi à la destruction des cellules bêta pancréatiques. La destruction des cellules bêta induit une insulino-pénie entraînant ainsi une hyperglycémie lorsqu'environ 80-90% des cellules bêta sont détruites<sup>4</sup>. Le DT1 est mortel en l'absence de traitement.

## 1.2 Le traitement du diabète de type 1

Depuis la découverte de l'insuline en 1921 par Frederick Banting et Charles Best <sup>5</sup>, le DT1 est passé du stade de maladie mortelle au stade de maladie chronique. Dès 1923, les laboratoires débutent la production d'insuline à partir d'extraits de pancréas porcine ou bovine avec une faible pureté. Ce fut dans les années 80 qu'arriva sur le marché, la première insuline humaine synthétique. Depuis, l'arsenal thérapeutique des insulines n'a cessé de s'élargir. Le traitement du DT1 est désormais basé sur l'administration sous-cutanée pluriquotidienne d'insuline soit de manière discontinue (stylos à insuline) ou soit de manière continue (pompe à insuline). Avec l'évolution des technologies, sont récemment arrivés dans l'arsenal thérapeutique, les dispositifs de boucles semi-fermée<sup>6</sup> (et très prochainement les boucles fermées<sup>7</sup>) qui permettent une administration semi-automatique d'insuline par la pompe à insuline en fonction des données issues de capteur de glucose.

Malgré ces nouvelles opportunités de prise en charge, un traitement insulinaire optimal et une éducation thérapeutique bien conduite, un bon nombre de patients ne parviennent pas à obtenir un contrôle glycémique optimal et restent exposés au risque de complications du diabète.

### **1.3 Les complications du diabète**

#### **1.3.1 Les complications chroniques**

Malgré un traitement insulinique intensif les patients avec un DT1 peuvent conserver un équilibre glycémique non optimal les exposant à un risque de complications micro-angiopathiques (néphropathie, rétinopathie, neuropathie) et macro-angiopathiques (évènements cardiovasculaires, ulcères, amputations).

Dans les différentes études de suivi de cohorte de DT1<sup>8-12</sup>, il est rapporté différentes prévalences pour ces complications :

- Néphropathie : 14% à 33%
- Rétinopathie : 36% à 56%
  - o Panphotocoagulation dans 27% des cas
  - o Cécité 7%
- Neuropathie : 32% à 57%
- Evènements cardiovasculaires : 8 à 16%
- Dyslipidémie : 48% à 57%
- Hypertension artérielle : 42% à 46%
- Ulcères : 4%
- Amputation : 3%

#### **1.3.2 Les complications métaboliques aiguës**

Au sein des complications aiguës, on peut distinguer des situations liées à l'hyperglycémie et des situations liées à l'hypoglycémie.

En absence d'insuline, le glucose ne peut pas être stocké, ce qui induit une hyperglycémie. Une autre source d'énergie doit donc être trouvée : les acides gras libres. Leur métabolisme au niveau du foie engendre la production de corps

cétoniques, on parle alors de décompensation acido-cétosique du diabète. Cette crise acido-cétosique se caractérise par : hyperglycémie majeure, cétonémie, cétonurie, glycosurie, déshydratation, nausées, polyurie, confusion, coma et finalement la mort. Associé à cette crise acido-cétosique on peut également retrouver un syndrome cardinal (polyuro-polydipsie, perte de poids malgré polyphagie)<sup>13</sup>.

Les patients diabétiques sont à risque d'être exposés à des épisodes hypoglycémiques. Ceux-ci se manifestent par différents symptômes : transpiration, fatigue, tremblements et vertiges. La plupart des hypoglycémies peu sévères peuvent être corrigées par une prise orale de sucre rapide. Mais en cas d'hypoglycémie sévère et prolongée, des convulsions peuvent survenir et la situation peut évoluer jusqu'au coma et au décès du patient<sup>13</sup>. Ces hypoglycémies sévères font parties des critères définissant un diabète instable associé à une très grande variabilité glycémique et des conséquences sur la qualité de vie de ces patients<sup>14</sup>.

#### **1.4 Transplantation de pancréas et greffe d'îlots**

Pour ces patients particuliers, porteurs d'un diabète instable, une transplantation de pancréas organe ou une greffe d'îlots peut être proposée.

##### **1.4.1 La transplantation de pancréas organe**

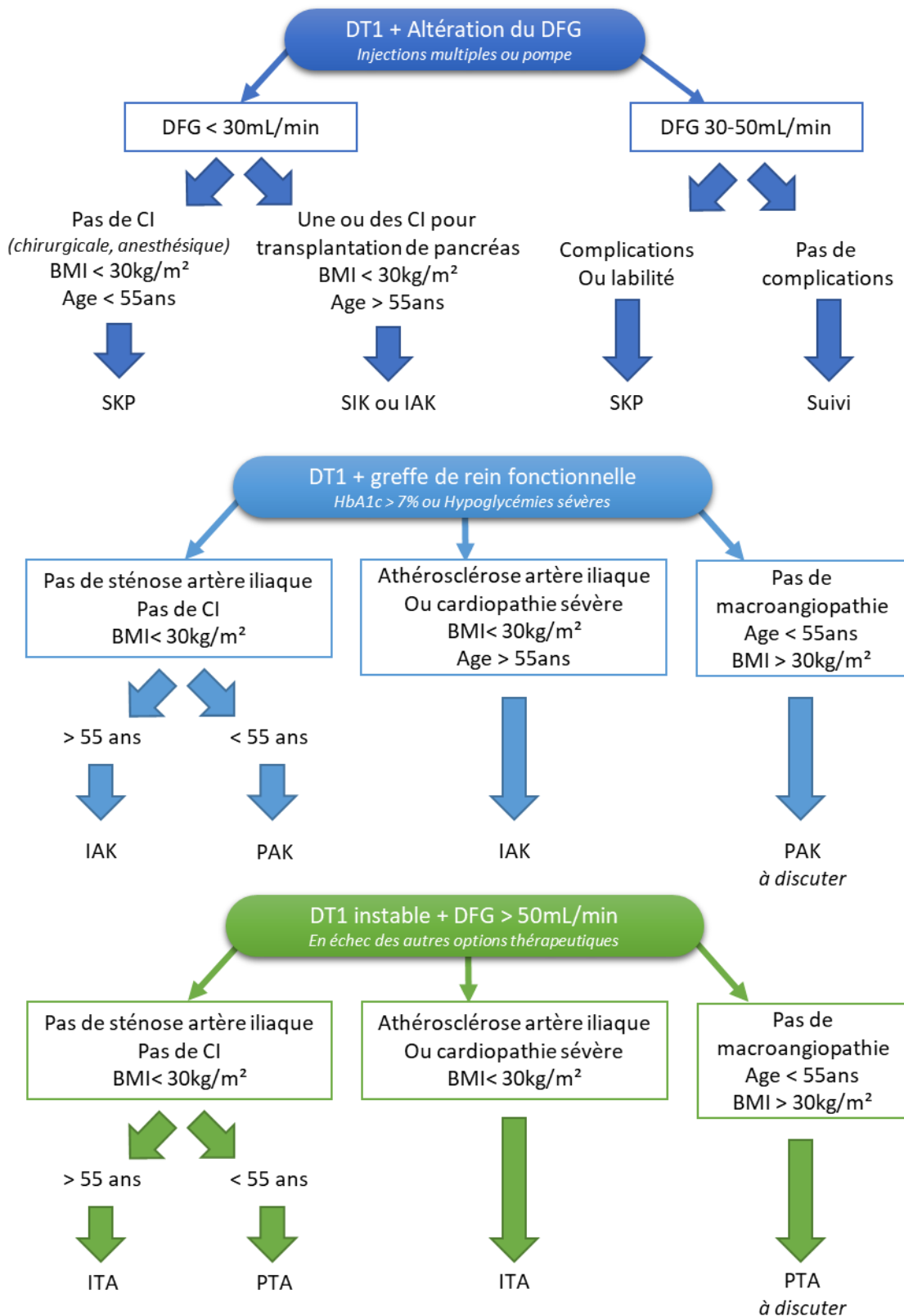
La première transplantation de pancréas a été réalisée en 1966 à Minneapolis<sup>15</sup>. Depuis, de nombreux progrès ont été réalisés sur la technique de transplantation et d'immunosuppression. Désormais, il est possible d'obtenir une insulino-indépendance à 5 ans dans 70% des cas<sup>16</sup>. Néanmoins, si la transplantation de pancréas organe permet l'obtention d'un taux d'insulino-indépendance élevé et durable, il n'en demeure pas moins qu'elle reste une opération chirurgicale majeure avec des risques péri- et

post-opératoires (hémorragie, thrombose de l'artère du greffon, rejet aigu, fistule, pancréatite) associés à une morbi-mortalité importante<sup>17,18</sup>. Cette transplantation ne peut donc pas être proposée à tous les patients, notamment aux patients les plus âgés et compliqués sur le plan cardio-vasculaire. Dans ce contexte, l'alternative peut être la greffe d'îlots de Langerhans.

#### **1.4.2 La greffe d'îlots pancréatiques**

Il s'agit d'une technique mini-invasive (procédure de radiologie interventionnelle ou mini-laparotomie chirurgicale) et présentant des risques modérés (hémorragies, thromboses, altération des enzymes hépatiques, hypertension portale) dans 3 à 10% des cas<sup>19-22</sup>.

Le groupe TREPID (Recommandation Et Promotion de la Transplantation de Pancréas et greffe d'îlots) a récemment publié leurs recommandations pour la transplantation de pancréas et la greffe d'îlots (associé ou non à une greffe de rein)<sup>23</sup> (Figure 1).



**Figure 1.** Arbre décisionnel pour l'orientation vers la transplantation pancréatique ou la greffe d'îlots. D'après A.Wojtuszczyński et al.<sup>23</sup>

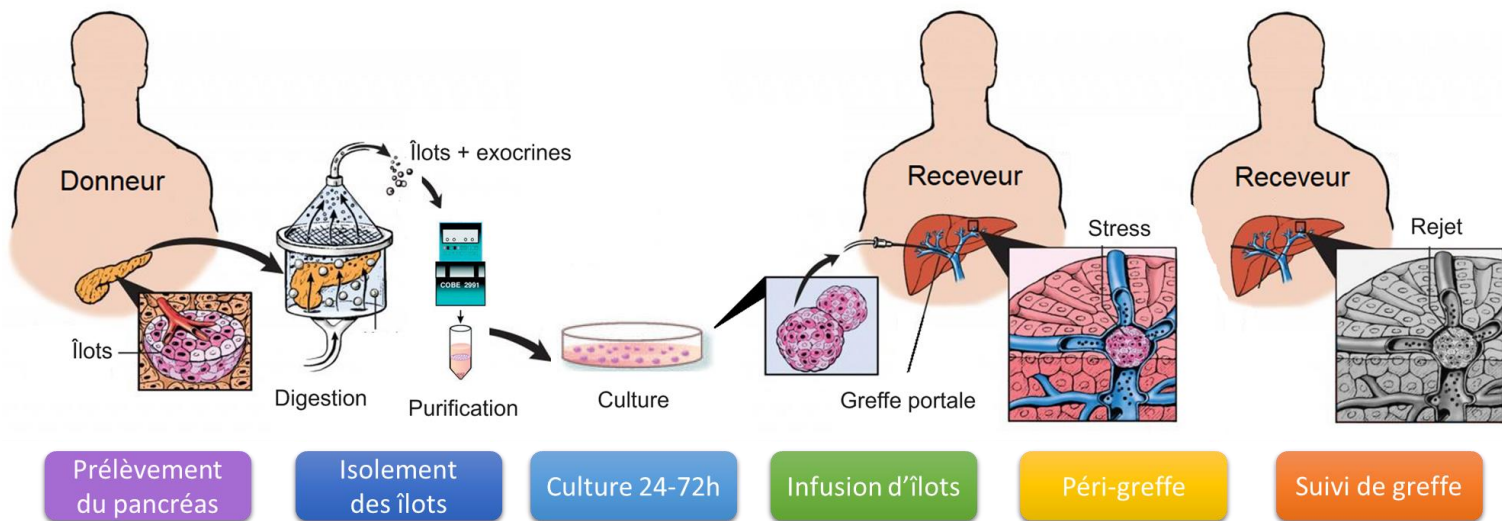
CI: Contre-indication, DFG : Débit de filtration glomérulaire, IAK : Islet after kidney transplantation, ITA : Islet transplantation alone, PAK : Pancreas after kidney transplantation, PTA : Pancreas transplantation alone, SIK : Simultaneous islet kidney transplantation, SKP : Simultaneous kidney pancreas transplantation



### 1.4.3 Isolement des îlots pancréatiques

Le processus d'isolement d'îlots regroupe plusieurs étapes complexes (Figure 2) :

- Acceptation, réception et préparation du pancréas provenant d'un donneur en état de mort encéphalique ou à cœur arrêté. Une fois le pancréas réséqué, il est acheminé en condition réfrigérée dans un centre d'isolement
- Digestion du pancréas : l'enzyme de digestion (collagénase) est perfusée de manière manuelle ou semi-automatique à 4°C. Ensuite, le pancréas est coupé en une dizaine de morceaux qui sont placés dans une chambre de digestion à 37°C avec des billes métalliques afin de subir une digestion enzymatique et mécanique (chambre de Ricordi)<sup>24</sup>
- Purification des îlots : la séparation du digestat exocrine des îlots est réalisée grâce à la densité différente de ces deux types de tissu. La séparation est faite par centrifugation dans un gradient de densité de sucre type Ficoll. Ensuite, la pureté de la préparation et le nombre d'îlots sont quantifiés
- Mise en culture / conservation des îlots : cette étape peut durer de 24h à 72h, ce qui laisse le temps nécessaire à l'organisation de la greffe et l'induction immunosuppressive du receveur en cas de première injection d'îlots
- Conditionnement des îlots et greffe : le jour de la greffe, les îlots sont placés dans une poche de transfert et leur greffe est réalisée au niveau de la veine porte par méthode chirurgicale mini-invasive ou en radiologie interventionnelle.



**Figure 2.** Méthode d'isolement des îlots humains pancréatiques  
D'après Diabetes Research Institute, Université de Miami, Robert Margulies, 2005

#### 1.4.4 Résultats clinico-biologiques de la greffe d'îlots et les évolutions

La première greffe d'îlots humains a été effectuée à Minneapolis en 1974<sup>25</sup>. Il fallut attendre les années 2000 pour avoir une première série de 7 patients insulino-indépendants à 1 an de leur greffe grâce à un nouveau protocole dit d'Edmonton<sup>26</sup>. Les principaux changements menés dans ce protocole par rapport aux précédents sont : une immunosuppression sans glucocorticoïde, l'ajout d'anti-inflammatoire (anti-TNF $\alpha$ ), la greffe d'une masse plus importante d'îlots à partir de plusieurs donneurs et la mise en place d'un procédé standardisé d'isolement. A ce jour, avec les améliorations entourant la technique d'isolement d'îlots (sélection du donneur et de l'organe<sup>27,28</sup>, les solutions de préservation d'organes<sup>29,30</sup>, les enzymes de digestion<sup>31,32</sup>, la technique de purification<sup>33,34</sup> et la culture des îlots<sup>35-37</sup>) et la prise en charge péri-implantatoire avec notamment la suppression des corticoïdes et l'adaptation des protocoles d'immunosuppression<sup>26</sup>, le taux d'insulino-indépendance à 5 ans peut atteindre jusqu'à 50%<sup>38,39</sup>. De plus, 73,9% des patients greffés d'îlots n'ont plus expérimenté d'hypoglycémie sévères à 10 ans<sup>40</sup>, 49 à 77% des patients ont une

HbA1c inférieure à 7% à 8 ans de leur greffe<sup>41</sup>, un bénéfice est également noté sur l'amélioration de la qualité de vie des patients après transplantation<sup>19</sup>.

En France, l'induction immunosuppressive correspond actuellement à un traitement par anticorps anti-thymocyte pour la première transplantation et anti-IL2 (basiliximab) pour les injections suivantes. L'immunosuppression de maintien repose en France actuellement sur l'association d'anti-calcineurine (tacrolimus, 9-12 ng/mL sur 3 mois, puis 6-9 ng/mL) associé à du mycophénolate mofétail (2g/j). Un protocole alternatif est l'utilisation et de forte dose d'inhibiteur de mTOR (sirolimus, 12-15 ng/mL sur 3 mois, 7-10 ng/mL la première année puis 5-6 ng/mL) associé à de faible dose de tacrolimus (3-6 ng/mL) sans corticoïdes (protocole dit EDMONTON)<sup>42</sup>.

#### **1.4.5 Les limites et enjeux**

Tout d'abord, il a récemment été démontré au cours d'une analyse de risques<sup>43</sup> que le processus d'isolement d'îlots pancréatiques humains regroupe de nombreux risques (contaminations bactériennes ou exocrines, altération de la viabilité des îlots, faible nombre d'îlots après isolement) et le taux de réussite des isolements est variable selon les centres (20 à 60%)<sup>44</sup> témoignant de la nécessité d'acquisition d'une réelle expertise. De plus, une des contraintes majeures de la greffe d'îlots est liée à la disponibilité des pancréas humains. En effet, ces organes proviennent de donneurs décédés qui doivent répondre à différents critères (âge, temps d'ischémie des organes, valeurs biologiques), ce qui rend leur disponibilité limitée<sup>28</sup> d'autant que la technique de greffe d'îlots est consommatrice en organe (2 à 3 pancréas pour greffer un donneur).

Par ailleurs, après l'isolement, les îlots perdent leur vascularisation. Une fois les îlots infusés au niveau portal et embolisés au niveau des capillaires sinusoides hépatiques,

leur revascularisation peut prendre plusieurs semaines<sup>45</sup> exposant les îlots pendant cette période à des phénomènes d'hypoxie et de carence en substrats énergétiques<sup>46</sup>. De plus, l'embolisation des îlots dans de multiples zones du foie rend les biopsies peu informatives et donc le diagnostic de rejet cellulaire incertain. Les îlots isolés, en plus de leur absence de vascularisation, sont déconnectés de leur matrice extracellulaire (MEC). La vascularisation et la MEC étant essentielles à la fonction et la survie<sup>47</sup> des îlots, leur absence induit la perte de nombreux îlots.

Enfin, le foie est actuellement le site préférentiel de greffe. Cet organe représente un environnement pro-inflammatoire propice à une réaction inflammatoire et pro-coagulante précoce (*IBMIR : instant blood mediated inflammatory reaction*) aboutissant à la destruction de 50 à 70% des îlots en post-greffe immédiat<sup>48-50</sup>. En plus de ce phénomène inflammatoire, les îlots peuvent également être la cible de la réponse immunitaire à l'origine de rejet allogénique ou d'une récurrence de l'auto-immunité<sup>51</sup>.

### **1.5 Pistes de recherche et viabilité du greffon**

Alors que plusieurs axes de recherches visent à trouver une source alternative d'îlots (xénotransplantation, cellules souches, *induced-pluripotent stem cells*, organoïdes) l'enjeu lié à la perte importante du nombre d'îlots au moment de la greffe et à la perte de fonction du greffon à court, moyen et long terme demeure. C'est ainsi que de multiples recherches sont menées visant à préserver la viabilité et fonctionnalité du greffon, et à diminuer la réponse inflammatoire (IBMIR) à l'origine d'une perte importante des îlots.

### 1.5.1 Immunosuppression de maintien

Une des limites est liée à l'iatrogénie du tacrolimus au niveau rénal et son potentiel diabétogène. C'est ainsi que d'autres traitements pour maintenir l'immunosuppression sont à l'étude :

- Alefacept : Blocage des lymphocytes T mémoires, peu d'effet dans le DT1, pas de recherche actuellement autour de la greffe d'îlots<sup>52</sup>
- Belatacept : (anti-CTLA4-Ig = inhibition de co-stimulation<sup>53</sup>) utilisé principalement en transplantation rénale a montré : une absence de toxicité rénale, une diminution des anticorps anti-HLA spécifiques du donneur, pas d'effets indésirables métaboliques (hypertension, dyslipidémie, hyperuricémie, diabète) et une absence de toxicité neurologique<sup>54,55</sup>. De plus, chez les patients transplantés rénaux avec un diabète préexistant ou cortico-induit, un switch des anti-calcineurines vers le belatacept a montré une amélioration de l'HbA1C<sup>56</sup>. Il convient de rester prudent quant au risque augmenté de rejet aigu dans la première année de traitement par belatacept, au risque plus important de lymphoprolifération EBV (*Epstein-Barr virus*) induite et de réplication du cytomégalovirus<sup>55</sup>.

### 1.5.2 L'encapsulation

L'enjeu est de diminuer le recours aux immunosuppresseurs tout en favorisant la viabilité des îlots en diminuant la durée d'hypoxie, en apportant des nutriments/O<sub>2</sub> pour limiter la carence en substrats et améliorer la revascularisation précoce des îlots.

#### ➤ Micro-encapsulation

La micro-encapsulation consiste à enrober de manière individuelle les îlots dans une capsule biocompatible de composition variable : alginate, polyéthylène glycol, agarose, cellulose<sup>57</sup>. L'objectif de cette approche est d'appliquer une barrière physique entre les îlots et le système immunitaire tout en reconstituant un microenvironnement favorable à leur survie et fonctionnement avec pour objectif final l'arrêt du recours aux immunosuppresseurs et l'amélioration de la survie du greffon à court/moyen et long terme. L'enjeu majeur de cette technique repose sur la capacité de survie des îlots au sein des microcapsules. Les capsules ont donc été progressivement enrichies avec différents éléments en vue d'augmenter l'efficacité de cette technique :

- Les relargueurs d'oxygène<sup>58-60</sup>
- Les nanoparticules ou peptides pouvant relarguer différents facteurs de contrôles (TGF- $\beta$  anti-inflammatoire, LIF (leukemia inhibitory factor) pour favoriser la différenciation des lymphocytes T en LTreg, rapamycine anti-inflammatoire)<sup>61</sup>
- Les cellules endothéliales progénitrices ou des cellules souches mésenchymateuses pouvant être incluses dans la composition de la capsule, ou dans la composition des îlots afin de promouvoir la vascularisation au pourtour de la capsule et induire une immuno-modulation<sup>62</sup>

➤ Macro-encapsulation

Les dispositifs de macro-encapsulation sont conçus pour immuno-isoler les îlots et leur procurer un microenvironnement favorable. Contrairement à la technique précédente, l'ensemble des îlots sont présents au sein d'un même dispositif de type macro-chambre.

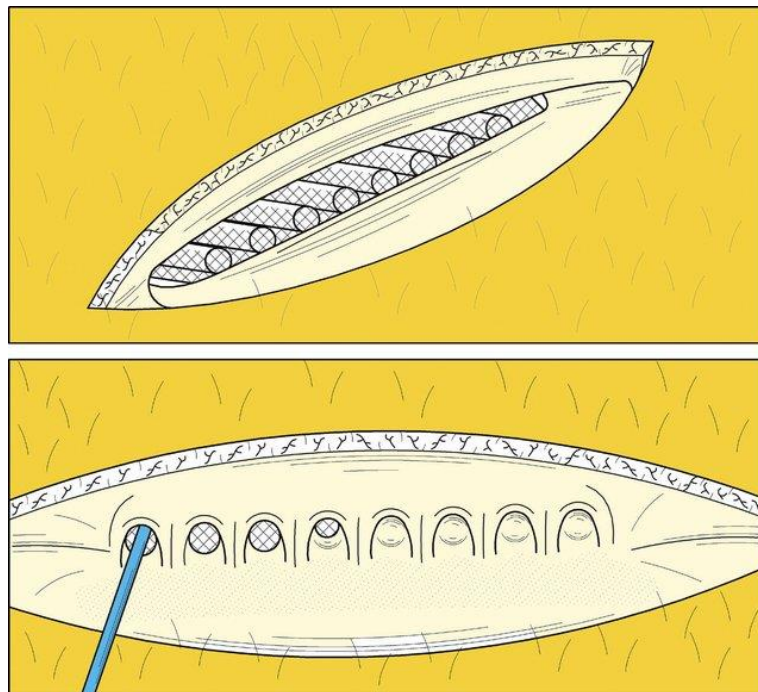
Le dispositif de la compagnie Beta-O<sub>2</sub> Technologies, appelé *β-air bio-artificial pancreas* (Figure 3) en essais de phase I/II (NCT02064309) regroupe trois technologies : l'utilisation d'îlots humains pancréatiques encapsulés dans deux types d'alginate, puis un macro-dispositif en Teflon recouvert d'alginate avec un réservoir sous-cutané approvisionné en oxygène et permettant l'oxygénation du dispositif <sup>63</sup>.



**Figure 3.** Dispositif  $\beta$ -air bio-artificial pancreas

Dans l'essai clinique de preuve de concept<sup>64</sup>, 4 patients ont reçu 1 à 2 dispositifs et ont été suivi sur 3 à 6 mois. L'implantation du dispositif était sûre et les îlots survivaient dans le dispositif. Cependant le niveau de C-peptide était très faible et sans impact sur le contrôle métabolique des patients. Cela était attribué à la présence d'un tissu fibrotique riche en cellules immunitaires autour de la capsule et une formation de tissu amyloïde dans le tissu endocrinien.

La société Sernova a développé le Cell Pouch System<sup>TM</sup> (Figure 4) actuellement en essai de phase I/II (NCT03513939) qui regroupe 2 technologies : un dispositif à base de polymère et mimant la MEC afin de favoriser un espace de vascularisation dans lequel sera injecté la préparation cellulaire Sertolin<sup>TM</sup> (coculture d'îlots humains pancréatiques et de cellules de Sertoli)<sup>57</sup>.



**Figure 4.** Illustration du Cell Pouch System<sup>TM</sup> et de l'injection d'îlots<sup>65</sup>

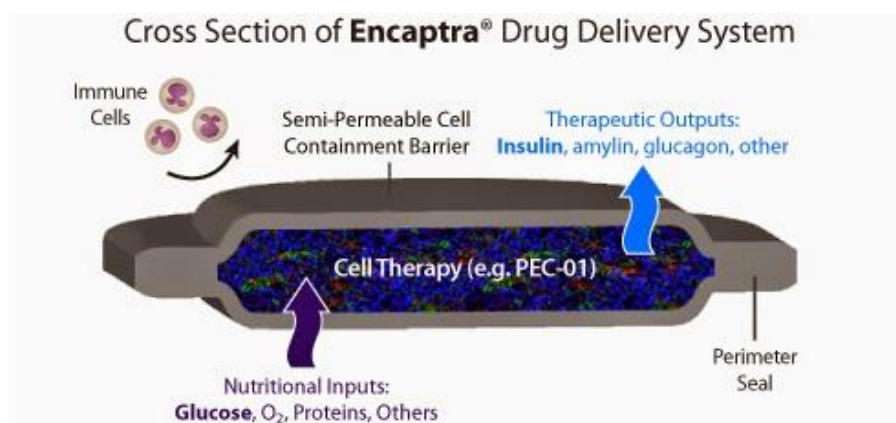


Dans leur communication la plus récente au congrès de l'ADA (American Diabetes Association) le 24 juin 2023 à San Diego, le laboratoire a rapporté les résultats des patients traités dans l'étude de phase I/II par le Cell Pouch System™.

Avec le 1<sup>er</sup> dispositif à 8 canaux, 5 des 6 patients ont atteint l'insulino-indépendance durant 6 à 38 mois. Avec le 2<sup>ème</sup> dispositif à 10 canaux (capable de recevoir 50% d'îlots en plus), 5 patients sont déjà enrôlés et 3 ont reçu des îlots. L'utilisation d'immunosuppresseurs reste nécessaire avec cette technologie, le bénéfice qui était attendue portait donc sur la prise de greffe et la promotion d'une survie à plus long terme.

Le 1<sup>er</sup> médicament de thérapie innovante de la compagnie Vertex (initialement ViaCyte), PEC-Encap™ (ou VC-01™) était en étude de phase I/II (NCT02239354 et NCT03163511), il regroupe trois technologies :

- La différenciation de cellules souches embryonnaires humaines en progéniteurs pancréatiques (PEC-01)
- Une micro-encapsulation dans un hydrogel
- Une macro-encapsulation dans le dispositif Encaptra™<sup>63</sup> (Figure 5).



**Figure 5.** Illustration du dispositif PEC-Encap™ proposé par Vertex

L'essai pilote a montré la performance du dispositif en terme d'immuno-isolation (absence d'immunisation des patients). Néanmoins, aucune sécrétion de C-peptide n'a été détectée et le contenu cellulaire présentait une nécrose centrale majeure et malheureusement l'ensemble de ces cellules étaient mortes.

Le 2<sup>ème</sup> médicament investigué par Vertex est le VX-880 (NCT04786262). Ce médicament de thérapie innovante consiste en l'administration de cellules souches allogéniques complètement différenciées en cellules productrices d'insuline. L'objectif de ce médicament est de proposer une alternative à la greffe d'îlots allogéniques (leur disponibilité étant réduite). Cependant, l'utilisation d'immunosuppresseurs est toujours nécessaire avec ce médicament. Dans leur communication la plus récente au congrès de l'ADA le 23 juin 2023 à San Diego, le laboratoire a rapporté les résultats des 6 patients traités dans l'étude de phase I/II par le médicament. Les 6 patients ont montré après traitement :

- Une sécrétion d'insuline endogène (avec un c-peptide positif)
- Une amélioration de l'HbA1c
- Une insulino-indépendance
- Une amélioration du temps passé dans les cibles glycémiques

Dans la première partie de l'étude, 2 patients ont reçu plusieurs injections jusqu'à atteindre la dose cible. Ils ont été suivis pour une durée de 12 mois sans survenue d'hypoglycémie sévère, une insulino-indépendance maintenue avec une diminution de 1,6 et 3,3% d'HbA1c avec plus de 95% du temps passé dans les cibles. Dans une deuxième partie, 3 patients ont reçu l'intégralité de la dose de médicament en une fois. Ils ont été suivis entre 29 et 90 jours et montrent une sécrétion d'insuline endogène, une réduction de l'HbA1c (pas de données chiffrées), une amélioration du temps passé

dans les cibles (pas de données chiffrées) et une réduction des doses d'insuline administrées quotidiennement. D'un point de vue sécurité, aucun effet indésirable majeur n'a été rapporté, il s'agissait essentiellement de déshydratations, diarrhées, hypomagnésémie et éruption cutanée. Aucun signe quant à un potentiel d'oncogénération n'a été détecté à ce stade.

Le 3<sup>ème</sup> médicament de thérapie innovante en essais clinique (NCT05791201) est le VX-264. Il regroupe les technologies du VX-880 mais avec une encapsulation dans un dispositif à canaux pour protéger les cellules du système immunitaire et donc l'affranchir de l'utilisation d'immunosuppresseurs.

## 2. La lumière au service de la clinico-biologie

### 2.1 Historique et photothérapie

La lumière du soleil fut la première utilisée il y a plus de 3000 ans dans l'Égypte antique, dans la médecine traditionnelle indienne (Ayurveda) et chinoise pour son rôle sur la régulation du cycle circadien et dans de nombreuses autres pathologies notamment cutanées. Ce n'est qu'à partir du 19<sup>ème</sup> siècle avec l'invention de l'ampoule électrique en 1879 par Joseph Swann que d'autres sources de lumière furent utilisées. L'intérêt autour de la lumière est matérialisé par plus de 20.000 publications à visée médicale (base MEDLINE) depuis le début du 21<sup>ème</sup> siècle. Ainsi, la photothérapie a émergé dans différentes utilisations médicales :

- L'héliothérapie : utilisation de la lumière du soleil ou d'une lumière artificielle polychromatique pour les fluctuations de moral saisonnières<sup>66</sup> et des pathologies cutanées<sup>67</sup>
- La thérapie par lumière UVA ou UVB pour le traitement du lupus qui a conduit le Dr Niels Ruberg à remporter un prix NOBEL, les affections cutanées<sup>68</sup>, la jaunisse néonatale<sup>69</sup>
- La thérapie photodynamique : activation de cellules, particules ou substances aboutissant à la libération de principes actifs ; particulièrement appliqué en cancérologie<sup>70</sup>
- La photophorèse extracorporelle : pour les lymphomes T cutanés<sup>71</sup>, la maladie du greffon contre l'hôte<sup>72</sup> et dans la prise en charge du rejet chronique en transplantation pulmonaire<sup>73</sup>. Les cellules du patient sont soumises à un rayonnement UV qui active différentes cellules de l'immunité
- La thérapie par laser : photocoagulation dans la rétinopathie diabétique<sup>74</sup> ou la photovaporisation pour l'ablation de prostate<sup>75</sup> par exemple.

## 2.2 Principes physiques de la photobiomodulation

### 2.2.1 Définition

La photobiomodulation (PBM) appelée initialement thérapie par laser de faible énergie (*Low level laser therapy* (LLLT)) correspond à l'absorption sélective de longueurs d'ondes donnée par des chromophores endogènes induisant des modifications biologiques.

Historiquement, la PBM a été décrite pour la première fois par Endre Mester en 1968 qui observa une repousse de poils plus rapide chez des rongeurs exposés à un laser de basse énergie de longueur d'onde de 694 nm. Dans les mêmes années, elle fut également développée par la *National Aeronautics and Spatial Administration* (NASA) pour accélérer la cicatrisation et la régénération des cellules musculaires des astronautes. Depuis ces dernières décennies, la recherche biomédicale relative à la PBM est en augmentation constante indiquant un intérêt grandissant concernant son potentiel thérapeutique. Ces trois dernières années (2020-2022), environ 700 articles ont été publiés annuellement et référencés dans MEDLINE.

## 2.2.2 Principes physiques de la PBM

De nombreux paramètres dosimétriques doivent être pris en compte :

- La source de lumière
- La longueur d'onde ( $\lambda$  en nm)
- La durée d'exposition
- Le nombre d'exposition et leur fréquence
- La densité de puissance (« *irradiance* » W/cm<sup>2</sup>)
- La densité d'énergie (« *fluence* » J/cm<sup>2</sup>)
- La méthode d'illumination (continue ou pulsée)
- Les caractéristiques des matériaux (absorbance) et tissus (présence de chromophores)
- Le moment de la journée où est appliqué la thérapie

## 2.2.3 La source de lumière

Initialement, ce sont les lasers qui ont été utilisés (d'où l'appellation initiale de LLLT).

En effet, les lasers ont été inventés en 1960 par Théodore Maiman<sup>76</sup> alors que les diodes électroluminescentes (LED) l'ont été en 1962 par Nick Holonyak.

Depuis lors, les deux sources sont utilisées avec leurs avantages et limites respectives<sup>77</sup> :

- Laser (essentiellement avant le 21<sup>ème</sup> siècle) :
  - Emet une lumière cohérente qui stimulerait mieux les mitochondries<sup>78</sup>, même si la cohérence n'est pas nécessaire pour la lumière rouge pour obtenir une action biologique<sup>79,80</sup>
  - Pénètre plus profondément dans les tissus
  - Longueur d'onde très sélective
  - Toutes les longueurs d'ondes ne sont pas disponibles
  - Le système consomme beaucoup d'énergie et chauffe
- LED :
  - Possibilité d'avoir un dispositif planaire irradiant de grande surface
  - Dégage très peu de chaleur
  - Plus sûr que l'utilisation des lasers
  - Moins onéreux que l'utilisation des lasers
  - Pouvoir irradiant plus fiable et durée de vie plus courte que les lasers
  - Longueur d'onde moins spécifique (spectre plus large)

#### 2.2.4 La longueur d'onde

En PBM, on retrouve deux plages principales de longueur d'onde utilisées<sup>81,82</sup> :

- Dans le rouge : entre 600 et 700nm, à préférer pour les pathologies superficielles.
- Dans le proche infra-rouge : entre 780 et 1100 nm, à préférer pour les pathologies avec atteinte plus profonde.

La plage de 700 à 780nm est décrite comme non efficace car ne correspondant pas au spectre d'absorption du chromophore biologique qu'est la Cytochrome C Oxydase (CCO), cible principale de la PBM qui sera détaillée ultérieurement.

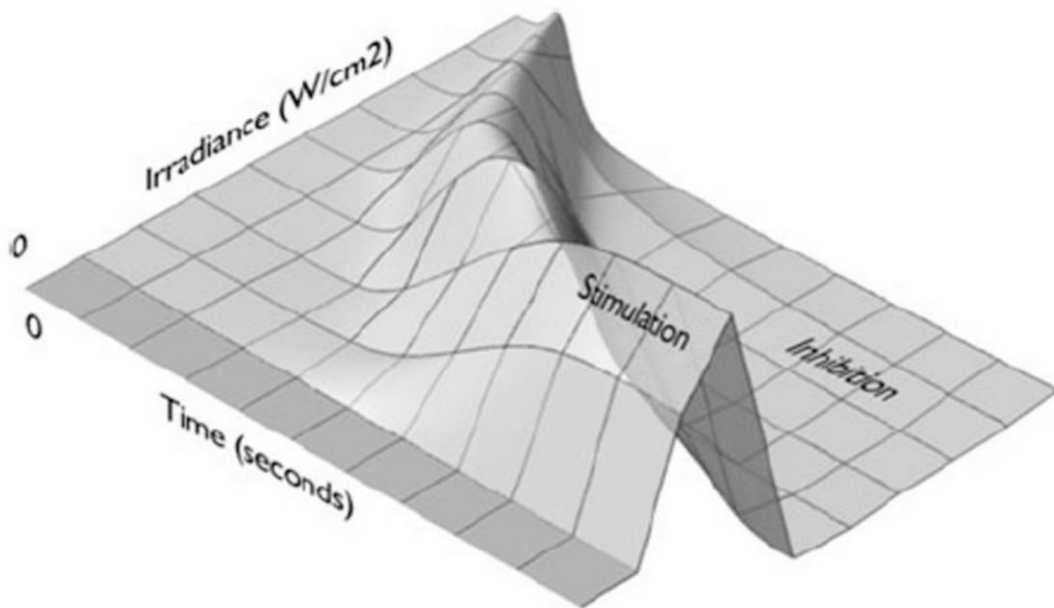
#### 2.2.5 Les paramètres d'exposition, de puissance et d'énergie

Les paramètres retrouvés dans la littérature sont très hétérogènes et varient selon les modèles utilisés :

- Les densités d'énergie : 0,04 à 50 J/cm<sup>2</sup>
- Les densités de puissance : 0,5 mW à 5 W/cm<sup>2</sup>
- Les temps d'exposition : exposition unique de quelques secondes à des expositions multiples de plusieurs minutes

Le principe de dose-réponse à la PBM serait biphasique (loi d'Ardnt-Schultz)<sup>81</sup> (Figure 6). De trop faibles doses n'auront aucun effet et de trop fortes doses conduiront au même résultat, voir une possible toxicité<sup>83,84</sup>. Cela est notamment lié à un excès de ROS et une suractivation de NFkB<sup>85</sup> à l'origine d'une cytotoxicité utilisée en cancérologie<sup>86</sup>.





**Figure 6.** Illustration de la réponse biphasique de la PBM (loi d'Arndt-Schultz)

Une autre possibilité serait que la PBM réponde à la loi de Roscoe-Bunsen : le paramètre le plus important est la quantité de photons reçue, peu importe la rapidité (exemple : 5 mW/cm<sup>2</sup> sur 100 secondes serait équivalent à 0,5 W/cm<sup>2</sup> sur 1 seconde)<sup>82</sup>. Cependant, plusieurs études ont démontré que cette loi ne s'appliquait pas pour la PBM<sup>87-89</sup> et que la densité d'énergie et la densité de puissance ont toutes deux leur importance.

Actuellement, il n'a pas été mis en évidence de supériorité entre l'illumination pulsée ou continue<sup>82</sup>. En cas d'utilisation de lumière pulsée, il conviendra de tenir compte de différents paramètres tel que sa fréquence (Hz) et sa durée (ms).

### 2.3 Cibles biologiques de la PBM

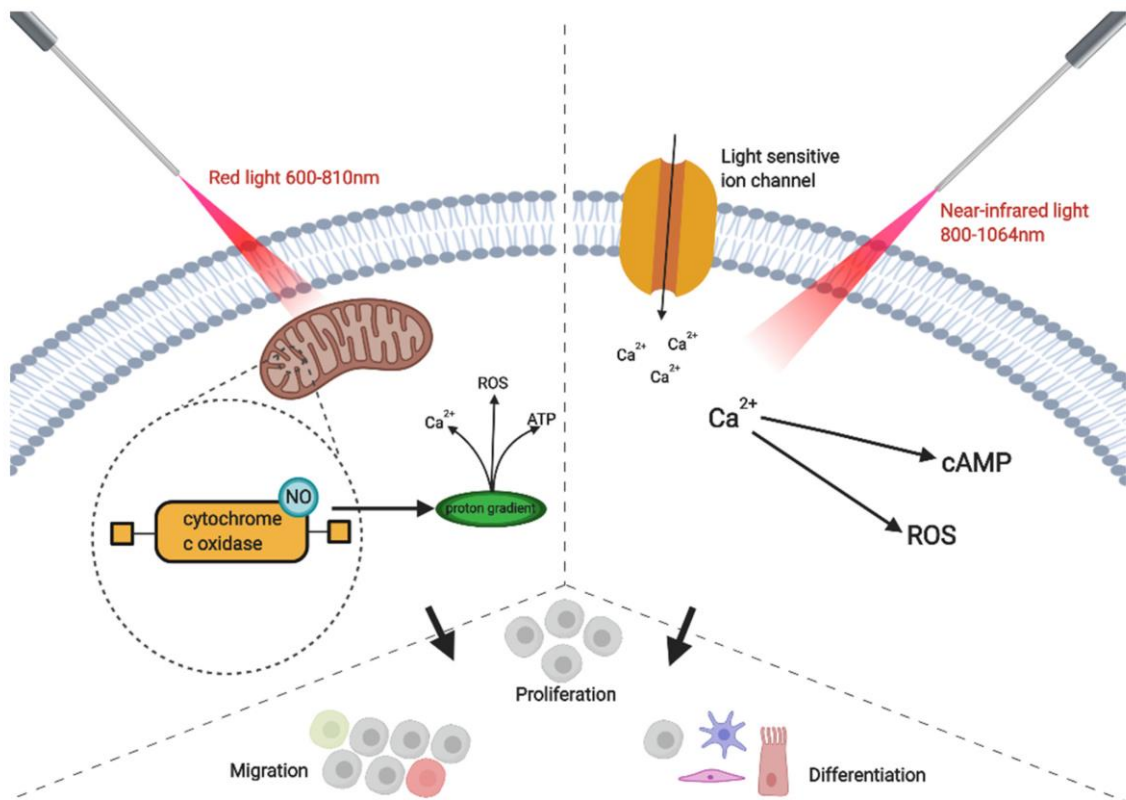
Différentes cibles hypothétiques sont envisagées pour la PBM :

- Réaction photochimique : la mitochondrie contient des chromophores capables d'absorber les photons issus de la lumière rouge et proche infrarouge
- Action directe des photons au niveau de l'ADN et du génome
- Augmentation de l'activité plasmique des membranes et des TRP (*transient receptor potential*)

L'impact de la PBM apparaît d'autant plus important que les cellules concernées ont un nombre important de mitochondries et donc une activité métabolique importante (comme les îlots pancréatiques avec une forte activité de synthèse hormonale)<sup>90</sup>.

L'impact biologique de la PBM concerne : la chaîne respiratoire mitochondriale, l'expression génique, la signalisation cellulaire, la synthèse de facteurs de croissance, la modulation inflammatoire et microbienne<sup>91</sup>. En conséquence, le mécanisme biologique d'action de la PBM (Figure 7) proposé est le suivant :

- Activation de la CCO (augmentation de la biodisponibilité des électrons pour la réduction des molécules d'oxygène)
- Augmentation du potentiel de membrane mitochondriale
- Augmentation de la production d'ATP, d'AMPc, variation de la production de radicaux libres de l'oxygène (ROS = *Reactive oxygen species*), de la concentration d'oxyde nitrique (NO)<sup>81</sup>
- Communication noyau / mitochondrie induisant un changement de l'ultrastructure des mitochondries (équilibre fusion-fission)<sup>92</sup>
- Augmentation du Ca<sup>2+</sup> intracellulaire<sup>81</sup>



**Figure 7.** Illustration du mécanisme d'action de la PBM<sup>93</sup>

## 2.4 La mitochondrie

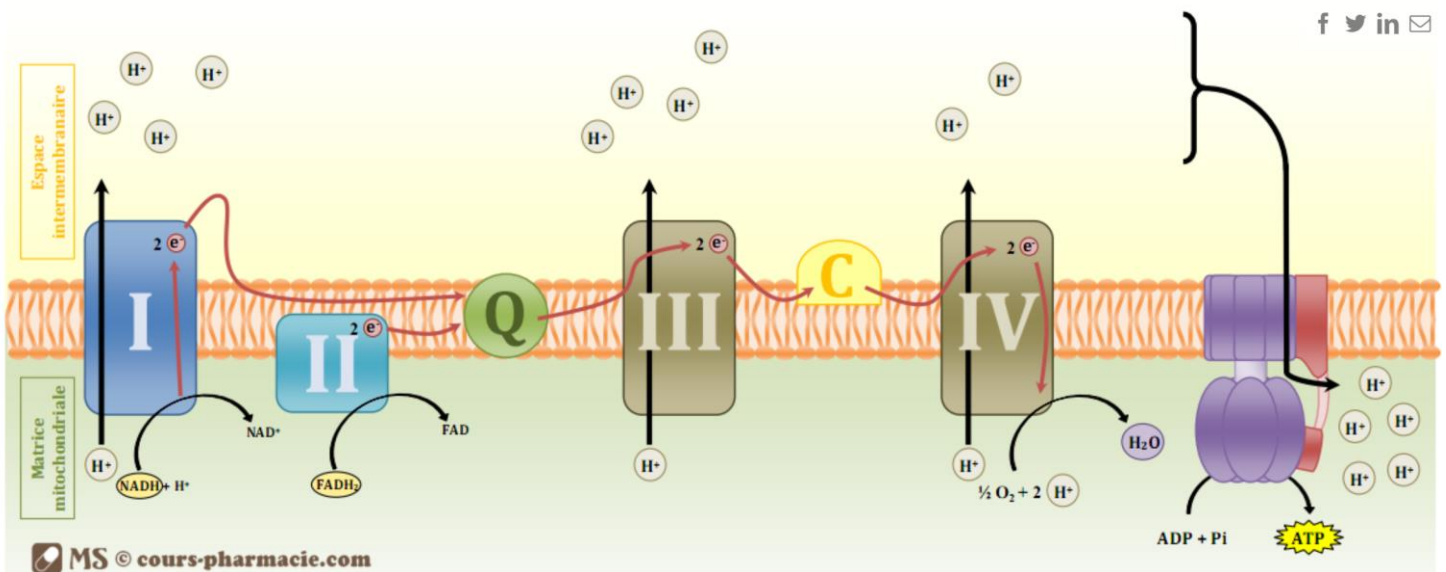
### 2.4.1 Généralité

La mitochondrie est un organe intracellulaire (environ 1  $\mu\text{m}$ ) présent dans le cytoplasme des cellules eucaryotes. Elle est composée de deux membranes (interne et externe) permettant de délimiter un espace intermédiaire. La membrane interne, riche en cardiolipine, est fortement imperméable aux ions et est le lieu de l'oxydation phosphorylante.

Un des rôles de la mitochondrie est de convertir l'oxygène et les nutriments en ATP qui est une source d'énergie majeure pour les cellules.

## 2.4.2 Chaîne respiratoire mitochondriale

La chaîne respiratoire mitochondriale se situe au niveau de la membrane interne de la mitochondrie. Le transfert l'électrons le long de la chaîne respiratoire jusqu'à l'accepteur final ( $O_2$ ) va permettre la création d'un gradient de protons ( $H^+$ ). Elle est composée de 4 complexes, 2 éléments mobiles et l'élément final de la chaîne (complexe V) (Figure 8).



**Figure 8.** Chaîne respiratoire mitochondriale

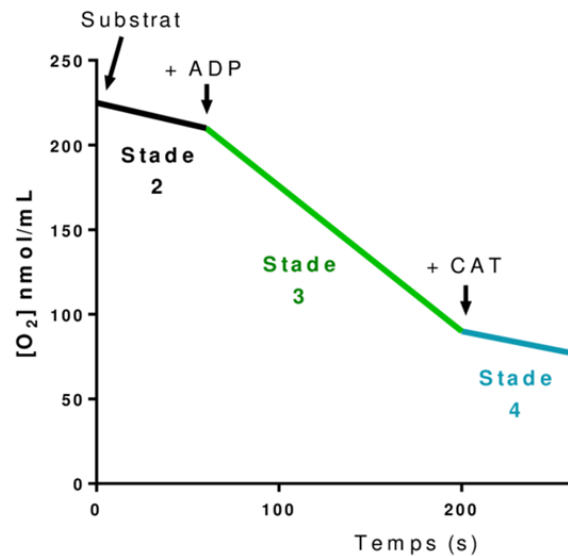
- Complexe I (NADH déshydrogénase) : oxyde le  $NADH, H^+$  en  $NAD^+$  et transfère deux électrons à l'ubiquinone. Cette oxydation est associée à une translocation de 4 protons ( $H^+$ ) vers l'espace intermembranaire. Ce complexe est inhibé par la roténone.
- Complexe II (Succinate déshydrogénase) : oxyde le succinate en fumarate, induisant ainsi la réduction du  $FAD$  en  $FADH_2$ . Le  $FADH_2$  est ensuite oxydé pour permettre le transfert de 2 électrons à l'ubiquinone.

- Élément mobile 1 = Ubiquinone (UQ) : elle reçoit les électrons des complexes I et II et se retrouve réduite en ubiquinol (UQH<sub>2</sub>). Elle diffuse ensuite vers le complexe III.  
Ce complexe est inhibé par l'antimycine A.
- Complexe III (Cytochrome bc1) : oxyde l'ubiquinol en ubiquinone et transfère les électrons au cytochrome C. Cette oxydation est associée à une translocation de 4 protons (H<sup>+</sup>) vers l'espace intermembranaire.  
Ce complexe est inhibé par l'antimycine A.
- Élément mobile 2 = Cytochrome C : il reçoit les électrons du complexe III. Il permet le transfert des électrons vers le complexe IV.
- Complexe IV (Cytochrome C Oxydase) : oxyde le cytochrome C et réduit l'O<sub>2</sub> en H<sub>2</sub>O, cette oxydation est associée à la translocation de 2 protons (H<sup>+</sup>) vers l'espace intermembranaire.  
Ce complexe est inhibé par le cyanure.
- Complexe V (ATP synthase) : par un changement de conformation, l'ATP synthase va permettre l'oxydation phosphorylante de l'ADP en ATP grâce au gradient de protons (H<sup>+</sup>).  
Ce complexe est inhibé par l'oligomycine.

Les complexes I, III et IV jouent le rôle de pompes à protons (H<sup>+</sup>) permettant la réalisation d'un gradient transmembranaire. Ils couplent l'oxydation du NADH, H<sup>+</sup> et FADH<sub>2</sub> à la réalisation d'une différence transmembranaire de la concentration en ions H<sup>+</sup>. Ce gradient de H<sup>+</sup> va définir un potentiel de membrane de la mitochondrie et permettre la synthèse d'ATP.

### 2.4.3 Consommation d'oxygène et mitochondrie

L'oxygraphie consiste à mesurer la quantité d'oxygène d'un échantillon au cours du temps en présence de différents substrats ou inhibiteurs pour étudier l'activité des complexes mitochondriaux ou la respiration cellulaire (Figure 9).



**Figure 9.** Graphique d'oxygraphie

On peut différencier plusieurs stades pour les complexes I et II :

- Stade 2 : Consommation résiduelle d'oxygène en présence des substrats des complexes I (glutamate/malate) ou II (succinate)
- Stade 3 : Consommation d'oxygène en condition de phosphorylation oxydative, les mitochondries sont saturées en ADP, la consommation d'oxygène est couplée à la phosphorylation de l'ADP en ATP grâce à la force protonotrice
- Stade 4 : Consommation d'oxygène non liée à la synthèse d'ATP.

Pour chaque stade il serait alors défini la consommation d'oxygène par unité de temps.

Il est ensuite possible de calculer le RCR (*respiratory control ratio*) qui correspond à la qualité du couplage de la mitochondrie. Plus le RCR est haut, moins les pertes sont

importantes (plus le stade 4 est bas). Autrement dit, plus le RCR est haut, plus la consommation d'oxygène par la mitochondrie est couplée à la production d'ATP.

L'activité du complexe III ne peut pas être réalisée en oxygraphie, il est possible de la réaliser en spectrométrie mais sa mesure reste complexe et peu réalisée.

Pour le complexe IV d'autres substrats sont utilisés :

- Le TMPD permet de donner des électrons à la CCO
- Le DNP dépolairise complètement la mitochondrie, ce qui permet d'obtenir la  $V_{max}$  du complexe et donc d'en mesurer indirectement l'activité

Il est également possible de mesurer la « respiration en routine » des cellules vivantes. Cela correspond à la respiration des cellules en lien avec leur activité physiologique dans des conditions où l'ADP n'est pas excès. La cellule ne peut donc se servir que de ces substrats endogènes.

#### **2.4.4 Radicaux libres et stress oxydant**

La présence de radicaux libre est corrélée au stress oxydant, mais ils sont également générés (en quantité physiologiquement faible) par la chaine respiratoire mitochondriale.

En effet, la réduction de l' $O_2$  par 4 électrons induit la formation d' $H_2O$ . Mais cette réduction s'effectue en plusieurs étapes successives où sont présents des intermédiaires partiellement réduits de l'oxygène. Ces radicaux libres sont capables de causer des dommages oxydatifs aux cellules.

Il existe différentes sources d'espèces réactives de l'oxygènes : NAD(P)H oxydase, respiration mitochondriale, xanthine oxydase et peroxysomes.

Il existe également différentes sources exogènes : radiations, champs électriques, xénobiotiques et cytokines.

Il existe donc des enzymes antioxydantes permettant de limiter l'apparition de ces espèces réactives de l'oxygène (superoxyde dismutase et catalase).

## 2.5 La cytochrome C oxydase

Il a été démontré que la CCO était un chromophore absorbant dans le rouge et le proche infrarouge<sup>94</sup>. Les arguments supportant que la CCO soit le site d'action de la PBM sont :

- Les cellules Rho-zéro (viable sans mitochondrie) ne sont pas affectées par la PBM<sup>86</sup>
- La CCO serait plus affine pour son substrat en présence de la PBM<sup>80</sup>
- Le NO est un inhibiteur de la CCO et est particulièrement présent dans les cellules stressées<sup>95,96</sup>. Dans ce cas, la PBM a induit une photodissociation du NO de la CCO<sup>95</sup> (action indirecte de la PBM)
- Le sodium azide (inhibiteur de la CCO) inhibe les effets de la PBM<sup>97</sup>

Cependant, alors que de très nombreux articles extrapolent les variations observées du potentiel de membrane mitochondriale et de la production d'ATP liés à la PBM comme dépendante de la CCO, une seule étude a démontré l'implication de la CCO dans les effet mitochondriaux de la PBM<sup>94</sup>. Des CCO isolées de cœur de cheval et exposées à un laser 632,8 nm à une puissance de 10 mW/cm<sup>2</sup> sur 200 secondes (2 J/cm<sup>2</sup>) a montré une augmentation de la consommation d'oxygène. Ces résultats n'ont pas été reproduit que ce soit dans des modèles de pré-illumination puis mesure de la consommation d'oxygène ou lors d'une illumination simultanée à la mesure<sup>98-100</sup>.



## 2.6 Les médiateurs et facteurs de transcription

### 2.6.1 ATP

Il a été montré que la PBM augmente la production d'ATP dans différents modèles : cellules HeLa<sup>101</sup>, lymphocytes<sup>102</sup>, cellules hépatiques<sup>103</sup>, neurones<sup>92</sup>, cellules d'adénocarcinomes<sup>104</sup>, et cardiomyocytes<sup>105</sup> notamment. Il a également été montré que la PBM augmente la production d'AMPc dans différents modèles : cellules adipocytaires<sup>106</sup>, inflammation pulmonaire induite par lipopolysaccharide<sup>107</sup>. Mais le lien entre ces deux augmentations n'est à ce jour pas démontré, bien qu'intuitif.

### 2.6.2 ROS

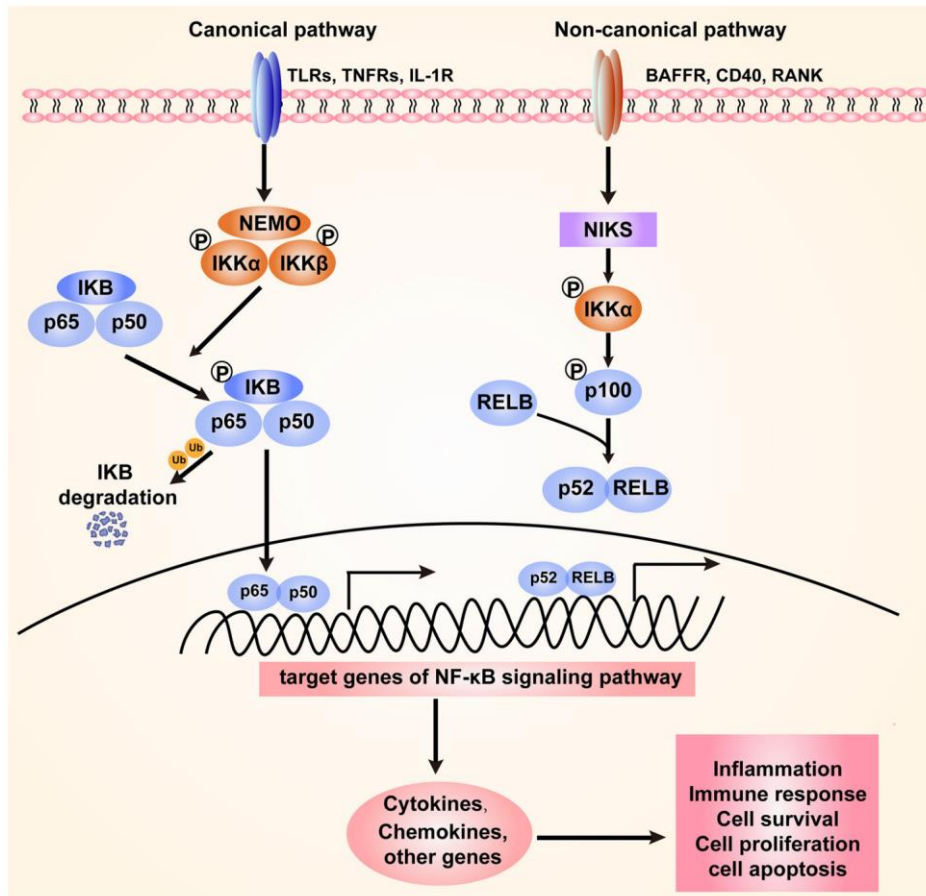
Sur des cellules non stressées, la PBM augmente le potentiel de membrane mitochondriale et induit une augmentation transitoire de la production de ROS<sup>108,109</sup>. A noter que les ROS en faible quantité sont bénéfiques<sup>110</sup>.

Sur des cellules avec un potentiel de membrane mitochondriale altéré par un stress oxydatif, la PBM restaure le potentiel de membrane mitochondriale et diminue la production de ROS<sup>109</sup>.

### 2.6.3 Autres facteurs de transcription

La PBM agit également sur d'autres facteurs de transcription :

- NFκB : la voie NFκB est activée par différents médiateurs (Figure 10) comme le TNFα (*Tumor necrosis factor*), l'IL-1β et les ROS<sup>111</sup>. La PBM active NFκB en lien avec l'augmentation de ROS observée sur des cellules non stressées. En revanche sur des cellules stressées la PBM va diminuer l'activation de la voie NFκB<sup>112</sup>.



**Figure 10.** Voie de signalisation de NFκB<sup>111</sup>

- RUNX-2 : la PBM augmente son activation et participe au remodelage tissulaire<sup>113</sup>
- VEGF et Métalloprotéinase 2 (MMP2) : la PBM augmente leur transcription, concentration et participe à l'angiogénèse<sup>114</sup>

## 2.7 Les effets cellulaires, métaboliques et cliniques de la PBM

Il existe de nombreuses revues sur les effets et mécanismes de la PBM sur des modèles cellulaires, pré-cliniques ou cliniques. Sont rapportés ici principalement les études avec des résultats positifs, mais du fait d'une grande variabilité d'application des paramètres d'illumination, il existe également de nombreuses études avec des bénéfices moins nettes de la PBM.

### 2.7.1 Les effets cellulaires de la PBM

La PBM favorise la régénération cellulaire de par son action sur :

- La prolifération cellulaire (les fibroblastes<sup>115-118</sup> (pouvant être associé à une augmentation du collagène<sup>119,120</sup>), les ostéoblastes<sup>121-123</sup>, les cellules musculaires<sup>122</sup>, les cellules souches adipocytaires<sup>124</sup>, les cellules souches mésenchymateuses<sup>125-127</sup> et les cellules souches cardiaques<sup>126</sup>)
- La différenciation cellulaire (les cellules souches de la moelle osseuse en neurones ou lignées ostéoblastiques<sup>128</sup>, les cellules souches mésenchymateuses en neurones<sup>129</sup> ou lignée ostéoéblastique<sup>130,131</sup>, et les cellules souches adipocytaires en cellules musculaires lisses<sup>132</sup>)
- La migration cellulaire (les ténocytes en cas d'atteinte du tendon d'Achilles<sup>133</sup>, les mélanocytes<sup>134</sup>, les cellules souches épidermique pour favoriser l'épithélialisation en cas de plaie<sup>135</sup> et les cellules neuronales lors du développement du cerveau<sup>136</sup>)
- La viabilité cellulaire (les fibroblastes<sup>118,137</sup>, les cellules souches mésenchymateuses<sup>138</sup>, les cellules souches endothéliales (HUVEC)<sup>139</sup>, les spermatozoïdes<sup>140</sup>, les cellules souches adipocytaires<sup>141</sup> et les substituts cutanés cultivés in vitro<sup>142</sup>).

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L'action de la PBM au niveau inflammatoire est également un point d'attention. Elle induit une inhibition de la prostaglandine E2 associée à une diminution de la phospholipase A2, une diminution de COX1 et COX2, une inhibition de NFkB<sup>143</sup>, une polarisation des macrophages par modification des histones<sup>89</sup>, une augmentation de l'activité antioxydante (superoxyde dismutase et catalase), une augmentation de l'IL-10<sup>144</sup> et une régulation des populations Th1 et Th2<sup>145</sup>.

L'action de la PBM est également rapportée comme étant antalgique. Le mécanisme associé proposé est le suivant<sup>146</sup> : absorption de photons par les mitochondries, vasodilatation (par photodissociation du NO de la CCO), stimulation de la division cellulaire, augmentation du cortisol avec synthèse protéique, augmentation des enzymes antioxydantes et l'implication des récepteurs aux opioïdes périphériques<sup>147</sup>.

## 2.7.2 La PBM en pré-clinique

### 2.7.2.1 Régénération cellulaire et cicatrisation

La PBM améliore la cicatrisation :

- Des ulcères de pression chez la souris<sup>148</sup>
- Des lésions ischémiques chez le rats<sup>122</sup>
- Des lésions cutanées chez le rats<sup>149</sup>
- Des lésions musculaires chez le crapaud<sup>150</sup>
- Des lésions type brûlure chez le rat<sup>151</sup> et la souris<sup>152</sup>
- Des lésions de mucites buccales chimioinduites chez le hamster<sup>153</sup> et le chien<sup>154</sup>.

La PBM favorise :

- L'intégration et la viabilité de greffons cutanés chez le rat<sup>155</sup>
- La pousse des poils dans un modèle de souris avec alopecie<sup>156</sup> et de rats avec alopecie chimioinduite<sup>157</sup>
- La régénération des hépatocytes post-hépatectomie partielle chez le rat<sup>158</sup>
- La biosynthèse de cartilage et améliore histologiquement l'os dans un modèle de rat avec ostéoarthrite<sup>159</sup>

La PBM diminue la perte de tissus myocardiques post-infarctus chez le rat<sup>160</sup>.

### 2.7.2.2 Neurologie et psychologie

La PBM améliore la conduction neuronale chez le rat<sup>161</sup>. Elle prévient la dégénérescence des neurones dopaminergiques :

- Dans un modèle de rat avec dégénérescence neuronale<sup>162</sup>
- Dans un modèle de souris parkinsoniennes<sup>163,164</sup>

En cas de lésions neurologiques sur un traumatisme crânien, la PBM :

- Stimule la neurogénèse chez les souris <sup>165</sup>
- Améliore la mémoire, stimule la neuroprotection et la neurogénèse chez les souris<sup>166</sup>
- Améliore la cicatrisation des lésions et le *Neurological Severity Score* chez les souris<sup>167</sup>
- Améliore les performances fonctionnelles et sensitives, diminue le volume des lésions et l'inflammation chez le rat avec atteinte de la moelle épinière<sup>168,169</sup>

Sur les douleurs neuropathiques chez le rat, la PBM permet de les diminuer en cas de compression nerveuse<sup>170</sup> et en cas d'atteinte médullaire<sup>171</sup>.

Dans le cadre de la dépression et de l'anxiété, la PBM transcrânienne améliore les symptômes chez les souris soumises à un stress chronique<sup>172</sup> et a un effet comparable au Citalopram dans un modèle de rat en dépression et stress chronique<sup>173</sup>. Elle permet de protéger de la toxicité oculaire du méthanol chez le rat<sup>174</sup>.

### 2.7.2.3 Muscle et exercice

La PBM permet :

- De prévenir la tétanie musculaire liée à la fatigue chez le rat<sup>87</sup>
- De diminuer les dommages musculaires post-exercices et d'améliorer les performances musculaires chez le rat<sup>175,176</sup>

### 2.7.2.4 Inflammation et auto-immunité

La PBM diminue l'inflammation :

- Dans un modèle de rat avec arthrite inflammatoire<sup>177</sup>
- Dans un modèle de rat avec arthrite liées à des cristaux<sup>178</sup>
- Dans un modèle de souris avec douleurs inflammatoires<sup>144</sup>
- Dans un modèle de souris asthmatique par diminution de l'hyper-réactivité bronchique<sup>145,179</sup>

La PBM permet d'améliorer les fonctions musculaires et de diminuer la neurodégénérescence au niveau de la moelle épinière dans un modèle de souris avec encéphalite auto-immune<sup>180</sup>. Néanmoins, une différence d'effet de la PBM est observée selon le niveau inflammatoire des cellules. En cas d'inflammation, la PBM présente des effets anti-inflammatoires, alors qu'en absence d'inflammation, la PBM présente des effets pro-inflammatoires pour favoriser le remodelage tissulaire<sup>146</sup>. Il semblerait donc que l'effet de la PBM soit dépendant du niveau de stress des cellules<sup>83</sup>.

## 2.7.3 La PBM en pratique clinique

### 2.7.3.1 Régénération cellulaire et cicatrisation

La PBM permet d'améliorer la cicatrisation des plaies ischémiques<sup>122</sup>. Dans les suites d'une plaie par brûlure, la PBM :

- Améliore la cicatrisation chez l'enfant<sup>181</sup>
- Fait aussi bien que la greffe de peau chez l'enfant<sup>182</sup>

La PBM permet de prévenir l'apparition de cicatrices post-acné ou post chirurgie<sup>81,183</sup>.

La PBM est efficace dans le traitement des lichens buccaux et pourrait être utilisée en alternative aux corticoïdes<sup>184</sup>. Concernant les mucites chimioinduites, elle permet :

- De les prévenir et les traiter chez l'enfant<sup>185</sup>
- D'en diminuer les douleurs associées<sup>186</sup>

La PBM présente un effet antalgique :

- Sur la pulpe dentaire avec une efficacité comparable à la lidocaïne<sup>187</sup>
- Sur les douleurs orthodontiques liées à l'application d'un élastique<sup>188</sup>

Dans des utilisation annexes, la PBM permet d'améliorer la structure cutanée des patients avec des rides<sup>189</sup> et stimule la pousse des cheveux particulièrement en cas d'alopécie androgénique<sup>190</sup>.

Actuellement la PBM est recommandée dans la prise en charge des mucites chimioinduites par différentes sociétés savantes : ESMO (*European Society for Medical Oncology*), MASCC (*Multinational Association of Supportive Care in Cancer*), ISOO (*International Society of Oral Oncology*).

### **2.7.3.2 Neurologie et psychologie**

Après traumatisme crânien, la PBM :

- Améliore les fonctions cognitives<sup>191-194</sup>
- Améliore la vascularisation de la zone traumatique<sup>192</sup>

Dans le cadre du syndrome du canal carpien, alors que quelques études rapportaient des résultats prometteurs sur la douleur et la fonctionnalité des doigts<sup>195,196</sup>, une méta-analyse récente conclut à l'absence de bénéfice notable de la PBM par rapport à une attelle<sup>197</sup>.

Enfin, la PBM améliore les symptômes de la dépression<sup>198</sup>, ainsi que de nombreux symptômes de la fibromyalgie (douleurs, fatigue, dépression, raideurs matinales et l'anxiété)<sup>199</sup>.

### **2.7.3.3 Muscles et exercice**

La PBM améliore la capacité fonctionnelle cardiaque et diminue les symptômes chez les patients avec une atteinte sévère des artères coronaires<sup>200</sup>.

Le pré-conditionnement par PBM permet d'améliorer les performances, diminuer les marqueurs de souffrance et de fatigue musculaire, et donc d'améliorer la récupération chez :

- Les personnes avec une activité physique intense<sup>201</sup>
- Les rugbymen<sup>202</sup>
- Les coureurs<sup>203</sup>
- Les footballeurs<sup>204</sup>



#### **2.7.3.4 Inflammation et auto-immunité**

La PBM n'a montré qu'un très faible niveau de preuve dans la prise en charge des tendinopathies du tendon d'Achille<sup>205</sup>. La PBM a permis de diminuer les douleurs et l'impotence liées à l'arthrite rhumatoïde<sup>206</sup> et à l'ostéoarthrite du genou<sup>207</sup>.

Chez les patients asthmatiques, la revue la plus récente<sup>208</sup> rapporte un effet de la PBM sur la fréquence des exacerbations, la fréquence de l'iatrogénie médicamenteuse et l'amélioration des paramètres respiratoires.

Enfin, dans le cas de thyroïdite auto-immune, la PBM a permis de :

- Diminuer les doses de lévothyroxine des patients avec amélioration de la fonction thyroïdienne et diminution de l'auto-immunité<sup>209,210</sup>
- Améliorer la vascularisation du parenchyme thyroïdien<sup>211</sup>
- Diminuer l'inflammation (augmentation du TGF- $\beta$ 1)<sup>212</sup>
- Démontrer des résultats rassurant au long cours (6 ans)<sup>213</sup>

#### **2.7.4 La PBM et le diabète**

Vu les applications cliniques de la PBM dans le champ de la cicatrisation, des désordres neurologiques et de la récupération musculaire post-exercice, la PBM a été évaluée chez des patients diabétiques qui présentent le potentiel de regrouper l'ensemble de ces problématiques. L'ensemble des données sont détaillées dans une revue de la littérature, en cours de reviewing et disponible en annexe 1.

Brièvement, cette revue regroupe 82 études pré-cliniques et 87 essais cliniques qui investiguent les effets de la PBM dans le diabète (neuropathie, rétinopathie, métabolisme glucidique, récupération post-exercice, cicatrisation/ulcères, cicatrisation osseuse et parodontites).

Les paramètres d'illumination étaient très variables :

- Longueurs d'ondes entre 425 et 1064 nm
- Temps d'expositions de moins d'une seconde à 50 minutes
- 79% d'utilisation de laser, contre 28% de LED (2% non défini)
- 80% d'utilisation d'illumination continue contre 16% de pulsée (4% non défini)
- 29% des études ont au moins un des paramètres d'illumination insuffisamment décrits

Les données observées en clinique sont cohérentes avec celles observées dans les études pré-cliniques, excepté pour la récupération post-exercice (peu d'études pré-cliniques et cliniques disponibles). Les principales données de cette revue sont illustrées dans le résumé graphique ci-dessous (figure 11).

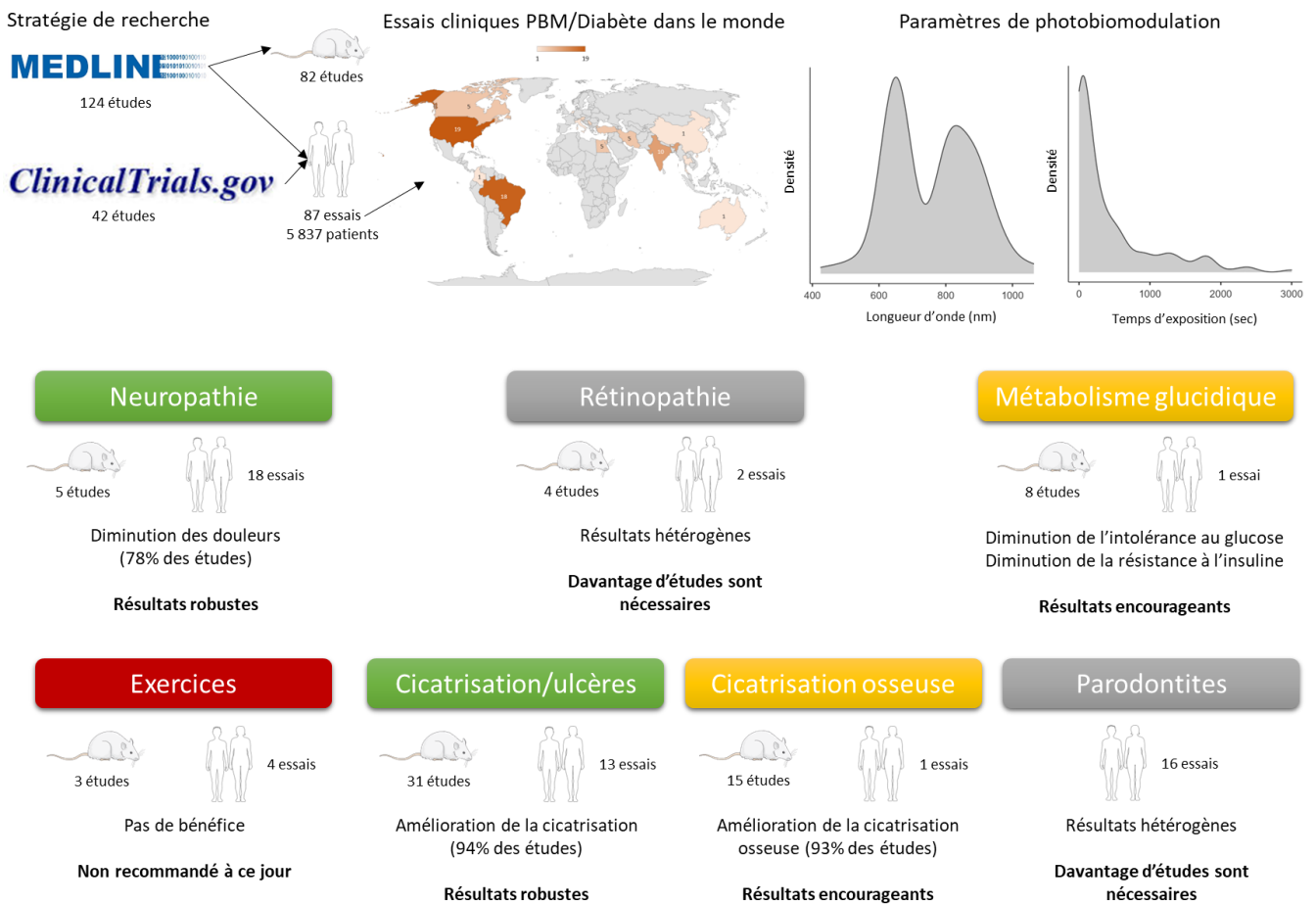


Figure 11. Résumé graphique des effets de l'utilisation de la PBM en diabétologie

## **Photobiomodulation et îlots : toxicité, cellules bêta, îlots de rongeur, modèle pré-clinique et îlots humains**

L'amélioration de l'insulino-sensitivité et la diminution de l'intolérance au glucose était un des champs d'intérêt prometteur de l'utilisation de la PBM dans le diabète (annexe 1). Diverses équipes de recherche se sont penchées sur l'impact de la PBM sur les cellules bêta et les îlots. Liebman et al.<sup>214</sup> ont opté pour l'utilisation d'un laser de 810 nm, avec une densité de puissance de 150 mW/cm<sup>2</sup>, durant 60 secondes (9 J/cm<sup>2</sup>), ciblant spécifiquement les cellules bêta (BTC6). Leurs investigations ont démontré une hausse discernable de la production de superoxyde et d'oxyde nitrique entre les laps de temps de 5 à 15 minutes consécutifs à l'application de la PBM, associé une élévation significative de la sécrétion d'insuline en bas glucose. De manière similaire, Huang et al.<sup>215</sup> ont utilisé un laser de 633 nm avec une densité de puissance de 1,7 mW/cm<sup>2</sup>, durant 9 secondes (15,6 J/cm<sup>2</sup>) et 18 secondes (31,3 J/cm<sup>2</sup>) sur des îlots porcins. Bien que cette intervention ait provoqué une augmentation modeste de la sécrétion d'insuline en bas glucose, il est important de noter que l'indice de stimulation de l'insuline n'a pas été impacté. Dans une démarche similaire, Irani et al.<sup>216</sup> ont soumis des îlots de rat (groupe de 10 îlots) à des lasers émettant à des longueurs d'onde de 630 nm et 810 nm, avec des fluences respectives de 1, 3 et 5 J/cm<sup>2</sup>. Les périodes d'application se sont étendues de 8 à 42 secondes. Les îlots ont initialement exprimé une réaction limitée au glucose, caractérisée par un indice de stimulation de l'insuline s'approchant de 1, ou ont été soumis à des contraintes induisant un stress. Toutefois, l'administration de PBM, avec une fluence de 1 J/cm<sup>2</sup>, a été en mesure de rétablir la sécrétion d'insuline en réponse au glucose, atteignant

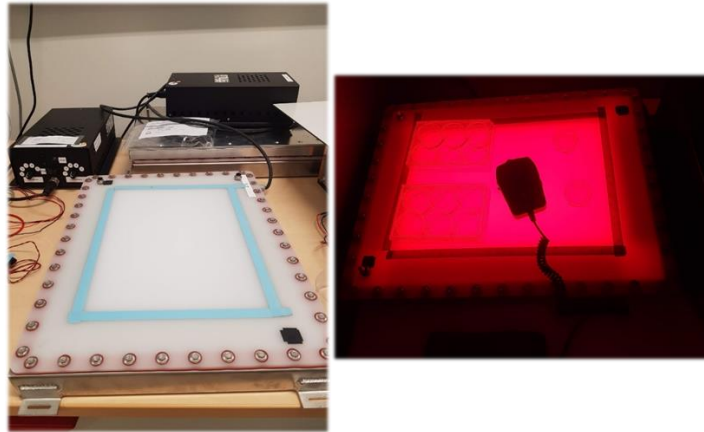
ainsi un niveau approprié, se rapprochant de 2. Ces données bien qu'encourageantes restent limitées et les mécanismes en lien peu explorés.

C'est dans ce contexte que le projet NIRILO a vu le jour avec pour objectif d'analyser l'impact de PBM sur la cellule bêta et sur un modèle de greffe d'îlots. Ce projet s'appuie sur des expertises locales maintenant bien développées :

- Expertise clinique en diabétologie et greffe d'îlots avec l'équipe CHUGA-UGA des Pr Benhamou et Pr Lablanche
- Expertise en physiologie de l'îlot et des mitochondries avec l'équipe de recherche du Pr Lablanche (LBFA – Inserm 1055)
- Capacités technologiques d'innovation et expertise en vascularisation du CEA
- Expertise en PBM de Clnatec (notamment dans la maladie de Parkinson)
- Expertise de la pharmacie et de la direction de la recherche clinique du CHUGA dans la promotion d'étude de phase 1

Le projet NIRILO a obtenu un financement de la région Rhône-Alpes Auvergne pour une durée de 2 ans. L'articulation se fait en différents workpackages :

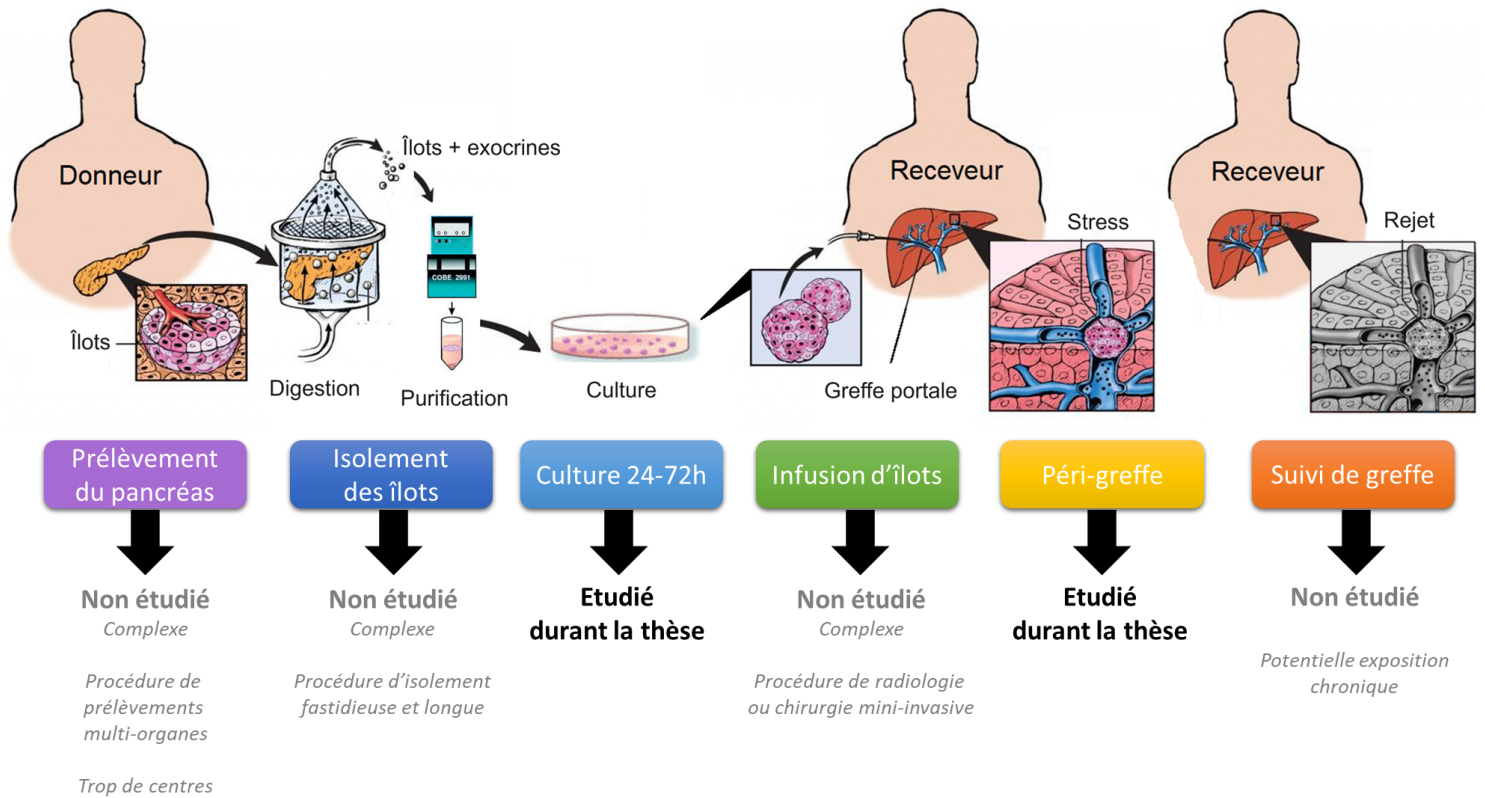
- Module illumination (Clnatec) : mise au point des caractéristiques du dispositif et des protocoles d'illumination. Le dispositif LED 670 nm utilisé sur le projet est identifié sur la figure 12



**Figure 12.** Illuminateur éteint et allumé avec monitoring de la puissance

- Module vascularisation (CEA) : évaluation de l'impact de la PBM sur l'architecture et la stabilité des réseaux vasculaires. Cela se base sur l'expertise du CEA sur le projet PANACHE visant à maintenir les îlots sur plusieurs semaines dans des puces reconstituants un environnement complexe autour des îlots
- Module biologique (LBFA) qui a fait l'objet des travaux de thèse pour :
  - Valider l'absence de toxicité de la PBM sur l'îlot
  - Analyser l'impact in vitro de la PBM sur l'îlot de rongeur et humain à l'occasion de différents stress
  - Analyser l'impact in vivo de la PBM sur un modèle de greffe d'îlots sous la capsule rénale de rats diabétiques
- Module réglementaire : afin d'évaluer les tests nécessaires avant un essai clinique, conduite d'évaluation des risques, étude sur la brevetabilité des découvertes
- Module architecture des études cliniques : le livrable final du projet sera de proposer un protocole optimisé d'illumination et une maquette du dispositif utilisable en clinique

La PBM pourrait être envisagée sur les différentes étapes liées à la greffe d'îlots (figure 13). Par soucis de simplicité d'application, le choix a été fait d'évaluer l'effet de la PBM durant la culture cellulaire (avant les stress) ou en péri-greffe immédiat (durant les stress).



**Figure 13.** Etude de la PBM dans le processus de greffe d'îlots

## Article n° 1

### Longue exposition de lignée cellulaire bêta et îlot pancréatique de rat à la photobiomodulation: existe-t-il un risque?

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**Key Words:** Diabetes, Photobiomodulation, Beta cells, insulinosecretion, Viability, Fonctionnalité

**Abbreviations:**

AA: Antimycine A

CCCP: Carbonylcyanide m-chlorophenyl hydrazone

FBS: Foetal bovine serum

GSIS: Glucose stimulated insulin secretion

T1D: Type 1 diabetes

KRB: Kreb's ringer buffer

MIN6: Mouse insulinoma cells

MTG: Mitotracker Green

PBM: Photobiomodulation

TMRM: Tetramethyl rhodamine methyl ester

## Abstract

**Introduction.** Type 1 diabetes (T1D) is characterized by the loss of insulin-producing beta cells, requiring daily insulin administration. In cases of brittle type 1 diabetes, marked by severe glycaemic fluctuations, islet transplantation becomes a potential therapy. Photobiomodulation (PBM), a process involving selective absorption of specific wavelengths to induce biological changes, has shown positive effects on various tissues. This study evaluates the safety and potential benefits of PBM on pancreatic beta cells and islets.

**Methods.** PBM was applied to insulin-secreting cell lines (MIN6) and rat pancreatic islets using a 670 nm light source, continuous output, with a power density of 2.8 mW/cm<sup>2</sup>, from few seconds (5s) to several hours (24h). Viability, insulin secretion, mitochondrial function, ATP content and cellular respiration were assessed.

**Results.** Results demonstrate that short and prolonged PBM exposure did not affect beta cell neither islets viability nor insulin secretion. While short-term PBM (2h) increase mitochondrial membrane potential and increase superoxide ion production, ATP content and mitochondrial respiration were slightly decreased. These effects were not observed for long exposure to PBM (24h).

**Discussion.** Notably, long-term PBM exhibited safety in terms of cell viability, insulin secretion, and energetic profiles. Further investigation is warranted to explore PBM's protective effects under conditions of stress, aiding in the development of innovative approaches for cellular therapy. If positive results were observed, PBM may have potential applications in enhancing islet transplantation outcomes for T1D therapy.



## Introduction

Type 1 diabetes (T1D) results from loss of beta cells that produces insulin. As a result, patients with T1D must have daily administration of insulin. However, despite optimal medical care, some patients experience severe hypo- or hyper-glycaemia leading to unstable diabetes. In this case, islet transplantation can be proposed as a therapeutic option. To briefly describe the technique, human islets are obtained from brain-dead donors through pancreas sampling. Then, pancreas undergoes a complex process of islet isolation followed by islet transplantation in diabetic recipients<sup>1</sup>. Pancreatic islet transplantation demonstrated its positive effect on metabolic parameters<sup>2-4</sup>. However, to achieve this control of metabolic parameters, 2-3 grafts are needed due to high loss of islet after injections<sup>5,6</sup>.

Photobiomodulation (PBM) (also known as low level laser therapy) corresponds to the selective absorption of given wavelengths by chromophores inducing biological modifications. PBM has been extensively explored, with over 800 publications per year indexed in Medline since 2020. However, the underlying pathophysiological mechanisms leading to PBM effects remain unclear but may involve mitochondria. PBM has impact on mitochondrial membrane potential, reactive oxygen species (ROS), adenosine triphosphate (ATP) and by extrapolation impact on the mitochondrial respiratory chain especially cytochrome C oxidase (CCO) that has its absorption peak in the range used in PBM<sup>7,8</sup>. The principle of PBM is likely governed by the Arndt-Schultz law<sup>9</sup>, where low doses do not produce an effect and high doses are detrimental<sup>10,11</sup>. For instance, Flores Luna et al<sup>12</sup>. reported a decrease in the viability of human fibroblasts exposed to continuous 660 nm laser illumination with an irradiance of 21.5 mW/cm<sup>2</sup> for 147s (fluence: 3.16 J/cm<sup>2</sup>). Schalch et al<sup>13</sup>. observed reduced viability of oral squamous cell carcinoma under continuous laser illumination at: 1) 660 nm with an irradiance of 214 mW/cm<sup>2</sup> for 25.3s (fluence: 4 J/cm<sup>2</sup>), and 2) 780 nm with a power of 220 mW/cm<sup>2</sup> for 24.7s (fluence: 4 J/cm<sup>2</sup>). While numerous preclinical and clinical studies have investigated the potential of PBM in diabetes-related complications (retinopathy<sup>14</sup>, neuropathies<sup>15</sup>, wound healing<sup>16</sup>), only a few have focused on insulin secretion<sup>17</sup>, or its direct effects on beta cells<sup>18</sup> and islets<sup>19,20</sup>. In the preliminary

evaluation of PBM in a preclinical and clinical objective, we therefore wanted to see if PBM had no deleterious effect, or even positive results on pancreatic beta cells and pancreatic islets on viability and functionality in basal conditions.

## Materials and methods

- Biological materials

The present study was performed in parallel either on insulin-secreting cell line or primary rodent pancreatic islets. The cell line and primary cells were maintained in a controlled atmosphere at 37°C and 5% CO<sub>2</sub>.

- MIN6 cells (AddexBio, San Diego, California, USA) (immortalized murine pancreatic insulin-secreting  $\beta$ -cells) were used and cultured in DMEM 24.8 mM glucose (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts, USA) supplemented with 15% foetal bovine serum (FBS), 1% Penicillin-Streptomycin, and 50 $\mu$ M 2- $\beta$  Mercapto-ethanol. Cells were stored 48h before experimentation and each condition comprised 400,000 cells.
- Rat pancreatic islets were obtained as previously described<sup>21</sup>. Briefly, Wistar rat (Charles River, Lyon, France) pancreases were harvested after injection of a 10 mg/mL collagenase IX solution. The pancreas was then enzymatically and mechanically digested by shaking for 11min in a water bath at 37°C. The isolate was then purified by a discontinuous gradient of different densities. Islets were stored for 24h before experimentation in RPMI 1640, 11 mM glucose (Thermo Fisher Scientific, Bourgoin-Jallieu, France) supplemented with 10% (v/v) FBS, 1% (v/v) sodium pyruvate, 1% (v/v) L-Glutamine, 1% (v/v) Penicillin - Streptomycin.

- Photobiomodulation procedure

The illumination was made by a PBM device (EFFI-BL-IP69K, Effilux, Les Ulis, France), wavelength of 670 nm (largest wavelength used in literature), continuous output power (Table 1). Short illuminations (few seconds to- several minutes, see Table 1) were made at room temperature. Long illuminations (more than 2h) were made in a humidity incubator (37°C, 5% CO<sub>2</sub>). As the illumination bench generates heat, a chiller was added to keep the incubator temperature stable at 37°C. PBM irradiation parameters were monitored and adjusted using a power and optical energy meter (PM100D, Thorlabs, Newton, New Jersey) with a photodiode power sensor (S120C, Thorlabs). The cells (MIN6) were illuminated 72h after passage and islets were illuminated 24h after isolation.

- MIN6 cells viability assay

MIN6 cells were detached with 2X trypsin-EDTA, centrifuged for 3min at 1200rpm and rinsed with phosphate buffered saline (PBS). The pellet was re-suspended in 100 µL of specific 1X buffer. 10 µL of Annexin V-FP488 (Interchim, Montluçon, France) were added before incubation for 20min at room temperature. The suspension was then supplemented with 900 µL of PBS and 10 µL of Propidium Iodide (PI) (stock solution at 1 mg/mL, Thermo Fisher Scientific) just before analysis with a BD LSR Fortessa™ flow cytometer (Beckton-Dickinson Biosciences, Pont-de-Claix, France). It was equipped with two excitation lasers: a Coherent Compass 532 nm laser and a Coherent Sapphire 488 nm laser. The corresponding emission filters are: 610/20 nm for PI and 525/50 nm for annexin-FP488. The data acquisition and processing software were BD FACSDiva™ (Beckton Dickinson Biosciences). Results were reported as percentage of viability, 10,000 events were recorded per condition. Positive control of death was obtained with cells supplemented before analysis with digitonin 2% (v/v). Results were normalized to the control value.

- Islet viability assay

Islet viability was analysed by confocal microscopy on whole and intact islets, previously cultured in petri dish with glass bottom (Greiner Bio-One GmbH, Austria). Islets were incubated in 2 mL of fully supplemented RPMI 1640 media with 1  $\mu$ M Syto13 (Thermo Fisher Scientific) for 20min in a humid atmosphere (37°C, 5% CO<sub>2</sub>) and protected from light<sup>22</sup>. Briefly, just before analysis, 10  $\mu$ L of PI (Thermo Fisher Scientific) was added. The 488 and 532 nm lasers were used at 2% for excitation; the fluorescence emission was collected between 500-530 nm for Syto13 and 585-665 nm for PI. Images were obtained using a Leica TCS CSU SP8 confocal microscope (LEICA, Microsystems Heidelberg, Germany) equipped with a Fluotar 10x/0.30 HC PL objective driven by the LasX software. Images were acquired in z-stack with a z-step of 10  $\mu$ m, a pinhole of 1 (Airy units) for all channels. Green Syto13 fluorescence labelled live cells while dead cells were labelled by a positive PI nuclear label in red. Quantification was done with a macro on ImageJ software (version 8). Results were expressed as percentage of viability =  $100 * \text{number of green fluorescent cells} / (\text{total number of green and red cells})$ , at least 50 islets equivalent were analysed. Results were normalized to the control value.

- Glucose Stimulated Insulin Secretion (GSIS) assay

GSIS was performed in static incubation using Krebs-Ringer bicarbonate buffer medium (125 mM NaCl, 4.74 mM KCl, 1 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 5 mM NaHCO<sub>3</sub>, 25 mM HEPES, pH 7.4) supplemented with 0.1% bovine serum albumin (BSA) (w/v) according to the following protocol: wash in 2.8 mM glucose, pre-incubation 1h at 37°C in 2.8 mM glucose, incubation 1h at 37°C in 2.8 mM glucose (basal condition), then incubation 1h at 37°C in 16.7 mM glucose (stimulated condition). All incubation were made in a humid atmosphere (37°C, 5% CO<sub>2</sub>). Insulin content was extracted by overnight incubation at -20°C in ethanol acid solution (375 mL absolute ethanol + 7.5 mL HCl 12.7 M + 117.5 mL distilled water). Measurement of insulin content allows normalization of secretion results by total insulin content. The incubation supernatants were collected and frozen for later analysis. Insulin assays were performed on incubation supernatants from basal, stimulated, and acid-ethanol extracts.

The assays were done by chemiluminescence enzyme-linked immunosorbent assay (Eurobio Scientific, Les Ulis, France) using the CLARIOstar plate reader (BMG Labtech, Champigny-sur-Marne, France). The results were expressed as percentage of secreted insulin in relation to the total (contained and secreted), and the insulin stimulation index was  $100 \times (\text{insulin secreted in high glucose} / \text{insulin secreted in low glucose})$ . At least 100 islets equivalent were used for GSIS assay.

- Mitochondrial superoxyde production

Superoxide production measurement was performed by flow cytometry on trypsinized MIN6 cells. The cells were incubated for 20min in 1 mL of full supplemented DMEM medium containing 1  $\mu\text{M}$  of MitoSOX<sup>TM</sup> Red (Invitrogen, Carlsbad, California, USA), in a humid atmosphere (37°C, 5% CO<sub>2</sub>) and protected from light. Fluorescence was measured by 488 nm laser with PE emission filter (BP 575/26), analysis software remains unchanged, results were expressed as average fluorescence intensity arbitrary unit.

- Mitochondrial membrane potential

Mitochondrial membrane potential measurement of MIN6 was performed by flow cytometry. After trypsinization, cells were incubated either in 1 mL of full supplemented DMEM medium with 100 nM Tetramethyl rhodamine methyl ester (TMRM) (Life Technologies, Carlsbad, California, USA), or in 1 mL of full supplemented DMEM medium with 100 nM Mitotracker Green<sup>TM</sup> (MTG) (Life Technologies) in a humid atmosphere (37°C, 5% CO<sub>2</sub>) protected from light. Cell suspensions were analysed by FACS with excitation at 488 nm or 532 nm and emission bandpass filters 530/30 nm for MTG or 585/15 nm for TMRM, respectively. Mitochondrial mass is estimated by quantification of MTG labelling (fluorescence average). TMRM-labelled cells were then incubated for 15min with 250  $\mu\text{M}$  carbonyl cyanide m-chlorophenyl hydrazone (CCCP) (Sigma-Aldrich, Saint-Louis, Missouri, USA). Mitochondrial membrane potential was calculated as a difference in TMRM fluorescence before and after the addition of CCCP and then divided by mitochondrial mass.

- Mitochondrial and cell oxygen consumption

This was performed as previously described<sup>23</sup>. Briefly, the rate of oxygen consumption ( $JO_2$ ) was measured at 37°C using a Clark-type  $O_2$  electrode in a 1 mL-chamber filled with 500  $\mu$ l respiration buffer: KCl 125 mM, EGTA 1 mM, Tris HCl 20 mM, pH 7.2. MIN6 cells were detached with 2X trypsin-EDTA, centrifuged for 3min at 1200rpm count and used for the experiment. Cells were quantified with automated cell counter (LUNA-II®, Logos Biosystems, Villeneuve d'Ascq, France) after blue trypan exclusion labelling to quantified dead cells.

Complexes I and II MIN6 cells activity: cells (5 million of total cells) were permeabilized with 2% digitonin, measurement was firstly made in the presence of different substrates (state 2): glutamate 5 mM / malate 2.5 mM (for Complex I) or succinate 5 mM / rotenone 1  $\mu$ M (for Complex II), after addition of adenosine di-phosphate (ADP) 0.5 mM (state 3) and followed by addition of oligomycin 2  $\mu$ g/mL (state 4). The respiratory control ratio (RCR) was obtained by dividing state 3 (ADP) by state 4 (oligomycine). The RCR reflected how the respiratory was coupled to the ATP production, a decrease in RCR translates into a decrease in ATP production for a given amount of oxygen consumed.

Complex IV MIN6 cells activity: cells (2.5 million of total cells) were permeabilized with 2% digitonin, measurement was made in the presence of antimycin A (AA) 1  $\mu$ M / Ascorbate 4 mM, followed by TMPD 0.5 mM / Ascorbate 0.25 mM and finally DNP 150  $\mu$ M.

MIN6 cells oxygen consumption (5 million of total cells): measurement was made in DMEM medium (routine respiration of intact cells (ROUT)), followed by addition of oligomycin 2  $\mu$ g/mL and finally AA 1  $\mu$ M. Results were expressed for 5 million of live cells.

- ATP/ADP content

After experiment, MIN6 cells were rinsed 3 times with PBS. Then, attached cells were incubated for a minute in perchloric acid 2.5% EDTA 6.25 mM solution and then detached mechanically, centrifuged (2min, 13,000rpm, 4°C), neutralized by addition of KOMO solution (KOH 2N and 3-morpholinopropane-1-sulfonic acid 0.3 M), and centrifuged again (10min, 13,000rpm, 4°C). Protein-free extract was separated on a C18 HPLC column (Polaris 5C18-A, S250\*4.6 Repl, Varian, France) in pyrophosphate buffer (28 mM, pH 5.75) at 1 mL/min flow rate and 30°C. ATP and ADP eluted at 3 and 5min, respectively. Elution peaks were integrated with the STAR software (Varian, France). Measurement of proteins was made with Pierce kit (Thermo Scientific). Results were expressed in  $\mu\text{mol}$  of ATP or ADP per mg of proteins.

- Statistical Analyses

Results are expressed as mean value  $\pm$  SEM (standard error of mean), the number of experimentations was indicated in the legend of each figure. All statistical tests were performed by Jamovi software (version 2.25). The different groups were compared by ANOVAs (or an equivalent non-parametric test if the application conditions were not met). A value is considered significant if  $p < 0.05$ .

## Results

- PBM did not alter MIN6 cells nor rat islet viability

No difference was observed between control and illuminated conditions for viability ( $p = 0.408$ , Fig. 1A and 1B for MIN6 cells, and  $p = 0.791$ , Fig. 1C and 1D for islet).

- PBM did not alter MIN6 cells and rat islet insulin secretion

No difference was observed between control and illuminated condition for insulin secretion in low glucose 2.8 mM ( $p = 0.707$ , Fig. 2A for MIN6 cells, and  $p = 0.760$ , Fig. 2B for islet), in high glucose 16.7 mM ( $p = 0.847$ , Fig. 2A for MIN6 cells, and  $p = 0.470$ , Fig. 2B for islet) or insulin stimulation index ( $p = 0.700$ , Fig. 2C for MIN6 cells, and  $p = 0.718$ , Fig. 2D for islet).

- Superoxide production and mitochondrial membrane potential were altered with PBM

MIN6 superoxide production was significantly higher than control after 2h of PBM ( $829 \pm 155$  vs.  $620 \pm 80$ ,  $p = 0.023$ ) but did not differ from control after 24h of PBM ( $569 \pm 92$ ,  $p = 0.634$ , Fig. 3A).

The mitochondrial mass (MTG) did not differ between control and illuminated condition ( $p = 0.824$ , Fig. 3B). The mitochondrial membrane potential was not significantly different from control ( $1.52 \pm 0.45$ ) after 2h ( $2.06 \pm 0.06$ ,  $p = 0.069$ ) or 24h of PBM ( $1.49 \pm 0.45$ ,  $p = 0.989$ , Fig. 3C).



- Mitochondrial and cell respiration slightly affected by PBM

For Complex I, PBM significantly decreased RCR if applied during 2h ( $15.1 \pm 1.5$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.012$ ) or 24h ( $13.9 \pm 0.5$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.006$ ) compared to control ( $25.5 \pm 6.7$  nmol/O<sub>2</sub>/min/5 million of cells, Fig. 4A). For Complex II, PBM decreased RCR if applied during 2h ( $6.3 \pm 0.3$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.002$ ) compared to control ( $7.1 \pm 0.1$  nmol/O<sub>2</sub>/min/5 million of cells) but had no impact if applied for 24h ( $6.9 \pm 0.3$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.319$ , Fig. 4B). For Complex IV, PBM did not affect oxygen consumption in uncoupled conditions (DNP) if applied for 2h ( $26.5 \pm 9.4$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.998$ ) or 24h ( $33.1 \pm 8.4$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.580$ ) compared to control ( $25.7 \pm 12.3$  nmol/O<sub>2</sub>/min/5 million of cells, Fig. 4C). For the whole cells, PBM did not affect ROUT if applied for 2h ( $14.5 \pm 4.5$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.390$ ) or 24h ( $17.3 \pm 6.4$  nmol/O<sub>2</sub>/min/5 million of cells,  $p = 0.860$ ) compared to control ( $19.0 \pm 1.4$  nmol/O<sub>2</sub>/min/5 million of cells, Fig. 4D).

- ATP content slightly affected by PBM

No significant difference was observed regarding ATP content ( $p = 0.193$ , Fig. 5A), ADP content ( $p = 0.839$ , Fig. 5B) or ATP/ADP ratio ( $p = 0.952$ , Fig. 5C).

## Discussion

In this study, we present the first report of the safety of PBM exposure on MIN6 beta cell line and rat islet with no deleterious effect on MIN6 and rat islet viability.

In contrast to studies mentioned in the introduction, our study revealed no toxic effects when cells were exposed to LED illumination at 670 nm, with an irradiance of 2.8 mW/cm<sup>2</sup> for durations ranging from 5s to 24h, corresponding to fluence of 14 mJ/cm<sup>2</sup> to 241.9 J/cm<sup>2</sup>. This result challenges the historical Roscoe-Bunsen law proposed for PBM<sup>24</sup>, where the total photon quantity received (fluence) matters more than the irradiance. Given the 40-fold higher fluence in our study compared to the existing literature, it is crucial to consider both the irradiance and fluence to optimize the illumination parameters, at least for the cells considered here.

Our work demonstrated that PBM did not influence insulin secretion in MIN6 cells nor in rat islets, regardless of glucose concentrations (2.8 mM or 16.7 mM) or insulin stimulation index. To date, only three studies have investigated the effects of PBM on insulin secretion in  $\beta$  cells or islets. Liebman et al.<sup>18</sup> reported a 29% increase in insulin secretion at low glucose (0.5 mM) in BTC6 cells (but not-significant effect in high glucose 3 mM). However, because these cells have different glucose stimulation thresholds than islets (2-3 mM at low glucose and 16.7-20 mM at high glucose), the results can't be directly extrapolatable. Irani et al.<sup>19</sup> reported a 2.4-fold increase in insulin secretion at a high glucose concentration (16.7 mM) under continuous 830 nm laser illumination (156 mW/cm<sup>2</sup> for 7s, fluence: 1 J/cm<sup>2</sup>) and a 3.4-fold increase under continuous 630 nm laser illumination (125 mW/cm<sup>2</sup> for 8s, fluence: 1 J/cm<sup>2</sup>). No effect on basal insulin secretion (2.8 mM glucose) was observed. These results may differ from our observations for several reasons. First, the results of the study by Irani et al. were not normalized with respect to the insulin content of the islets. Although each condition included 10 islets, their sizes vary significantly in rats (ranging from 50 to 500  $\mu$ m), and consequently, their insulin

content may also vary. Second, while our control islets exhibited an insulin stimulation index close to 2, their values were closer to 1, indicating poor islet secretion capability possibly due to islet stress. It has been demonstrated that the effects of PBM depend on the stress levels of cells. For example, in the presence of inflammation, PBM may have anti-inflammatory effects, whereas in the absence of inflammation, it may exhibit pro-inflammatory effects, which are necessary for tissue remodelling<sup>25</sup>. Finally, in a manner similar to our study, Huang et al.<sup>20</sup> reported no effect of continuous illumination with a 633 nm laser at 1.7 mW/cm<sup>2</sup> for 9 or 18sec (irradiance: 15.6 or 31.3 J/cm<sup>2</sup>) on insulin secretion in porcine islets, which are known to have low secretion capacity.

Regarding the potential mechanisms of PBM, mitochondrial action, particularly on CCO (Complex IV), is highly likely<sup>24</sup>. In this study, we evaluated various mechanisms involving the production of superoxide ions, mitochondrial membrane potential, cellular energy levels (ATP and ADP content), and mitochondrial respiration. Illumination of MIN6 cells for 2h led to an increase of superoxide content coherent with an increase of mitochondrial membrane potential (not statistically significant) and a decrease in the respiratory control ratio (RCR) of Complexes I and II. As previously mentioned, the effects of PBM may depend on cell stress levels<sup>26</sup>. In non-stressed cells (as in our study), PBM increased the mitochondrial membrane potential and associated ROS production. However, excessive ROS production, despite being beneficial in moderate quantities, may be deleterious<sup>11</sup>. While some studies have proposed an increase in the activity of the respiratory chain based on the combined effects of PBM on mitochondrial membrane potential, ATP production, and the absorption spectrum of CCO, only one study<sup>27</sup> demonstrated this hypothesis in isolated horse heart CCO exposed to a 632.8 nm laser at 10 mW/cm<sup>2</sup> for 200s and a fluence of 2 J/cm<sup>2</sup>. However, these results have not been replicated, regardless of the preconditioning or simultaneous application of PBM during oxygen consumption measurements in isolated CCO<sup>28-30</sup>. Several studies have reported an isolated increase in ATP production in different cell models (lymphocytes<sup>31</sup>, liver cells<sup>32</sup>, neurons<sup>33</sup>, and cardiomyocytes<sup>34</sup>). Chaudary et al<sup>35</sup>. found no effect of pulsed 635 nm laser illumination (40 mW/cm<sup>2</sup> for 10min, fluence:

24 J/cm<sup>2</sup>) on ATP production in myoblasts and fibroblasts. Interestingly, MIN6 cells exposed to continuous illumination for 24h showed no alteration in mitochondrial membrane potential, superoxide production and ATP content. This discrepancy might be attributed to the establishment of a new equilibrium (or transient alteration) in the mitochondrial membrane potential and superoxide after 24h of illumination, as variations in cellular models are known to be transient. Notably, no effect was observed on the oxygen consumption of Complex IV at 2h or 24h PBM.

While previous PBM studies have applied the technique for seconds to several minutes, our study is the first to report the long-term safety of PBM. These results mean that PBM can be considered with peace of mind (absence of toxicity after long exposure) in the islet isolation/transplantation process. Indeed, it has been reported that 50% of transplanted islets are rapidly destroyed by various mechanisms<sup>5,6,36</sup>, such as inflammatory and cytokine reaction (named instant blood mediated inflammatory reaction), nutrient deprivation and hypoxia. Since PBM has demonstrated interesting properties in inflammation and cellular protection<sup>25</sup>, and as islets are available *ex vivo* for a period of 24-72h, preconditioning them before transplantation and the occurrence of such stresses could be considered. Thus, this study represents an essential preliminary step before further investigation of this approach in the field of cellular therapy for T1D.

In conclusion, our study shows that long-term PBM exposure on pancreatic beta cells and islets is safe with regard to cell viability, insulin secretion, and energetic profile (mitochondrial membrane potential, superoxide ion production, and ATP/ADP ratio). Future research should focus on investigating the protective effects of PBM against various stresses that may occur after islet transplantation, which will determine whether this approach holds promise for the treatment of T1D.

## Acknowledgement

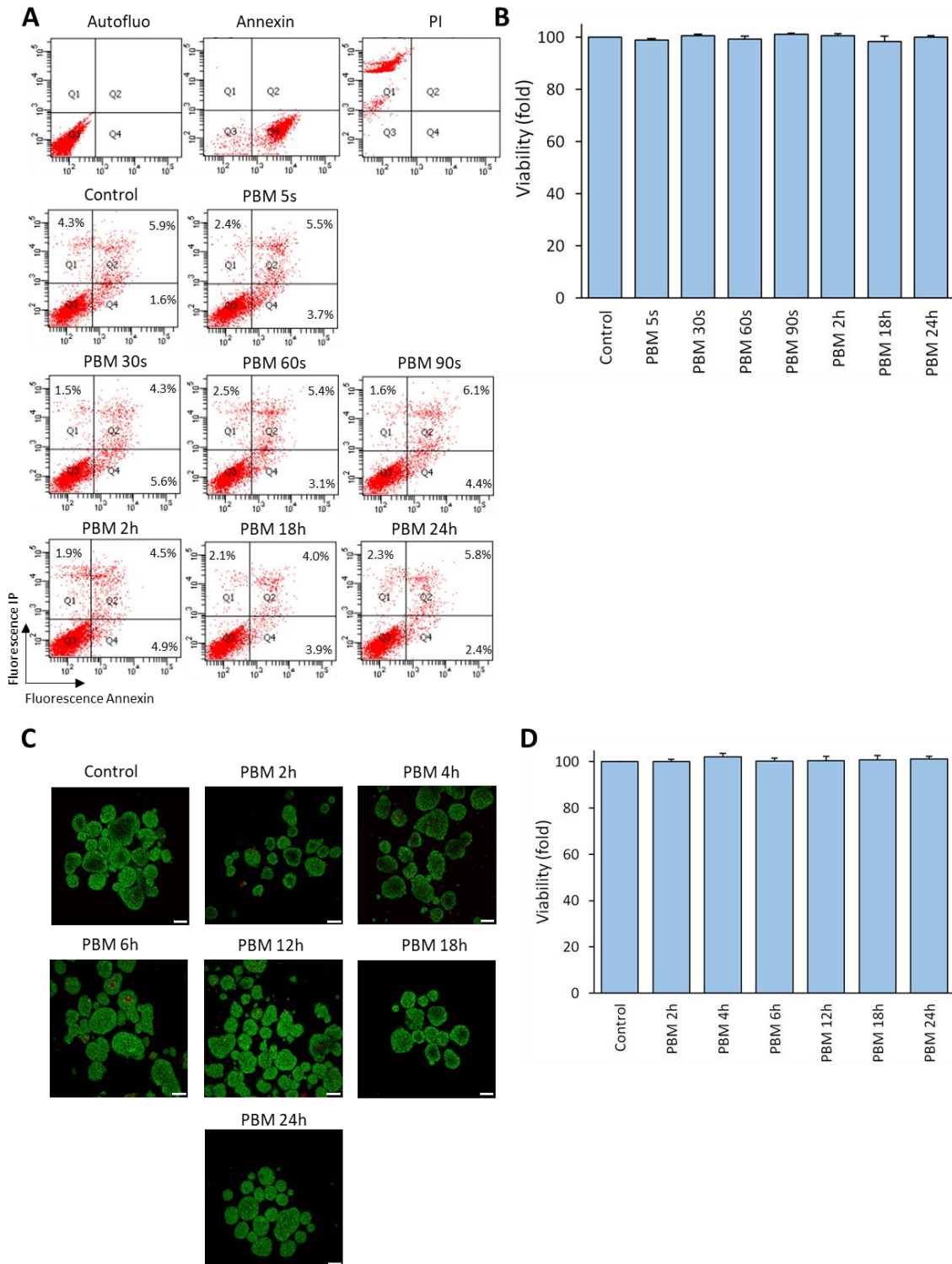
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**Table 1.** PBM parameters

Parameters	PBM
Type of light	LED, incoherent light
Wavelength	670 nm
Pulse structure	Continuous wave
Irradiance	2.8 mW/cm <sup>2</sup>
Back light panel area	20 * 30 cm <sup>2</sup> , 90% homogeneity

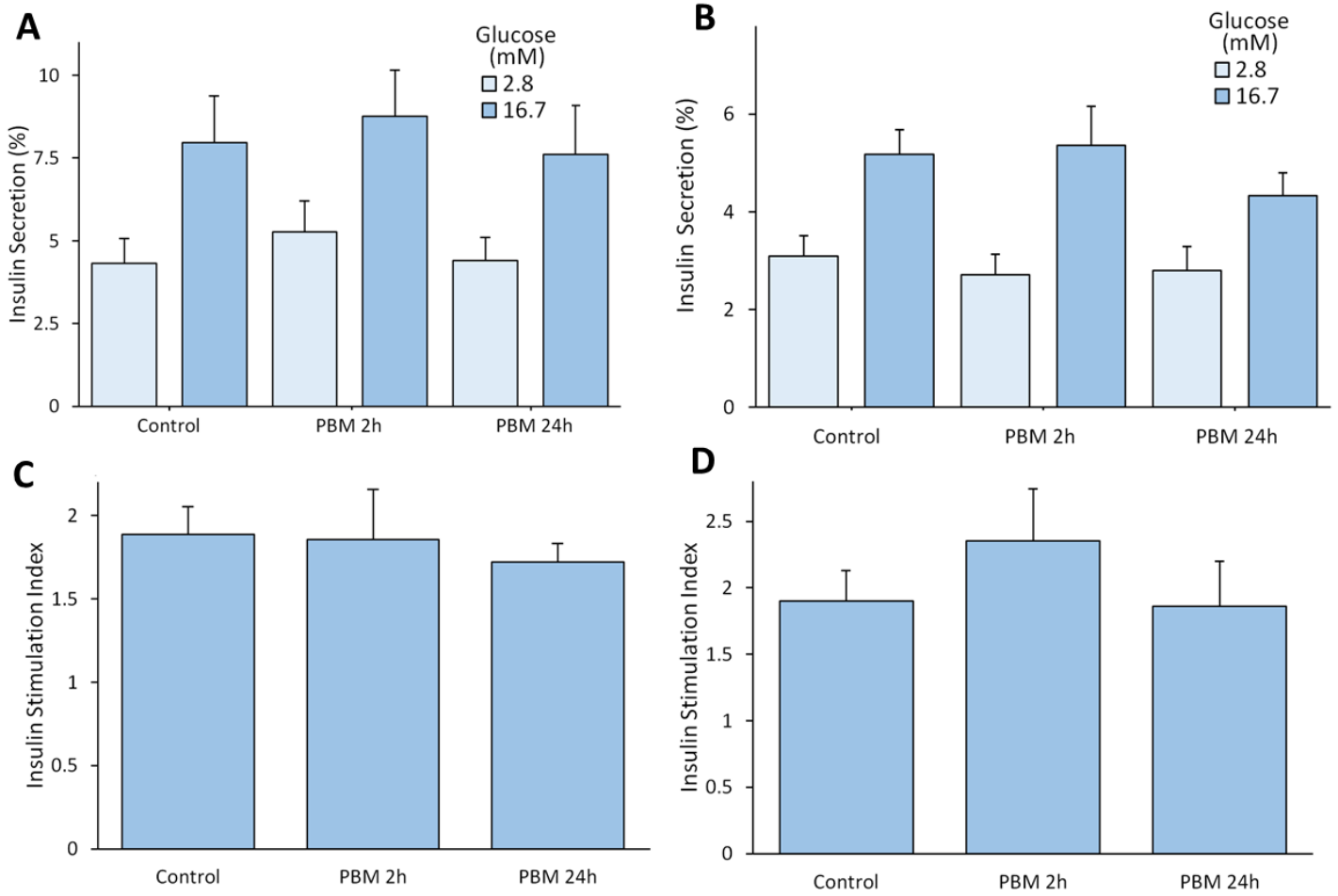
illumination time	Fluence
<b>Short</b>	5s 14 mJ/cm <sup>2</sup>
	30s 84 mJ/cm <sup>2</sup>
	60s 168 mJ/cm <sup>2</sup>
	90s 252 mJ/cm <sup>2</sup>
<b>Long</b>	2h 20.2 J/cm <sup>2</sup>
	4h 40.3 J/cm <sup>2</sup>
	6h 60.5 J/cm <sup>2</sup>
	12h 121 J/cm <sup>2</sup>
	18h 181.4 J/cm <sup>2</sup>
	24h 241.9 J/cm <sup>2</sup>

**Figure 1.** Effect of PBM on the viability of MIN6 cells and rat islets



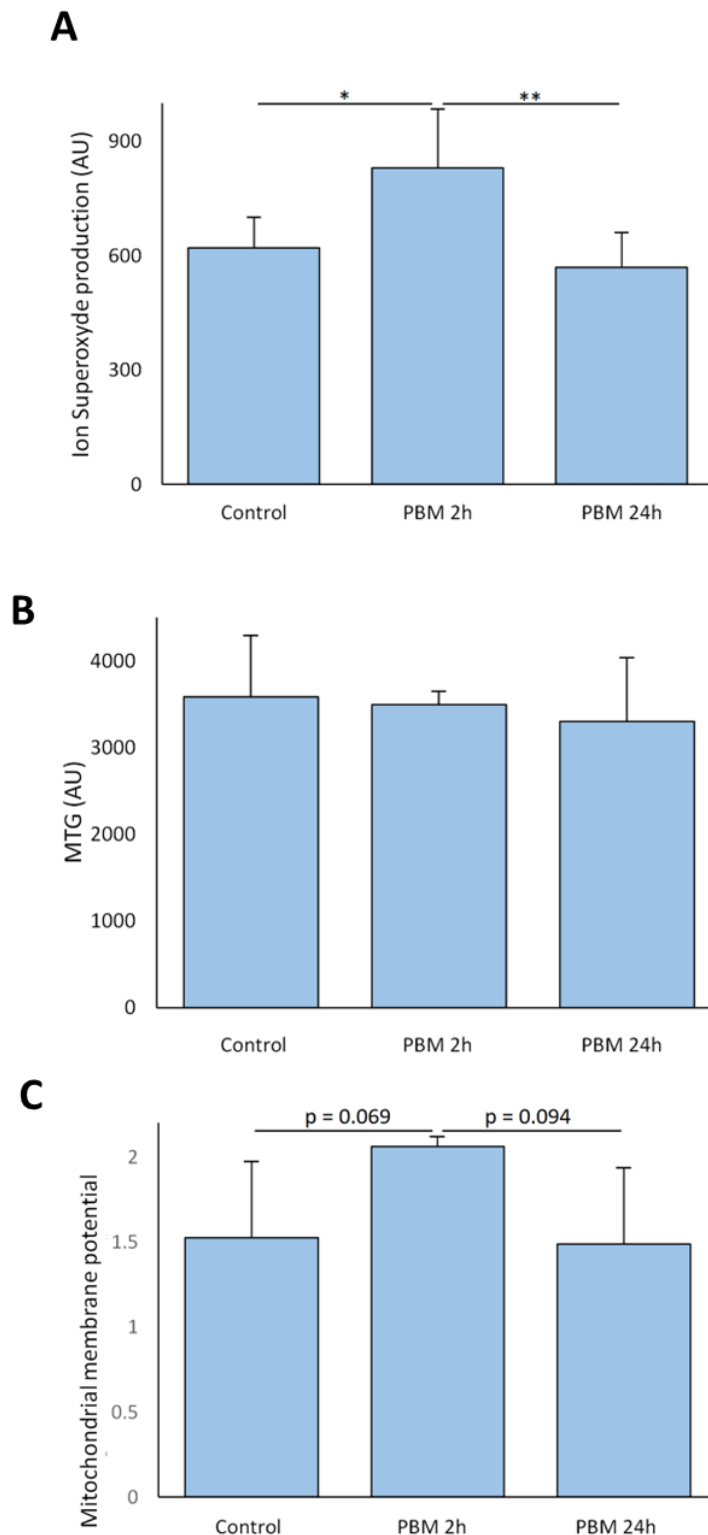
MIN6 cells and rat islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Results of a representative FACS experiment on viability of MIN6 cells after PBM. Live cells are in Q3 (annexin-negative and IP-negative). Annexin and PI conditions correspond to death control. **B.** Effect of PBM on MIN6 cells' viability. Results are normalized to control value. **C.** Representative images of islet viability experiment by confocal microscopy after PBM. Live cells are labelled with Syto13 (green) and dead cells are labelled with PI (red). **D.** Effect of PBM on rat islets' viability. Results are normalized to control value. PBM: photobiomodulation. Results are expressed as the mean  $\pm$  SEM,  $n = 4$ , Scale bars = 100  $\mu$ m, One-way ANOVA Kruskal–Wallis's test with pairwise comparison.

**Figure 2.** Effect of PBM on insulin secretion of MIN6 cells and rat islets



MIN6 cells and rat islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on MIN6 cells' insulin secretion in response to glucose ( $n = 8$ ). Results are normalized by total insulin content. **B.** Effects of PBM on islets' insulin secretion in response to glucose ( $n = 5$ ). Results are normalized by total insulin content. **C.** Effect of PBM on MIN6 cells' insulin secretion index. Secretion index is  $100 \times (\text{high glucose insulin secretion} / \text{low glucose insulin secretion})$ . **D.** Effect of PBM on islets' insulin secretion index. PBM: photobiomodulation. Results are expressed as mean  $\pm$  SEM, One-way ANOVA Welch's with Games Howell post-hoc test.

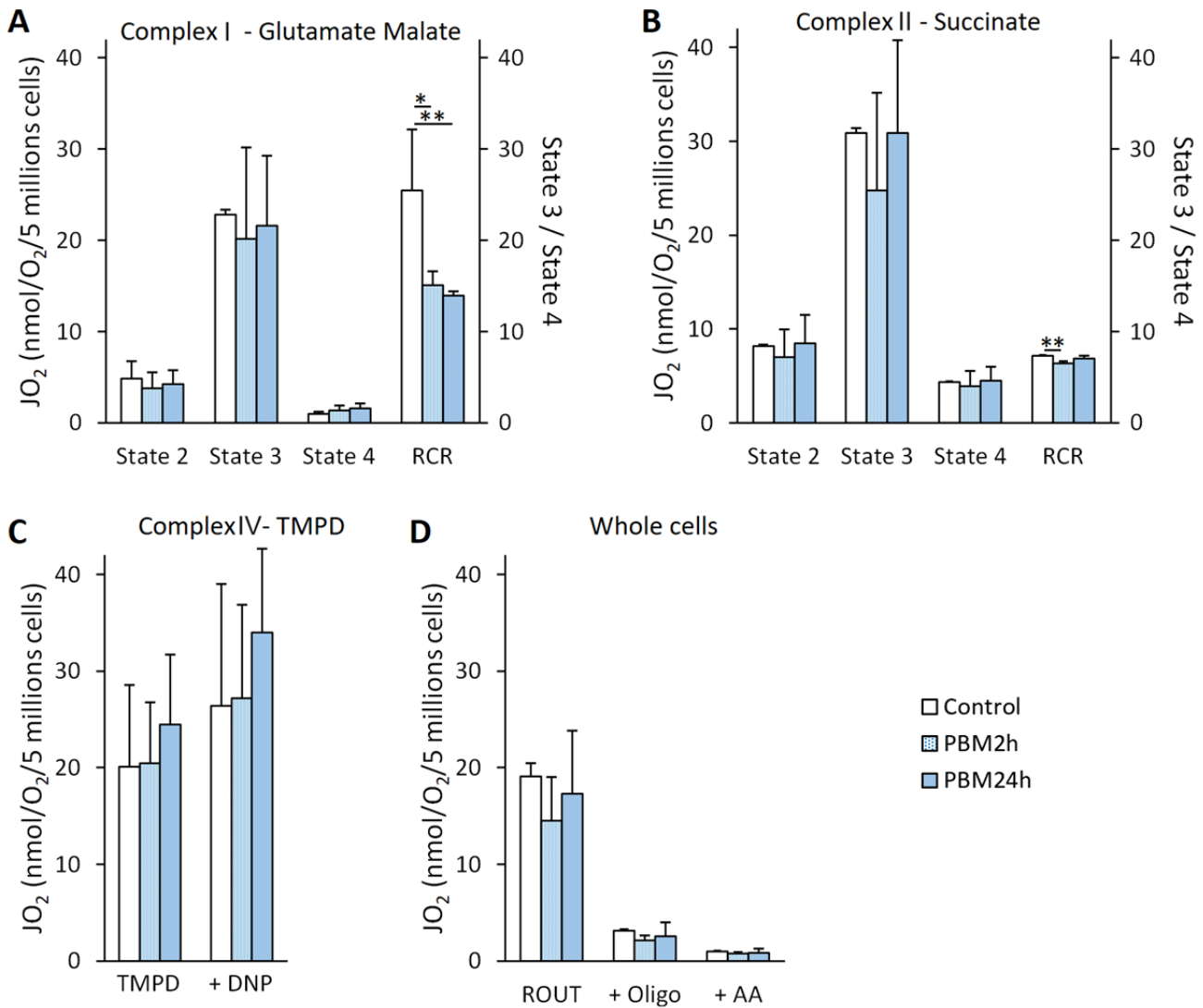
**Figure 3.** Effect of PBM on superoxide production and mitochondrial membrane potential of MIN6 cells



The MIN6 cells are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on MIN6 cells' superoxide production. Data are expressed as the mean fluorescence intensity. (n = 3 for PBM 2h, n = 6 for PBM 24h) **B.** Effect of PBM on the MIN6 cells' mitochondrial masse. **C.** Effect of PBM on the MIN6 cells' mitochondrial membrane potential (n = 3 for PBM 2h, n = 5 for PBM 24h). Data are expressed as the mean fluorescence intensity. The mitochondrial membrane potential is (TMRM-CCCP)/MTG. AU: Arbitrary unit; MTG: Mitotracker Green<sup>TM</sup>; PBM: photobiomodulation. Results are expressed as the mean  $\pm$  SEM, One-way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\* p < 0.01.

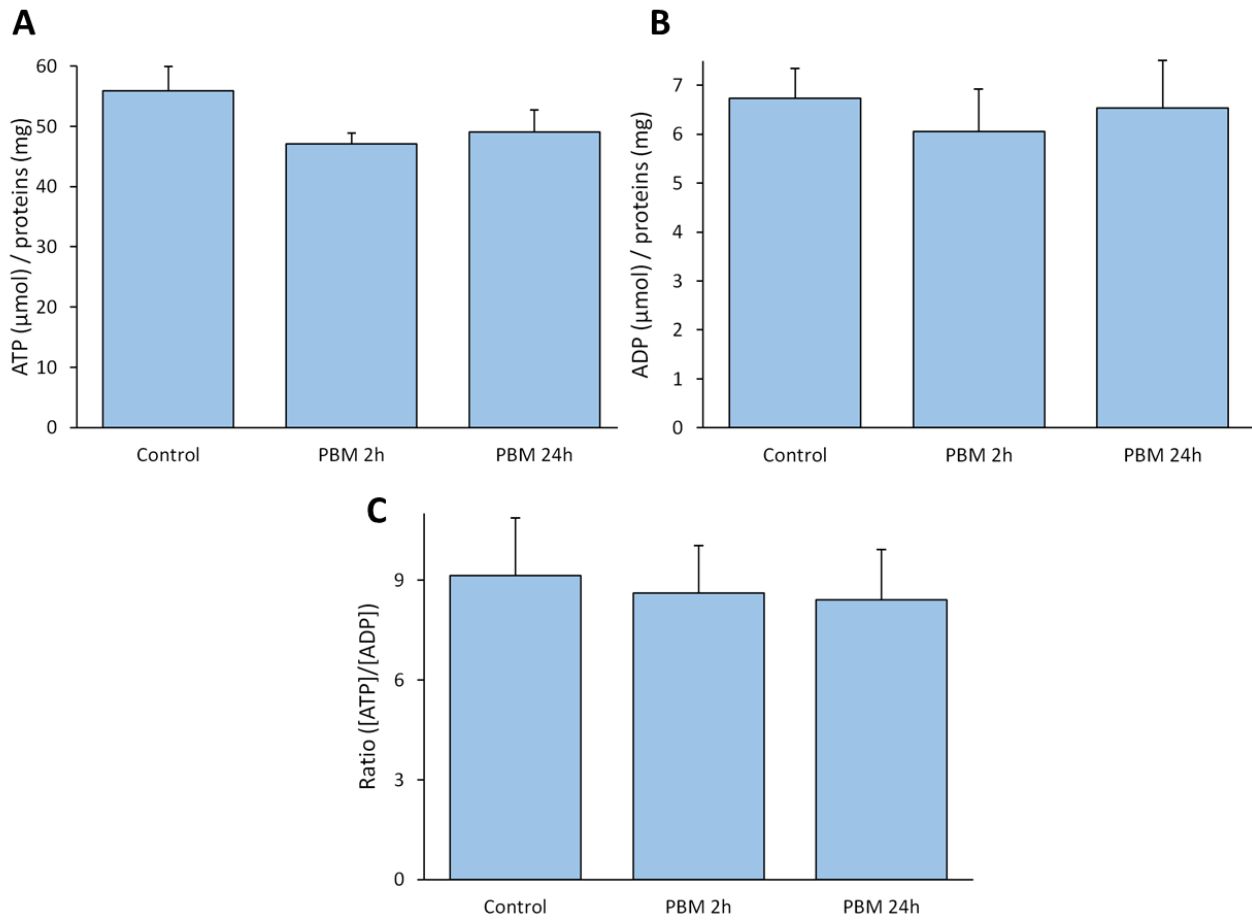


**Figure 4.** Effect of PBM on mitochondrial and cell respiration of MIN6 cells



The MIN6 cells are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on the MIN6 cells' Complex I (Glutamate malate) activity. **B.** Effect of PBM on the MIN6 cells' Complex II (succinate) activity. **C.** Effect of PBM on the MIN6 cells' Complex IV (TMPD) activity. **D.** Effect of PBM on respiration of the whole MIN6 cells. AA: antimycin A; Oligo: Oligomycine; PBM: photobiomodulation; RCR: respiratory control ratio (state 3/state 4) graduation is in the right y-axis; ROUT: routine respiration of intact cells. Data are expressed as nmol of oxygen consumed per minute per 5 million live cells. Results are expressed as the mean  $\pm$  SEM, n = 4, One-way ANOVA Fisher's with Tukey post-hoc test, \* p < 0.05, \*\* p < 0.01.

**Figure 5.** Effect of PBM on ATP and ADP concentrations in MIN6 cells



The MIN6 are exposed to  $2.8 \text{ mW/cm}^2$  PBM illumination. **A.** Effect of PBM on MIN6 cells' ATP content. **B.** Effect of PBM on MIN6 cells' ADP content. **C.** Effect of PBM on the MIN6 cells' ATP/ADP ratio. PBM: photobiomodulation. Data are expressed in  $\mu\text{mol}$  per mg of proteins, Results are expressed as the mean  $\pm$  SEM,  $n = 7$ , One-way ANOVA Welch's with Games-Howell post-hoc test, \*  $p < 0.05$ .

## References

1. Perrier, Q. *et al.* Failure mode and effect analysis in human islet isolation: from the theoretical to the practical risk. *Islets* **13**, 1–9 (2021).
2. Lablanche, S. *et al.* Ten-year outcomes of islet transplantation in patients with type 1 diabetes: Data from the Swiss-French GRAGIL network. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* **21**, 3725–3733 (2021).
3. Lablanche, S. *et al.* Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe hypoglycaemia or poorly controlled glycaemia after kidney transplantation (TRIMECO): a multicentre, randomised controlled trial. *Lancet Diabetes Endocrinol.* **6**, 527–537 (2018).
4. Vantyghem, M.-C., de Koning, E. J. P., Pattou, F. & Rickels, M. R. Advances in  $\beta$ -cell replacement therapy for the treatment of type 1 diabetes. *Lancet Lond. Engl.* **394**, 1274–1285 (2019).
5. Nilsson, B., Ekdahl, K. N. & Korsgren, O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr. Opin. Organ Transplant.* **16**, 620–626 (2011).
6. Toso, C. *et al.* Positron-emission tomography imaging of early events after transplantation of islets of Langerhans. *Transplantation* **79**, 353–355 (2005).
7. Glass, G. E. Photobiomodulation: A review of the molecular evidence for low level light therapy. *J. Plast. Reconstr. Aesthetic Surg. JPRAS* **74**, 1050–1060 (2021).
8. Silveira, P. C. L. *et al.* Effects of photobiomodulation on mitochondria of brain, muscle, and C6 astroglia cells. *Med. Eng. Phys.* **71**, 108–113 (2019).
9. de Freitas, L. F. & Hamblin, M. R. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J. Sel. Top. Quantum Electron. Publ. IEEE Lasers Electro-Opt. Soc.* **22**, 7000417 (2016).
10. Karu, T. Photobiology of low-power laser effects. *Health Phys.* **56**, 691–704 (1989).
11. Huang, Y.-Y., Sharma, S. K., Carroll, J. & Hamblin, M. R. Biphasic dose response in low level light therapy - an update. *Dose-Response Publ. Int. Hormesis Soc.* **9**, 602–618 (2011).
12. Flores Luna, G. L. *et al.* Biphasic Dose/Response of Photobiomodulation Therapy on Culture of Human Fibroblasts. *Photobiomodulation Photomed. Laser Surg.* **38**, 413–418 (2020).
13. Schalch, T. D. *et al.* Photobiomodulation is associated with a decrease in cell viability and migration in oral squamous cell carcinoma. *Lasers Med. Sci.* **34**, 629–636 (2019).
14. Eells, J. T. *et al.* 670 nm Photobiomodulation as a Therapy for Diabetic Macular Edema: A Pilot Study. *Invest. Ophthalmol. Vis. Sci.* **58**, 932 (2017).
15. Robinson, C. C. *et al.* Effects of monochromatic infrared phototherapy in patients with diabetic peripheral neuropathy: a systematic review and meta-analysis of randomized controlled trials. *Braz. J. Phys. Ther.* **21**, 233–243 (2017).
16. Dos Santos Mendes-Costa, L. *et al.* Photobiomodulation: systematic review and meta-analysis of the most used parameters in the resolution diabetic foot ulcers. *Lasers Med. Sci.* **36**, 1129–1138 (2021).
17. Scontri, C. M. C. B. *et al.* Dose and time-response effect of photobiomodulation therapy on glycemic control in type 2 diabetic patients combined or not with hypoglycemic medicine: a randomized, crossover, double-blind, sham controlled trial. *J. Biophotonics* e202300083 (2023) doi:10.1002/jbio.202300083.
18. Liebman, C., Loya, S., Lawrence, M., Bashoo, N. & Cho, M. Stimulatory responses in  $\alpha$ - and  $\beta$ -cells by near-infrared (810 nm) photobiomodulation. *J. Biophotonics* **15**, e202100257 (2022).
19. Irani, S. *et al.* Effect of low-level laser irradiation on in vitro function of pancreatic islets. *Transplant. Proc.* **41**, 4313–4315 (2009).
20. Huang, H.-H., Stillman, T. J., Branham, L. A. & Williams, S. C. The Effects of Photobiomodulation Therapy on Porcine Islet Insulin Secretion. *Photobiomodulation Photomed. Laser Surg.* **40**, 395–401 (2022).
21. Laporte, C. *et al.* Human mesenchymal stem cells improve rat islet functionality under cytokine stress with combined upregulation of heme oxygenase-1 and ferritin. *Stem Cell Res. Ther.* **10**, 85 (2019).
22. Laporte, C. *et al.* Improved human islets' viability and functionality with mesenchymal stem cells and arg-gly-asp tripeptides supplementation of alginate micro-encapsulated islets in vitro. *Biochem. Biophys. Res. Commun.* **528**, 650–657 (2020).
23. Vial, G. & Guigas, B. Assessing Mitochondrial Bioenergetics by Respirometry in Cells or Isolated Organelles. *Methods Mol. Biol. Clifton NJ* **1732**, 273–287 (2018).
24. Zein, R., Selting, W. & Hamblin, M. R. Review of light parameters and photobiomodulation efficacy: dive into complexity. *J. Biomed. Opt.* **23**, 1–17 (2018).

25. Hamblin, M. R. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys.* **4**, 337–361 (2017).
26. Huang, Y.-Y., Nagata, K., Tedford, C. E., McCarthy, T. & Hamblin, M. R. Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. *J. Biophotonics* **6**, 829–838 (2013).
27. Pastore, D., Greco, M. & Passarella, S. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. *Int. J. Radiat. Biol.* **76**, 863–870 (2000).
28. Ball, K. A., Castello, P. R. & Poyton, R. O. Low intensity light stimulates nitrite-dependent nitric oxide synthesis but not oxygen consumption by cytochrome c oxidase: Implications for phototherapy. *J. Photochem. Photobiol. B* **102**, 182–191 (2011).
29. Quirk, B. J. & Whelan, H. T. Effect of Red-to-Near Infrared Light on the Reaction of Isolated Cytochrome c Oxidase with Cytochrome c. *Photomed. Laser Surg.* **34**, 631–637 (2016).
30. Quirk, B. & Whelan, H. T. Effect of Red-to-Near Infrared Light and a Nitric Oxide Donor on the Oxygen Consumption of Isolated Cytochrome c Oxidase. *Photobiomodulation Photomed. Laser Surg.* **39**, 463–470 (2021).
31. Benedicenti, S., Pepe, I. M., Angiero, F. & Benedicenti, A. Intracellular ATP level increases in lymphocytes irradiated with infrared laser light of wavelength 904 nm. *Photomed. Laser Surg.* **26**, 451–453 (2008).
32. Passarella, S. *et al.* Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser. *FEBS Lett.* **175**, 95–99 (1984).
33. Wong-Riley, M. T. T. *et al.* Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. *J. Biol. Chem.* **280**, 4761–4771 (2005).
34. Zhang, R. *et al.* Near infrared light protects cardiomyocytes from hypoxia and reoxygenation injury by a nitric oxide dependent mechanism. *J. Mol. Cell. Cardiol.* **46**, 4–14 (2009).
35. Chaudary, S. *et al.* In vitro effects of 635 nm photobiomodulation under hypoxia/reoxygenation culture conditions. *J. Photochem. Photobiol. B* **209**, 111935 (2020).
36. Onaca, N., Takita, M., Levy, M. F. & Naziruddin, B. Anti-inflammatory Approach With Early Double Cytokine Blockade (IL-1 $\beta$  and TNF- $\alpha$ ) Is Safe and Facilitates Engraftment in Islet Allograft Transplantation. *Transplant. Direct* **6**, e530 (2020).

## Article n° 2

# Impact of photobiomodulation on beta cells and islets under stress condition in vitro and in vivo approach

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**Key Words:** Diabetes, Photobiomodulation, Beta cells, insulinosecretion, stress conditions, functionality, viability, in vivo, diabetic rat

### Abbreviations:

AA: Antimycin A

AUC: Area under the curve

CCCP: Carbonyl cyanide m-chlorophenyl hydrazone

ELISA: Enzyme-linked immunosorbent assay

GSIS: Glucose stimulated insulin secretion

IFN- $\gamma$ : Interferon- $\gamma$

IL-1 $\beta$ : Interleukin-1 $\beta$

MIN6: Mouse insulinoma cells

MTG: MitoTracker GreenFM

OGTT: Oral glucose tolerance test

SDS : Substrate deprivation stress

STZ: Streptozotocin

TMRM: Tetramethylrhodamine methyl ester

TNF- $\alpha$ : Tumor necrosis factor  $\alpha$

## Abstract

**Introduction.** There has been a historical progression of diabetes treatment, particularly type 1 diabetes, from insulin discovery to technological advancements, such as closed-loop systems. Cellular therapy using pancreatic islets has emerged as an option for severe cases. Challenges remain, including islet isolation and transplantation issues. PBM, a technique involving light absorption by chromophores to induce biological changes, is investigated for its potential to mitigate stress-induced damage in pancreatic beta cells.

**Materials & Methods.** MIN6 cells (pancreatic beta cell line) and rat pancreatic islets are subjected to different stress conditions, such as substrate deprivation (no glucose, no foetal bovine serum), cytokine exposure (TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ ), and hypoxia (1% O<sub>2</sub>). PBM is applied either before or during these stress conditions (LED device, 670 nm, 2.8 mW/cm<sup>2</sup>). Various assays are conducted to assess cell viability (Annexin/propidium iodide (PI) for MIN6 by FACS, or Syto13/PI for islets by confocal microscopy), insulin secretion (by glucose stimulated insulin secretion assay), mitochondrial function (superoxide production by MitoSOX and mitochondrial membrane potential by TMRM), and ATP/ADP contents (measured by HPLC). Additionally, a diabetic rat model is used for islet transplantation (pre-conditioning with PBM or not) experiments.

**Results.** Substrate Deprivation: PBM applied before or during substrate deprivation increased cell and islet viability, prevents insulin secretion decline, decreased superoxide production, and improves mitochondrial membrane potential. However, PBM does not impact mitochondrial and cell respiration or ATP/ADP ratios. Cytokine Stress: PBM applied during cytokine stress enhances cell and islet viability, prevents insulin secretion reduction, and decreases superoxide production. Yet, mitochondrial membrane potential remains altered, and ATP/ADP ratio unchanged. Hypoxic Stress: PBM does not protect cells and islets from the negative effects of hypoxia on viability and insulin secretion. In the islet transplantation model, both pre-illuminated and non-illuminated islets improved metabolic control in diabetic rats. No additional benefit of PBM is observed when islets are illuminated before transplantation.

**Discussion.** PBM may have beneficial effects on pancreatic beta cell viability and functionality under certain stress conditions. However, its effectiveness varies depending on the type of stress applied. Moreover, application of PBM prior to islet transplantation led to metabolic control comparable to non-illuminated islet. Further study with transplantation model closer to human islet transplantation must be done to highlight the long-term effects of PBM.

## Introduction

Since the discovery of insulin in 1921<sup>1</sup>, the treatment of type 1 diabetes (T1D) has continuously evolved, progressing from isolated and purified insulin to synthetic insulins, and subsequently incorporating technological innovations such as insulin pumps, continuous glucose monitoring, and more recently, closed loop systems<sup>2</sup>. From the early 2000s, cellular therapy based on pancreatic islets has emerged as a therapeutic option for patients with unstable T1D experiencing severe hypoglycaemic episodes<sup>3</sup>. Studies in this field have demonstrated the potential benefit of islet transplantation on patients' quality of life<sup>4</sup> and glycaemic control, including a reduction in severe hypoglycaemic events, despite a long-term insulin independence rate of around ten percent at ten years post-transplantation<sup>5</sup> and half patient with HbA1c under 7% 8 years after the islet transplantation<sup>6</sup>. However, challenges persist regarding the availability, isolation, and transplantation of human islets.

The isolation of human islets is a lengthy and intricate process<sup>7</sup> that disconnects the islets from their microenvironment, followed by a 24h to 72h culture period and then by an islet intra portal injection. Nonetheless, it is estimated that 50% of the islets are destroyed upon injection, primarily due to various stresses such as nutrient and substrate deprivation, hypoxia, and inflammatory stress (instant blood mediated reaction = IBMIR) particularly evident with human pancreatic islets<sup>8-10</sup>. Several techniques aim to mitigate islet damage caused by these stresses.

Photobiomodulation (PBM), also known as low level laser therapy, involves the selective absorption of specific wavelengths by chromophores, inducing biological changes. The biological mechanism of PBM is grounded in the hypothesis that cytochrome C oxidase (CCO) absorbs red and near-infrared wavelengths, altering cellular energy status by modifying mitochondria function, which affects adenosine triphosphate (ATP) production and reactive oxygen species (ROS) generation. This modulation also influences the expression of various factors such as cytokines and transcription factors to promote cell viability, functionality, and proliferation<sup>11,12</sup>.

Preclinical studies have demonstrated positive effects of PBM on inflammatory processes, immunomodulation, analgesia, wound healing, and cellular regeneration<sup>13,14</sup>. Clinical trials are even exploring its use in neurodegenerative conditions like Parkinson's disease. Given the established metabolic aspect of these pathologies, investigating PBM's impact on other metabolic disorders such as diabetes is warranted. Clinical studies have investigated the application of PBM in diabetes-related complications, including ulcers<sup>15</sup>, periodontitis<sup>16</sup>, retinopathy<sup>17</sup>, neuropathies<sup>18</sup>, and post-exercise recovery<sup>19</sup>, showing varied outcomes due to differences in illumination parameters. Limited in vitro research has directly examined the effect of PBM on porcine<sup>20</sup> and rodent islets<sup>21</sup>, or pancreatic beta cell lines<sup>22</sup>, yielding heterogeneous results.

The objective of our study is to assess the effect of prolonged preconditioning or simultaneous application of PBM under different stress conditions (substrate deprivation, cytokines and hypoxia) on the viability and functionality of pancreatic beta cell lines and rat islets. Furthermore, we planned to evaluate the impact of PBM preconditioning on transplanted rat islets in a diabetic rat model.



## Materials and methods

- Cell culture and rat islet isolation

MIN6 cells (AddexBio, San Diego, California, USA) were cultured in DMEM with 24.8 mM glucose (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts, USA) supplemented with 15% foetal bovine serum (FBS), 1% penicillin-streptomycin, and 50  $\mu$ M 2- $\beta$  mercaptoethanol. Cells were cultured for 48h before experimentation, and each conditions comprised 400,000 cells. Rat pancreatic islets were obtained as previously described from Wistar rats (Charles Rivers, Lyon, France) using mechanical and chemical digestion (collagenase IX), followed by purification<sup>23</sup>. Islets were stored for 24h before experimentation in RPMI 1640 with 11 mM glucose (Thermo Fisher Scientific, Bourguin-Jailleu, France) supplemented with 10% (v/v) FBS, 1% (v/v) sodium pyruvate, 1% (v/v) L-glutamine, and 1% (v/v) penicillin-streptomycin.

- Photobiomodulation procedure

The illumination (PBM) was performed by a PBM device (EFFI-BL-IP69K, Effilux, Les Ulis, France) LED, incoherent light, wavelength of 670 nm, continuous output power, 2.8 mW/cm<sup>2</sup> in a wet incubator (37°C, 5% CO<sub>2</sub>). As the illumination bench generate heat, a chiller was added to keep the incubator temperature stable at 37°C. PBM irradiation parameters were monitored and adjusted using a power and optical energy meter (PM100D, Thorlabs, Newton, New Jersey) with a photodiode power sensor (S120C, Thorlabs). The MIN6 were illuminated 48h after passage and islets were illuminated 24h after isolation.

- Substrate deprivation stress (SDS)

Substrate deprivation was achieved by replacing the complete medium with substrate-free DMEM medium (no glucose, no FBS). MIN6 cells were incubated with 2 mL of substrate-free DMEM. Substrate deprivation was maintained for 24h. After substrate deprivation, cells were cultured in complete medium for 2h. Analyses were carried out immediately afterwards. MIN6 cells were illuminated during substrate deprivation and 2h complete medium culture (during 26h) or before substrate deprivation (24h PBM). Pancreatic islets were incubated with 2mL of substrate-free DMEM. Substrate deprivation was maintained for 90min. After substrate deprivation, islets were cultured 2h in complete medium. Cells were illuminated during substrate deprivation and 2h complete medium culture (210min PBM) or before substrate deprivation (during 24h). Time to exposure to SDS was previously defined (data not shown) to obtain an alteration of viability between 20 and 40%. Analyses were performed immediately afterwards.

- Cytokine stress

MIN6 cells were incubated 24h with 2mL of DMEM complete medium containing 600 UI/mL mouse IL-1 $\beta$  (Sigma-Aldrich, Darmstadt, Germany), 6,000 UI/mL mouse TNF- $\alpha$ , (Sigma-Aldrich), and 6,000 UI/mL mouse IFN- $\gamma$  (Sigma-Aldrich) as previously described<sup>24</sup>. MIN6 cells illumination was performed during cytokine stress (24h PBM) or before cytokine stress (during 24h). Pancreatic islets were incubated 24h with 2 mL of DMEM complete medium containing 600 UI/mL human IL-1 $\beta$ , 6,000 UI/mL human TNF- $\alpha$ , and 6,000 UI/mL mouse IFN- $\gamma$ . Islets were illuminated during cytokine stress (during 24h) or before cytokine stress (24h PBM). Analyses were performed immediately afterwards.

- Hypoxic stress

Hypoxia was induced using a perfusion chamber (POC chamber, LaCom®, Erbach, Germany) coupled with an incubation system capable of regulating the oxygen concentration from 21% to 1% by injecting N<sub>2</sub> (O<sub>2</sub>-CO<sub>2</sub>-°C, PeCom®, Erbach). Hypoxia was induced in a humid environment at 37°C, 5% CO<sub>2</sub>, and 1% O<sub>2</sub>. MIN6 cells were incubated in 2 mL of hypoxic DMEM complete medium. Hypoxia was maintained for 24h. After hypoxia, cells were cultured 2h in non-hypoxic DMEM complete medium. Analyses were performed immediately afterwards. Cells were illuminated during hypoxia and 2h culture in non-hypoxic DMEM (26h PBM) or before hypoxia (24h PBM).

Pancreatic islets were incubated in 2 mL of hypoxic complete RPMI medium. Hypoxia was maintained for 16h. The pancreatic islets were then cultured 2h in non-hypoxic complete RPMI medium for 2h. Pancreatic islet illumination was performed during hypoxia and 2h culture in non-hypoxic complete medium (18h PBM) or before hypoxia (24h PBM). Analyses were performed immediately afterwards.

- Viability assays

MIN6 cell viability was performed as previously described<sup>23</sup>. Briefly, it was done using a BD LSR Fortessa™ flow cytometer (Beckton-Dickinson Biosciences, Pont-de-Claix, France) with Annexin V-FP488 (Interchim, Montluçon, France) and propidium iodide (PI) labelling (stock solution at 1mg/mL, Thermo Fisher Scientific). Results were reported as a percentage of viability. Islet viability (at least 50 islets equivalent per condition) was analysed by confocal microscopy as previously described<sup>23</sup>. Briefly, it was done using a Leica TCS CSU SP8 confocal microscope (LEICA, Microsystems Heidelberg, Germany) with 1 µM Syto13 (Thermo Fisher Scientific, labelled alive cells in green) and PI (Thermo Fisher Scientific, labelled necrotic cells in red). Results were expressed as a percentage of viability = 100 \* number of green fluorescent cells / (total number of green and red cells).

- Mitochondrial superoxide production

Superoxide production measurement was performed by FACS with 1  $\mu$ M MitoSOX™ Red (Invitrogen, Carlsbad, California, USA) as previously described<sup>23</sup>.

- Mitochondrial membrane potential

Mitochondrial membrane potential measurement of MIN6 was performed by FACS with 100 nM tetramethylrhodamine methyl ester (TMRM) (Life Technologies, Carlsbad, California, USA), 100 nM MitoTracker GreenFM (MTG) (Life Technologies), and 250  $\mu$ M carbonyl cyanide m-chlorophenyl hydrazone (CCCP) (Sigma-Aldrich) as previously described<sup>23</sup>. Mitochondrial membrane potential was calculated as the difference in TMRM fluorescence before and after the addition of CCCP and then divided by mitochondrial mass (MTG).

- Glucose stimulated insulin secretion (GSIS) assay

GSIS was performed as previously described<sup>23</sup> in static incubation using Krebs-Ringer bicarbonate buffer medium supplemented with 0.1% bovine serum albumin (BSA). After 1h of pre-incubation in 2.8 mM glucose, cells or islets were incubated for 1h at 37°C in 2.8 mM glucose (basal condition) and then incubated for 1h in 16.7 mM glucose (stimulated condition). Insulin content was extracted by overnight incubation at -20°C in an ethanol acid solution. The assays were done by enzyme-linked immunosorbent assay (Eurobio Scientific, Les Ulis, France). The results were expressed as a percentage of secreted insulin in relation to the total (contained and secreted), and the stimulation index was  $100 \times (\text{insulin secreted in high glucose} / \text{insulin secreted in low glucose})$ . 100 islets equivalent per condition were used for GSIS assay.

- Mitochondrial and cell oxygen consumption

This was performed as previously described<sup>23</sup>. The rate of oxygen consumption ( $J_{O_2}$ ) was measured at 37°C using a Clark-type  $O_2$  electrode in a 1 mL-chamber filled with 500  $\mu$ L of respiration buffer. MIN6 cells were detached with 2X trypsin-EDTA, centrifuged, and used for the experiment. For Complexes I and II activity of MIN6 cells: cells (5 million) were permeabilized with 2% digitonin, and the measurement was made in the presence of glutamate 5 mM/malate 2.5 mM (Complex I) or succinate 5 mM/rotenone 1  $\mu$ M (Complex II) as substrate (state 2), after the addition of adenosine diphosphate (ADP) 0.5 mM (state 3), followed by the addition of oligomycin 2  $\mu$ g/mL (state 4). The respiratory control ratio (RCR) was obtained by dividing state 3 (ADP) by state 4 (oligomycin). It reflected how the respiratory was coupled to the ATP production, an increase in RCR translates into an increase of ATP production for a given amount of oxygen consumed. For Complex IV activity of MIN6 cells: cells (2.5 million) were permeabilized with 2% digitonin, and the measurement was made in the presence of antimycin A (AA) 1  $\mu$ M/ascorbate 4 mM, followed by TMPD 0.5 mM/ascorbate 0.25 mM and finally DNP 150  $\mu$ M. For MIN6 cells oxygen consumption (5 million): the measurement was made in the basal condition (routine respiration of intact cells (ROUT)), followed by the addition of oligomycin 2  $\mu$ g/mL and finally AA 1  $\mu$ M. Results were expressed for 5 million of live cells.

- ATP/ADP content

MIN6 cells were incubated for a minute in a solution of 2.5% perchloric acid and 6.25 mM EDTA, then detached mechanically and centrifuged. The neutralization was done by adding a KOMO solution (KOH 2N and 3-morpholinopropane-1-sulfonic acid 0.3 M). Protein-free extract was separated on a C18 HPLC column in pyrophosphate buffer at a flow rate of 1 mL/min and 30°C. ATP and ADP eluted at 3 and 5min, respectively. Elution peaks were integrated with the STAR software. Results were expressed in  $\mu$ mol of nucleotides per mg of proteins (measured with Pierce kit (Thermo Scientific)).

- Animal model and groups

All animal experiments were authorized by the French Ministry of Higher Education and Research and approved by the Animal Experimentation Ethics Committee (APAFIS #41704-2023010617343060). A total of 32 male Lewis rats, aged 7 weeks and weighing 240 and 310 g, were obtained from Charles Rivers. All rats were housed under a 12-hour light/dark cycle at 24°C with ad libitum access to food and water. To induce diabetes, rats were injected intraperitoneally with 1% streptozotocin (STZ, Sigma Aldrich) at a dose of 60 mg/kg of body weight. The control group (n=8) was injected with diluent. Blood glucose levels were monitored daily, and glycemia above 16.5 mM for more than one day was defined as diabetes. It was estimated that two pancreases were necessary for single islet transplantation (1000 islet equivalent (IEQ) has been transplanted). After islets isolation (from other Lewis rat), they were stored overnight. Half of the islets were illuminated for 24h and transplanted under the kidney capsule. The groups were as follows: control (n=8, sham injection, sham surgery), diabetes (n=8, sham surgery), diabetes with islet transplantation (n=8, 1000 IEQ transplanted per rat), and diabetes with pre-illuminated islet transplantation (n=8, 1000 IEQ transplanted per rat). Two weeks after STZ injection, all animals were fasted for 12h and islet transplantation was performed as previously described<sup>25</sup>. Briefly, animals were anesthetized and maintained with isoflurane. After an incision was made at the right back site of the animal, the kidney was exposed, and the islets were placed under the kidney capsule. After the surgery, the animals received subcutaneous injections of 0.05 mg/kg of buprenorphine three times a day for two days. For the control group, sham surgery was performed without islets. Glycemia and weight were monitored regularly. Several blood samples were collected to check for C-peptide secretion. Blood was centrifuged at 4°C at 3000rpm for 15min and samples were then stored at -80°C until analysis. An oral glucose tolerance test (OGTT) was performed 35 days after surgery. The animals were fasted for 12h, and then fed a standard dose of 30% glucose (2 g/kg weight), and glycemia was monitored at different time points: 0, 5, 10, 15, 25, 40, 60, 90, and 120min. After the experiment, the area under the curve (AUC) was calculated. Euthanasia was performed after anaesthesia induction using isoflurane 42 days after the surgery.

- Statistical Analyses

Results are expressed as mean value  $\pm$  SEM (standard error of mean). All statistical tests were performed using Jamovi software (version 2.25). The different groups were compared using ANOVAs (or an equivalent non-parametric test if the assumptions were not met). A value was considered significant if  $p < 0.05$ .

## Results

- PBM prevents alteration of viability induced by SDS both on MIN6 cells and rat islets but had no significant effect on insulin secretion

Substrate deprivation decreased MIN6 (Fig. 1A), and islet (Fig. 1B) viability compared to control (for MIN6,  $61.3 \pm 1.7\%$  vs.  $86.6 \pm 1.0\%$ ,  $p < 0.001$ ; for islets,  $69.6 \pm 5.9\%$  vs.  $97.8 \pm 1.4\%$ ,  $p < 0.001$ ). PBM (applied before stress or during stress) enhanced MIN6 and islet viability compared to stress conditions (MIN6 viability when PBM applied before SDS:  $72.4 \pm 3.4\%$ ,  $p = 0.007$  vs. stress conditions; MIN6 viability when PBM applied during SDS:  $73.5 \pm 1.6\%$ ,  $p < 0.001$  vs. stress conditions; islet viability when PBM applied before:  $86.3 \pm 6.9\%$ ,  $p = 0.005$  vs. stress conditions; islet viability when PBM applied during SDS:  $85.8 \pm 6.8\%$ ,  $p = 0.006$  vs. stress conditions).

Substrate deprivation decreased MIN6 insulin stimulation index compared to MIN6 control ( $1.07 \pm 0.52$  vs.  $2.00 \pm 0.55$ ,  $p = 0.014$  vs. control). No significant difference was observed on insulin stimulation index of MIN6 exposed to PBM before stress ( $1.63 \pm 0.52$ ,  $p = 0.59$ ) or to PBM during stress ( $1.62 \pm 0.67$ ,  $p = 0.55$ ) as compared to control (Fig. 1C). Substrate deprivation did not alter GSIS of islets nor PBM ( $p = 0.725$ , Fig. 1D).

- PBM prevents increase of superoxide production and decrease of mitochondrial membrane potential induced by SDS

Substrate deprivation increased MIN6 superoxide production compared to control ( $4,369 \pm 904$  vs.  $865 \pm 308$ ,  $p < 0.001$ ). PBM applied before stress ( $3,054 \pm 753$ ,  $p < 0.001$ ) or during stress ( $2,454 \pm 405$ ,  $p < 0.001$ ) decreased superoxide ion production compared to stress conditions (Fig. 1E).

Substrate deprivation decreased MIN6 mitochondrial membrane potential compared to control ( $1.30 \pm 0.55$  vs.  $2.05 \pm 0.55$ ,  $p = 0.003$ ). PBM applied before stress ( $1.98 \pm 0.25$ ,  $p = 0.046$ ) or during stress ( $2.00 \pm 0.52$ ,  $p = 0.007$ ) enhanced mitochondrial membrane potential compared to stress conditions without change in the mitochondrial mass ( $p = 0.303$ , Fig. 1F).



- PBM had no impact on mitochondrial and cell respiration of MIN6 under SDS

For Complex I, SDS significantly decreased state 3 oxygen consumption compared to control ( $7.40 \pm 1.85 \text{ JO}_2/\text{min}/5$  millions on living cells vs.  $22.8 \pm 0.5 \text{ JO}_2/\text{min}/5$ - millions on living cells,  $p = 0.007$ ) and PBM did not prevent this alteration. Neither SDS nor PBM altered state 4 oxygen consumption ( $p = 0.321$ ). The RCR was also decreased by the stress ( $10.3 \pm 1.5$  vs.  $25.5 \pm 6.7$ ,  $p < 0.001$ ) (Fig. 2A). For Complex II, SDS significantly decreases oxygen consumption for the state 2 ( $3.27 \pm 0.84 \text{ JO}_2/\text{min}/5$  millions on living cells vs.  $8.15 \pm 0.17 \text{ JO}_2/\text{min}/5$  millions on living cells,  $p < 0.001$ ), the state 3 ( $11.3 \pm 1.7 \text{ JO}_2/\text{min}/5$  millions on living cells vs.  $30.9 \pm 0.5 \text{ JO}_2/\text{min}/5$  millions on living cells,  $p < 0.001$ ), and the state 4 ( $2.05 \pm 0.21 \text{ JO}_2/\text{min}/5$  millions on living cells vs.  $4.33 \pm 0.05 \text{ JO}_2/\text{min}/5$  millions on living cells,  $p < 0.001$ ), but not the RCR ( $6.78 \pm 1.21$  vs.  $7.13 \pm 0.10$ ,  $p < 0.001$ ) compared to control. PBM did not allow to prevent these alterations (Fig. 2B). Neither SDS nor PBM altered oxygen consumption of Complex IV (Fig. 2C). For ROUT, SDS significantly decreased oxygen consumption compared to control ( $8.7 \pm 1.7 \text{ JO}_2/\text{min}/5$  millions on living cells vs.  $18.1 \pm 1.3 \text{ JO}_2/\text{min}/5$  millions on living cells,  $p < 0.001$ ) and PBM did not prevent this alteration (Fig. 2D).

- PBM maintained ATP and ADP content in MIN6 cells submitted to SDS

SDS significantly decreased ATP (Fig. 3A) and ADP (Fig. 3B) contents of MIN6 cells compared to control (respectively:  $21.9 \pm 4.1 \text{ } \mu\text{mol}/\text{mg}$  of proteins vs.  $55.0 \pm 10.9 \text{ } \mu\text{mol}/\text{mg}$ ,  $p < 0.001$  and  $3.4 \pm 1.6 \text{ } \mu\text{mol}/\text{mg}$  vs.  $6.5 \pm 3.5 \text{ } \mu\text{mol}/\text{mg}$ ,  $p = 0.046$ ). PBM restored ATP and ADP contents if applied before the stress (respectively:  $44.9 \pm 15.1 \text{ } \mu\text{mol}/\text{mg}$ ,  $p = 0.450$  vs. control and  $7.9 \pm 5.0 \text{ } \mu\text{mol}/\text{mg}$ ,  $p = 0.99$  vs. control), or during stress (respectively:  $44.6 \pm 19.0 \text{ } \mu\text{mol}/\text{mg}$ ,  $p = 0.56$  vs. control and  $7.2 \pm 4.8 \text{ } \mu\text{mol}/\text{mg}$ ,  $p = 0.99$  vs. control). SDS neither PBM had impact on ATP/ADP ratio of MIN6 cells ( $p = 0.161$ , Fig. 3C).

- PBM prevents alteration of viability and insulin secretion induced by cytokine stress

Cytokines decreased MIN6 (Fig. 4A) and islet (Fig. 4B) viability compared to control ( $70.5 \pm 5.6$  % for MIN6 viability under cytokines stress vs.  $82.3 \pm 5.0$  % for control MIN6,  $p < 0.001$ ;  $63.5 \pm 6.0$  % for islet viability under cytokines stress vs.  $99.3 \pm 0.5$  % for control islet,  $p < 0.001$ ). PBM applied during stress enhanced MIN6 viability ( $78.1 \pm 5.1$  %,  $p = 0.011$ ) compared to stress condition. No difference with stress condition was observed if PBM was applied before stress ( $70.4 \pm 6.0$  %,  $p = 1$ ). PBM applied before or during stress enhanced islet viability (respectively  $73.3 \pm 6.4\%$ ,  $p = 0.01$ ; and  $75.2 \pm 3.9\%$ ,  $p = 0.002$ ) compared to stress conditions.

Cytokines decreased MIN6 (Fig. 4C) and islet (Fig. 4D) insulin stimulation index compared to control (respectively  $0.93 \pm 0.10$  for MIN6 stimulation index under cytokines stress vs.  $1.37 \pm 0.23$  for control MIN6,  $p = 0.027$ ;  $0.80 \pm 0.21$  for islet stimulation index under cytokines stress vs.  $1.56 \pm 0.46$  for control Islet,  $p = 0.044$ ). PBM exposition before or during stress restored completely MIN6 and islet insulin stimulation index (PBM applied before stress: MIN6 stimulation index:  $1.70 \pm 0.35$ ,  $p = 0.116$  as compared to control,  $p < 0.001$  as compared to stress condition; islet stimulation index:  $1.60 \pm 0.53$ ;  $p = 0.99$  as compared to control and  $p = 0.032$  as compared to stress conditions; PBM applied before the stress: MIN6 stimulation index:  $1.47 \pm 0.15$ ,  $p = 0.878$  as compared to control,  $p = 0.006$  as compared to stress condition; islet stimulation index:  $1.65 \pm 0.56$ ;  $p = 0.99$  as compared to control,  $p = 0.022$  as compared to stress conditions).

- PBM prevents increase of superoxide production but had not impact on alteration of mitochondrial membrane potential induced by cytokine stress

Cytokines increased MIN6 superoxide production compared to control ( $1,384 \pm 66$  vs.  $875 \pm 56$ ,  $p < 0.001$ ). PBM applied during stress ( $1,201 \pm 52$ ,  $p = 0.027$ ) decreased superoxide ion production compared to stress conditions, but no impact of PBM was observed if applied before the stress ( $1,358 \pm 71$ ,  $p = 0.95$ , Fig. 4E).

Cytokines decreased MIN6 mitochondrial membrane potential compared to control ( $1.51 \pm 0.31$  vs.  $2.17 \pm 0.34$ ,  $p < 0.001$ ). PBM did not prevent the alteration of mitochondrial membrane potential induced by cytokines (Fig. 4F).

- PBM and cytokines did not alter ATP/ADP ratio of MIN6 cells

Cytokines significantly decreased ATP content of MIN6 cells compared to control ( $30.3 \pm 11.5 \mu\text{mol/mg}$  vs.  $55.4 \pm 11.3 \mu\text{mol/mg}$ ,  $p = 0.005$ , Fig. 5A), but did not affect ADP content ( $p = 0.401$ , Fig. 5B). PBM did not prevent the alteration of ATP production and did not alter ADP content. Cytokines neither PBM had impact on ATP/ADP ratio of MIN6 cells ( $p = 0.930$ , Fig. 5C).

- PBM did not prevent alteration of viability and insulin secretion induced by hypoxic stress

Hypoxia decreased MIN6 and islets viability compared to control (respectively  $65.8 \pm 4.9 \%$  vs.  $82.5 \pm 2.0 \%$ ,  $p = 0.008$  and  $73.9 \pm 7.5 \%$  vs.  $96.7 \pm 1.7 \%$ ,  $p < 0.001$ ). PBM was not able to prevent alteration of MIN6 and islets viability (Fig. 6A and B).

Hypoxia altered MIN6 and islets GSIS and decreased the insulin stimulation index compared to control (respectively  $0.92 \pm 0.11$  vs.  $1.47 \pm 0.20$ ,  $p < 0.001$  and  $0.82 \pm 0.29$  vs.  $2.07 \pm 0.37$ ,  $p = 0.002$ ). PBM was not able to prevent alteration of MIN6 and islets insulin stimulation index (Fig. 6C and D).

- Islets transplantation in diabetic rat increase metabolic control either in case of preconditioning with PBM with non-incremental benefit of PBM

At the time of surgery (Day 0), both weight and blood glucose levels were comparable for diabetic groups ( $p > 0.6$  for both, Fig. 7A and B). Transplantation of control or pre-illuminated islets allowed improvement in glycemia after 3 days (Fig. 7B), body mass after 10 days (Fig. 7A) and C-peptide levels after 7 days (Fig. 7C) as compared to non-transplanted diabetic rats. Glycemia no longer differed from those of the control group after Day 28. C-peptide no longer differed from those of the control group after Day 28. Baseline glucose levels during the OGTT did not differ between the islet graft groups

(illuminated or not) and control groups ( $p > 0.2$ ) and were lower than those of the diabetic group ( $p < 0.05$ ) (Fig. 7D). In the diabetic group, 100% of the animals exhibited blood glucose levels  $> 500$  mg/dL during the OGTT, compared to one animal in the non-illuminated islet group, none in the pre-illuminated islet group. Higher glucose intolerance was observed in the diabetic group ( $AUC = 61.1 \pm 5.7$  mg/dL\*min\* $10^3$ ) compared to the control group ( $17.4 \pm 1.5$  mg/dL\*min\* $10^3$ ,  $p < 0.001$ ) and the non-illuminated islet group ( $34.4 \pm 13.7$  mg/dL\*min\* $10^3$ ,  $p = 0.003$ ) or pre-illuminated islet group ( $35.5 \pm 5.5$  mg/dL\*min\* $10^3$ ,  $p < 0.001$ ). No difference on AUC is observed between non-illuminated and illuminated islet (Fig. 7E).

## Discussion

We demonstrated that preconditioning or simultaneous application of PBM has a beneficial effect on the viability and functionality of pancreatic islets and beta cells with an effect dependent on the type of stress applied.

With regard to hypoxic stress, PBM did not demonstrate benefits in insulin secretion or viability in MIN6 cells and islets. Ischemia-reperfusion injuries seem to have little connection to mitochondrial metabolic impairment in beta cells (INS1), as indicated by minimal alterations in the mitochondrial membrane potential and ROS production<sup>26</sup>. These results, although discordant with the protective effect of PBM on myoblasts/fibroblasts subjected to hypoxia<sup>27</sup>, suggest that the effect of PBM could be depends on target tissues and cells.

Islets, such as neurons, lack glycogen reserves and are thus highly sensitive to substrate fluctuations. Lablanche et al<sup>26</sup>. previously demonstrated that pancreatic beta cells (INS1) are highly susceptible to substrate deprivation, which results in reduced viability, increased ROS production, and decreased in mitochondrial membrane potential. We observed similar alterations in the viability and functionality of MIN6 cells. Preconditioning or simultaneous application of PBM under substrate deprivation

demonstrated a protective effect on viability and complete restoration of the mitochondrial membrane potential, as ATP and ADP content of MIN6 cells. Additionally, superoxide ion production decreased by 30% in preconditioned conditions and by 44% with simultaneous PBM application under stress compared to stressed conditions only. We also investigated the effect of substrate deprivation on insulin secretion. In MIN6 cells, substrate deprivation abolished insulin secretion in response to glucose, whereas this response remained unaltered in rat islets. One hypothesis could be the importance of intercellular contact and paracrine communication among islet cells. Pseudo-islets composed only with beta cells exhibited significantly greater insulin secretion in response to glucose compared to pseudo-islets containing all islet cells<sup>28</sup>. These interactions appear to contribute to appropriate and regulated insulin secretion, potentially explaining the different responses to stress observed between individual beta cells and islets.

The common proposed mechanism of action of PBM is based on its absorption by CCO (Complex IV) and an associated increase in membrane potential, leading to enhanced ATP production and extrapolation of mitochondrial respiratory chain function<sup>12</sup>. While this has been demonstrated only in one article in a particular model of isolated cytochrome c oxidase from horse hearts<sup>29</sup>, it has not been replicated despite multiple attempts<sup>30-32</sup>. In our study, we did not observe any significant effects of PBM on CCO during SDS. Substrate deprivation resulted in impaired oxygen consumption in respiratory chain Complexes I and II state 3, as well as Complex II state 4. This inhibition displayed a pattern consistent with nitric oxide (NO) inhibition<sup>33</sup>. Notably, NO functions as an inhibitor of Complex IV. The complex interplay between ROS and NO led to the production of nitrite oxide, an inhibitor of Complex I. Another proposed mechanism underlying the action of PBM was the photo-dissociation of NO from CCO facilitated by PBM<sup>34</sup>. The lack of observable effects from PBM could potentially be attributed to an excessive quantity of NO within substrate-deprived stressed cells, consequently rendering the photo-dissociation of NO ineffective. Substrate deprivation triggers a significant energy stress, leading to a pronounced reduction in ATP and ADP content, while maintaining an unaltered

ATP/ADP ratio. This phenomenon represents a cellular safeguard response typically occurring prior to cell demise. In order for the remaining cellular ATP to be energetically effective, it becomes imperative to decrease the ADP quantity to re-establish a normalized ratio.

Islets are particularly sensitive to inflammatory stress<sup>35</sup>, which have been shown to impair viability, insulin secretion, and increase ROS production<sup>24</sup>. We replicated these findings and observed alterations in mitochondrial membrane potential and ATP content. TNF $\alpha$  and IL-1 $\beta$  (used during the cytokine stress) directly initiate the NF $\kappa$ B pathway, which is also activated by ROS<sup>36</sup>. Under physiological conditions, basal activation of this pathway supports cellular functionality. However, excessive activation can lead to cellular demise. PBM is also recognized for its capacity to modulate NF $\kappa$ B pathway activation, contingent upon the cellular energy stress level<sup>12,37</sup>. As a result, we found that PBM prevented and corrected alterations in insulin secretion in response to glucose and mitigated the impact of cytokine stress on the viability of MIN6 cells and rat islets. However, no effects were observed on energy status nor ATP and ADP contents.

In the diabetic rat model, no significant differences were observed in terms of glycaemia, body mass, glucose intolerance, and C-peptide secretion between the two groups of transplanted islets. In vitro, we demonstrated the beneficial effect of PBM on the viability and insulin secretion of stressed islets. These stressors are particularly pronounced during the transplantation of human islets into the portal system<sup>8-10</sup>. A research team showed that in rats, the site of transplantation that led to the best islet survival was the renal capsule, whereas the portal route yielded inferior outcomes<sup>39</sup>. This is attributed to the well-vascularized and oxygenated parenchyma of the rat renal capsule<sup>40</sup>, resulting in reduced substrate deficiency. Furthermore, the injection of islets into the portal system in humans exposes them directly to the bloodstream, subjecting them to major inflammatory stress (IBMIR) not observed at the renal capsule site. The renal capsule graft model is ideal for small animals because of its accessibility and potential for histological analysis. However, in humans, this graft location is not

suitable because of the potentially damaging volume of islets to be injected beneath the capsule, particularly considering pre-existing diabetic nephropathy in diabetic patients<sup>41</sup>. This in vivo model allowed us to study the mid-term safety of islet pre-conditioning prior to transplantation. It would be pertinent to employ an animal model with biological stresses that more closely resemble those of humans, such as pigs, where IBMIR is present, and the portal route is more accessible. Additionally, pig model could enable extended graft monitoring over several months, facilitating the investigation of the long-term effects of PBM preconditioning. It is worth noting that the parameters of PBM were established to counteract the impact of the stress modelled in vitro. The influence and combination of these in vivo stresses could necessitate adjustments to illumination parameters for optimal efficacy. The feasibility of directly illuminating the graft site is also a consideration, although this proves challenging if the portal route remains the chosen approach, given the deep-tissue nature and widespread distribution of islets within the liver. Therefore, the preconditioning of islets post-isolation appears to be an effective and less cumbersome option.

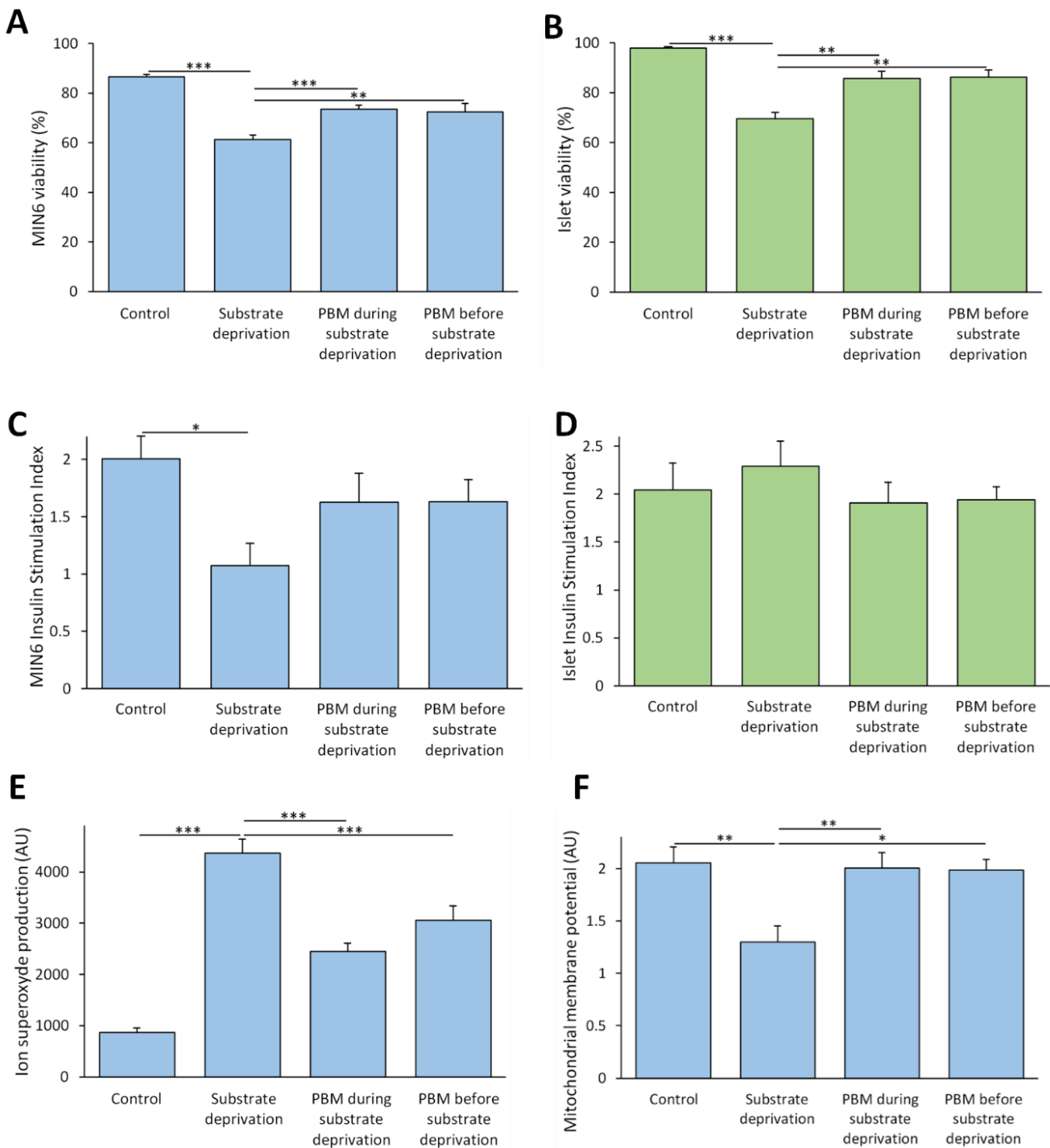
In conclusion, we have demonstrated that a 24h preconditioning of MIN6 cells and rat islets using 670 nm LED PBM at 2.8 mW/cm<sup>2</sup> for extended durations can prevent in vitro alterations in viability, insulin secretion in response to glucose, superoxide ion production, and restore mitochondrial membrane potential, ATP and ADP content depending on stress conditions. In vivo, preconditioned islets (in a non-stressed state) demonstrated complete safety with outcomes comparable to those of conventional islet transplantation but without incremental benefit. Further investigation is warranted to assess the effect of PBM preconditioning on islets in order to consider its potential clinical application in islet transplantation in diabetic patients.

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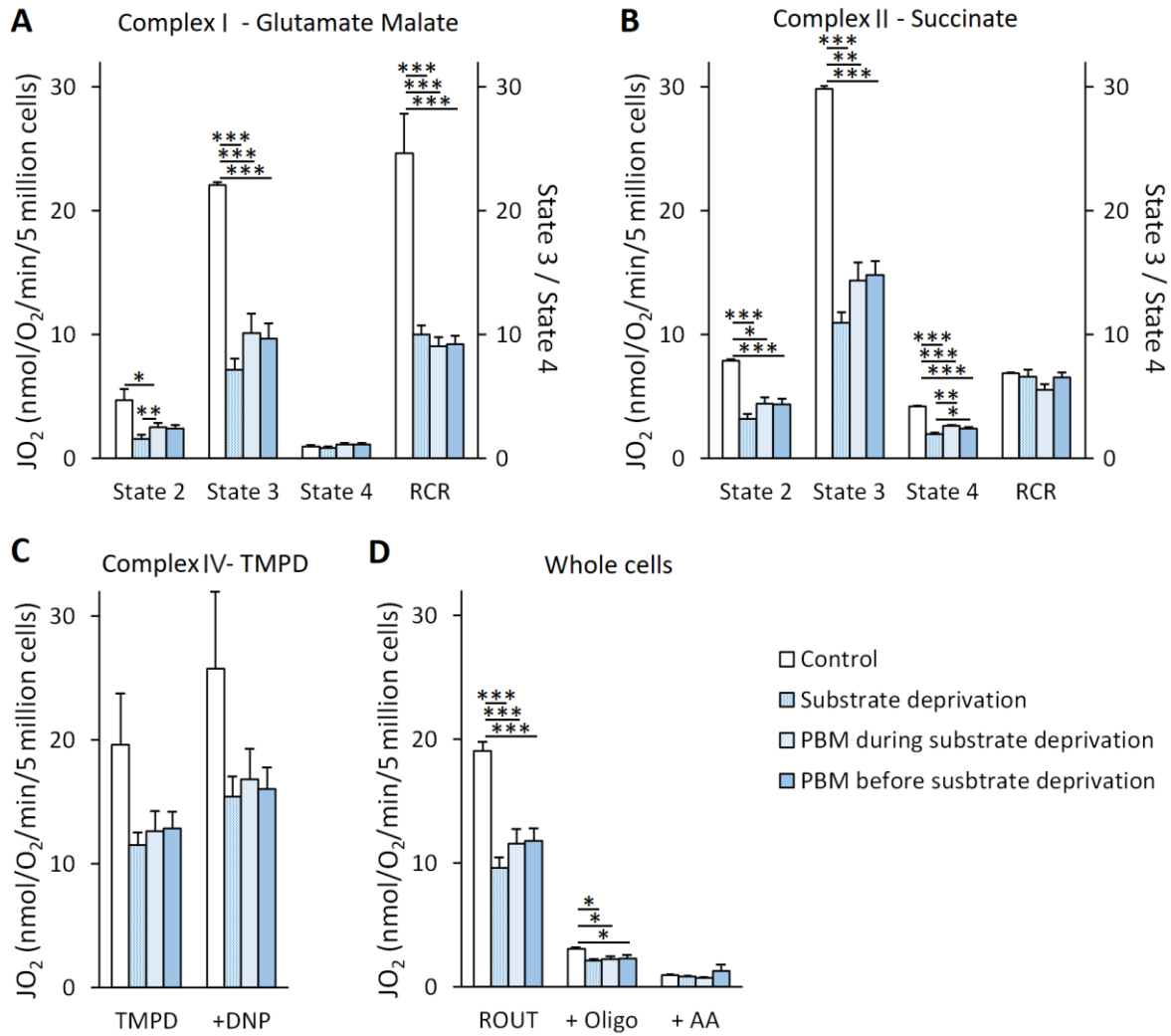


**Figure 1.** Effect of PBM on viability and insulin secretion from SDS MIN6 cells and rat islets



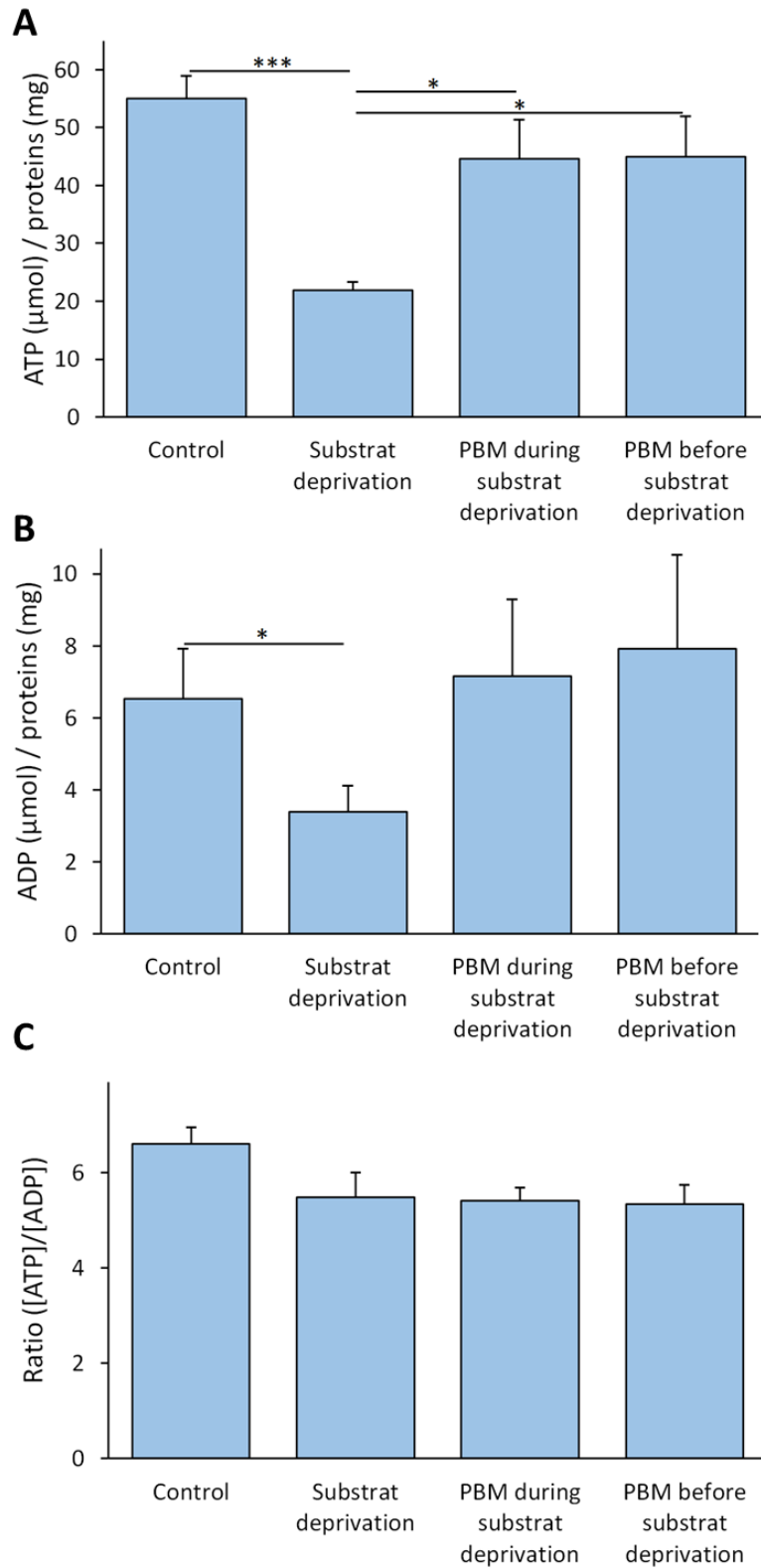
Substrate deprivation is achieved by the incubation of MIN6 cells for 24h and rat islets for 90 min in substrate-free DMEM medium (no glucose, no FBS). After substrate deprivation MIN6 cells and islets are cultured in complete medium. Cells and islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on viability from substrate deprivation stressed MIN6 cells (n = 7). **B.** Effect of PBM on viability from substrate deprivation stressed islets (n = 6). **C.** Effect of PBM on insulin stimulation index from substrate deprivation stressed MIN6. Stimulation index is 100\*(high glucose insulin secretion/low glucose insulin secretion) (n = 7). **D.** Effect of PBM on insulin stimulation index from substrate deprivation stressed islets (n = 6). **E.** Effect of PBM on MIN6 superoxide ion production under substrate deprivation stress (n = 7). Data are expressed as the mean fluorescence intensity. **F.** Effect of PBM on MIN6 mitochondrial membrane potential production under substrate deprivation stress. Data are expressed as the mean fluorescence intensity (n = 6). AU: arbitrary unit, PBM: photobiomodulation. Results are expressed as mean ± SEM, One-way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

**Figure 2.** Effect of PBM on mitochondrial and cell respiration from SDS MIN6 cells



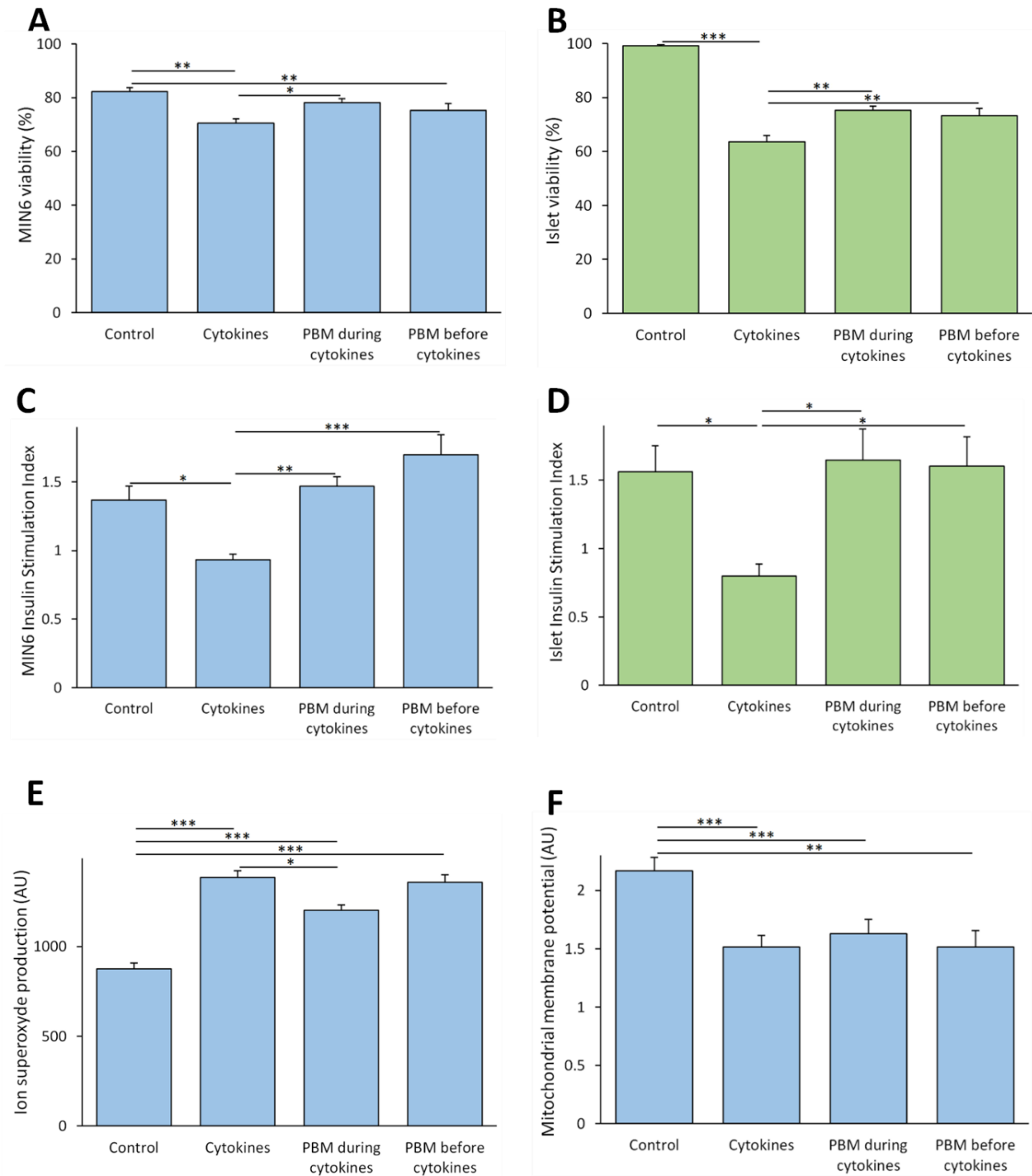
Substrate deprivation is achieved by the incubation of MIN6 cells for 24h and islets for 90 min in substrate-free DMEM medium (no glucose, no FBS). After substrate deprivation MIN6 cells and islets are cultured in complete medium. Cells and islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM and substrate deprivation on MIN6 cells' Complex I (glutamate malate) oxygen consumption. **B.** Effect of PBM and substrate deprivation on MIN6 cells' Complex II (succinate) oxygen consumption. **C.** Effect of PBM and substrate deprivation on MIN6 cells' Complex IV (TMPD) oxygen consumption. **D.** Oxygen consumption of the whole MIN6 cells' subjected to substrate deprivation stress  $\pm$  PBM. AA: antimycin A; Oligo: Oligomycine; PBM: photobiomodulation; RCR: respiratory control ratio (state 3/state 4) graduation is in the right y-axis; ROUT: routine respiration of intact cells. Data are expressed as nmol of oxygen consumed per minute per 5 million live cells. Results are expressed as mean  $\pm$  SEM, n = 4, One-way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

**Figure 3.** Effect of PBM on ATP/ADP content from SDS MIN6 cells



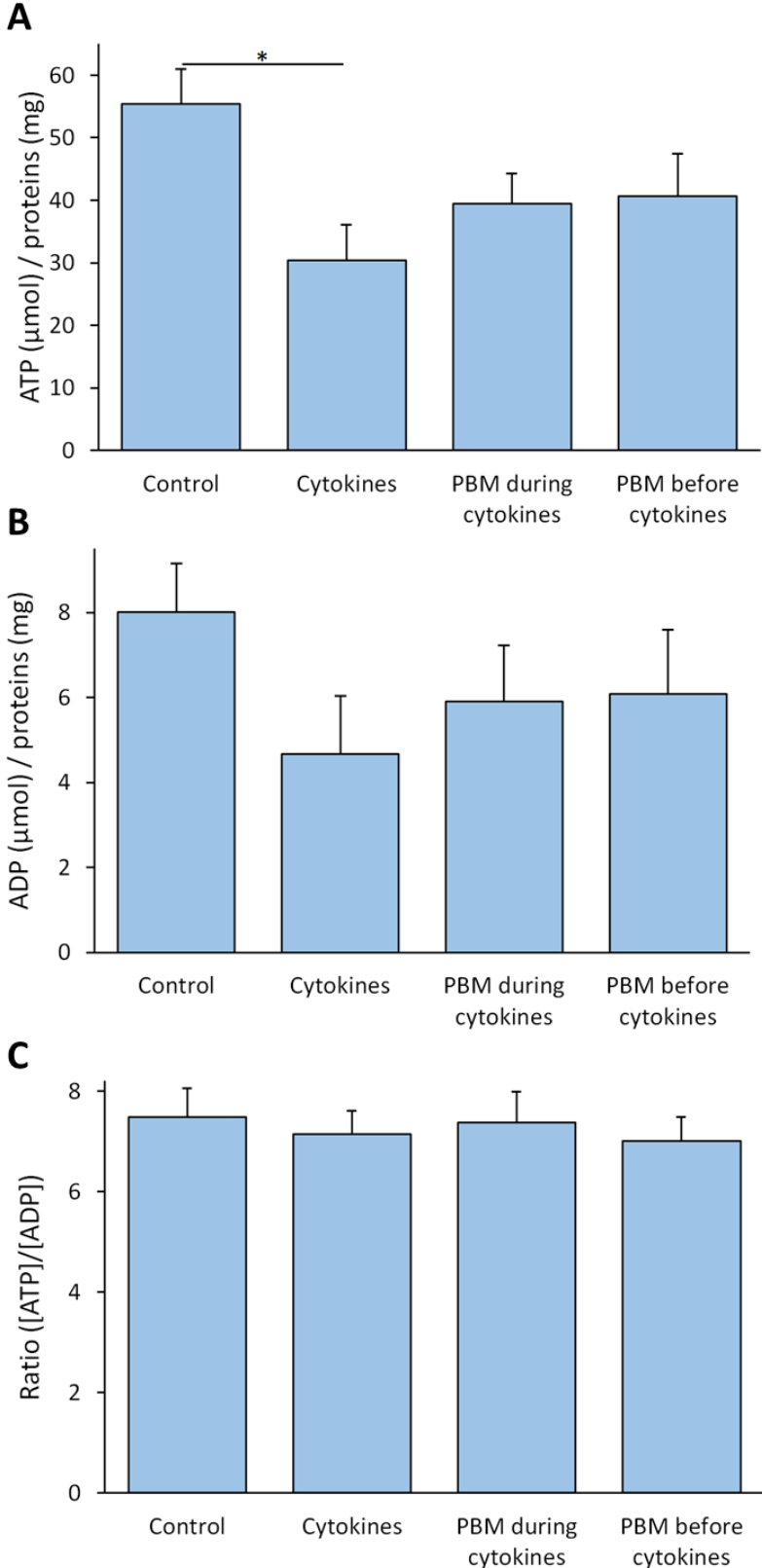
Substrate deprivation is achieved by the incubation of MIN6 cells for 24h and islets for 90 min in substrate-free DMEM medium (no glucose, no FBS). After substrate deprivation MIN6 cells and islets are cultured in complete medium. Cells and islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on ATP content from substrate deprivation stressed MIN6 cells. **B.** Effect of PBM on ADP content from substrate deprivation stressed MIN6 cells. **C.** Effect of PBM on ATP/ADP ratio from substrate deprivation stressed MIN6 cells. Data are expressed in  $\mu\text{mol}$  of nucleotides par mg of proteins, Results are expressed as mean  $\pm$  SEM, n = 6, One-way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\*\* p < 0.001.

**Figure 4.** Effect of PBM on viability and insulin secretion from cytokine stressed MIN6 cells and rat islets



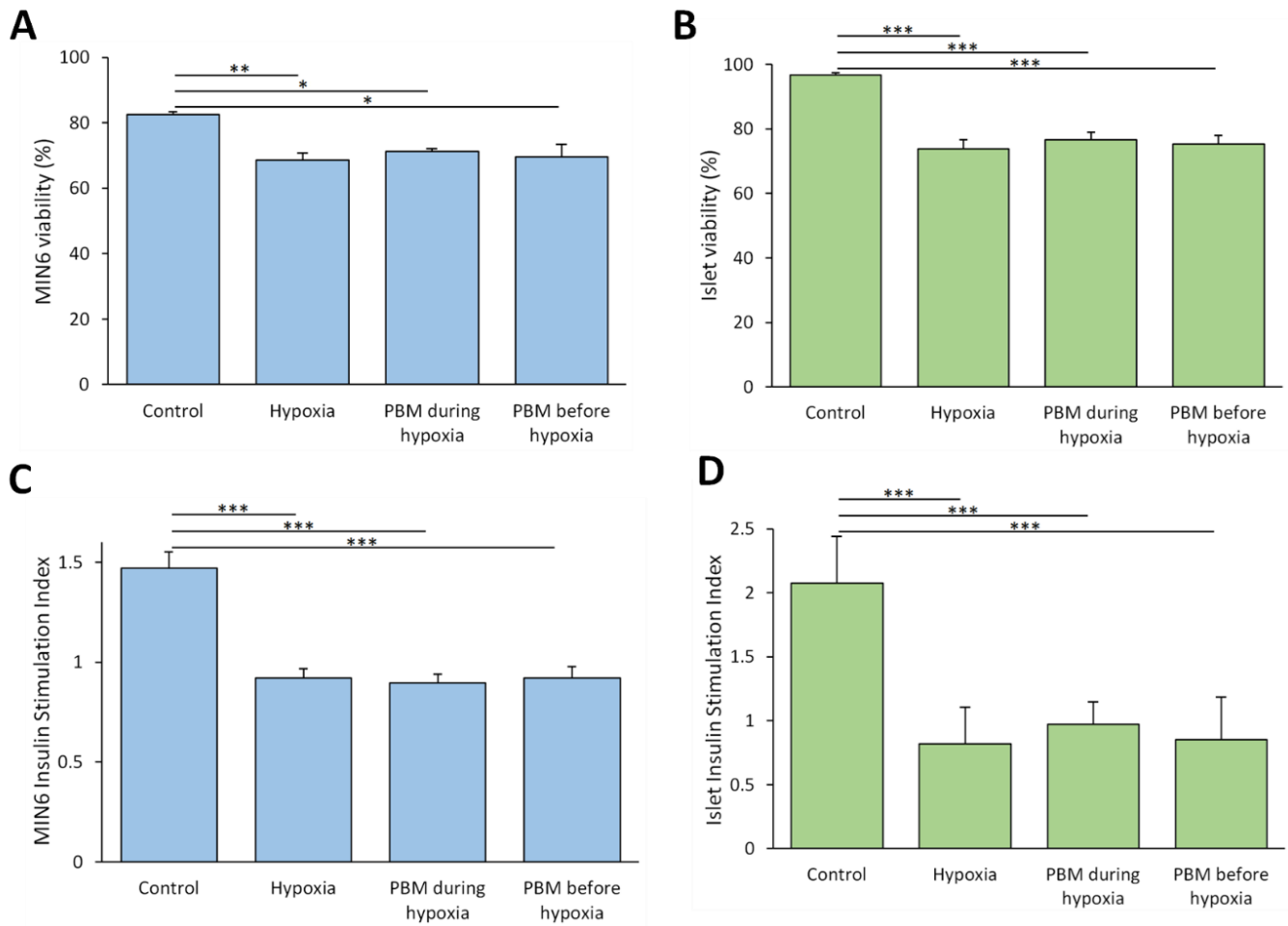
Cytokine stress is made with IL-1 $\beta$  600 UI/mL, TNF- $\alpha$  6,000 UI/mL, IFN- $\gamma$  6,000UI/mL for 24h. MIN6 cells and rat islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on viability from cytokine stressed MIN6 cells (n = 6). **B.** Effect of PBM on the viability from cytokine stressed islets (n = 6). **C.** Effect of PBM on the insulin stimulation index from cytokine stressed MIN6 cells (n = 5). Stimulation index is 100\*(high glucose insulin secretion/low glucose insulin secretion). **D.** Effect of PBM on insulin stimulation index from cytokine stressed islets (n = 6). **E.** Effect of PBM on superoxide ion production from cytokine stress MIN6 cells (n = 3). Data are expressed as mean fluorescence intensity. **F.** Effect of PBM on mitochondrial membrane potential production from cytokine stressed MIN6 cells. (n = 6). AU: Arbitrary unit, PBM: photobiomodulation. Results are expressed as mean  $\pm$  SEM, n = 6, One Way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

**Figure 5.** Effect of PBM on ATP/ADP content from cytokine stressed MIN6 cells



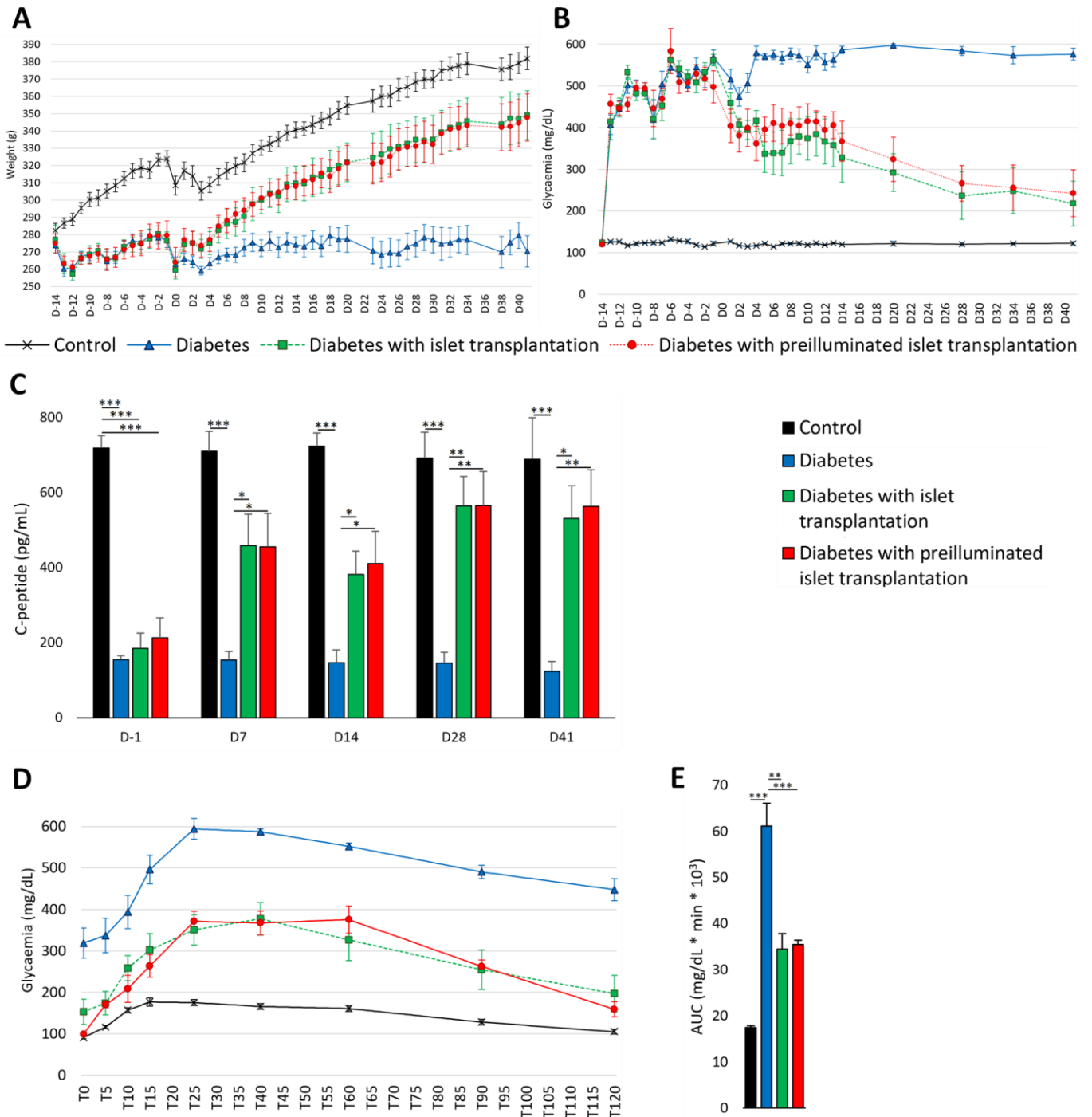
Cytokine stress is made with IL-18 600 UI/ML, TNF-α 6,000 UI/ML, IFN-γ 6,000 UI/mL for 24h. MIN6 cells are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on ATP content from cytokine stressed MIN6 cells. **B.** Effect of PBM on ADP content from cytokine stressed MIN6 cells. **C.** Effect of PBM on ATP/ADP ratio from cytokine stressed MIN6 cells. Data are expressed in μmol of nucleotides per mg of proteins, Results are expressed as mean ± SEM, n = 7, one-way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\* p < 0.01.

**Figure 6.** Effect of PBM on viability and insulin secretion from hypoxic stressed MIN6 cells and rat islets



MIN6 cells are placed in hypoxic medium (1% O<sub>2</sub>) for 24h, rat islets for 16h, followed by 2h of incubation in non-hypoxic medium (21% of O<sub>2</sub>). Cells and islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Effect of PBM on viability from hypoxic stressed MIN6 cells (n = 5). **B.** Effect of PBM on viability from hypoxic stressed islets (n = 7). **C.** Effect of PBM on the insulin stimulation index of hypoxic stressed MIN6 cells. The stimulation index is 100\*(high glucose insulin secretion/low glucose insulin secretion) (n = 6). **D.** Effect of PBM on insulin stimulation index from hypoxic stressed islets (n = 5). PBM: photobiomodulation. Results are expressed as mean ± SEM, One-way ANOVA Welch's with Games-Howell post-hoc test, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

**Figure 7.** Evolution of metabolic parameters after islet transplantation of diabetic rats



**A.** Evolution of body weight. **B.** Evolution of glycaemia. **C.** Evolution of C-peptide secretion. **D.** Glycaemia during OGTT, time is expressed in minutes. **E.** AUC of OGTT. Results are expressed as mean  $\pm$  SEM, One-way ANOVA Fisher's with Tukey post-hoc test,  $n = 8$  per group, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

## References

1. Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R. & Fletcher, A. A. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Can. Med. Assoc. J.* **12**, 141–146 (1922).
2. Benhamou, P.-Y. *et al.* The beneficial effects of closed-loop insulin delivery in patients with highly unstable type 1 diabetes eligible for islet transplantation are maintained over 6 months: An extension study of the DBLHU-WP10 trial. *Diabetes Obes. Metab.* **24**, 956–961 (2022).
3. Shapiro, A. M. *et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* **343**, 230–238 (2000).
4. Lablanche, S. *et al.* Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe hypoglycaemia or poorly controlled glycaemia after kidney transplantation (TRIMECO): a multicentre, randomised controlled trial. *Lancet Diabetes Endocrinol.* **6**, 527–537 (2018).
5. Lablanche, S. *et al.* Ten-year outcomes of islet transplantation in patients with type 1 diabetes: Data from the Swiss-French GRAGIL network. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* **21**, 3725–3733 (2021).
6. Rickels, M. R. *et al.* Long-term Outcomes With Islet-Alone and Islet-After-Kidney Transplantation for Type 1 Diabetes in the Clinical Islet Transplantation Consortium: The CIT-08 Study. *Diabetes Care* **45**, 2967–2975 (2022).
7. Perrier, Q. *et al.* Failure mode and effect analysis in human islet isolation: from the theoretical to the practical risk. *Islets* **13**, 1–9 (2021).
8. Onaca, N., Takita, M., Levy, M. F. & Naziruddin, B. Anti-inflammatory Approach With Early Double Cytokine Blockade (IL-1 $\beta$  and TNF- $\alpha$ ) Is Safe and Facilitates Engraftment in Islet Allotransplantation. *Transplant. Direct* **6**, e530 (2020).
9. Toso, C. *et al.* Positron-emission tomography imaging of early events after transplantation of islets of Langerhans. *Transplantation* **79**, 353–355 (2005).
10. Nilsson, B., Ekdahl, K. N. & Korsgren, O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr. Opin. Organ Transplant.* **16**, 620–626 (2011).
11. Glass, G. E. Photobiomodulation: A review of the molecular evidence for low level light therapy. *J. Plast. Reconstr. Aesthetic Surg. JPRAS* **74**, 1050–1060 (2021).
12. de Freitas, L. F. & Hamblin, M. R. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J. Sel. Top. Quantum Electron. Publ. IEEE Lasers Electro-Opt. Soc.* **22**, 7000417 (2016).
13. Dompe, C. *et al.* Photobiomodulation-Underlying Mechanism and Clinical Applications. *J. Clin. Med.* **9**, 1724 (2020).
14. Desmet, K. D. *et al.* Clinical and experimental applications of NIR-LED photobiomodulation. *Photomed. Laser Surg.* **24**, 121–128 (2006).
15. Dhlamini, T. & Houreld, N. N. Clinical Effect of Photobiomodulation on Wound Healing of Diabetic Foot Ulcers: Does Skin Color Needs to Be Considered? *J. Diabetes Res.* **2022**, 3312840 (2022).
16. Corbella, S. *et al.* Laser treatments as an adjunct to non-surgical periodontal therapy in subjects with periodontitis and type 2 diabetes mellitus: a systematic review and meta-analysis. *Clin. Oral Investig.* **27**, 1311–1327 (2023).
17. Eells, J. T. *et al.* 670 nm Photobiomodulation as a Therapy for Diabetic Macular Edema: A Pilot Study. *Invest. Ophthalmol. Vis. Sci.* **58**, 932 (2017).
18. Rastogi, A., Uppula, P., Saikia, U. & Bhansali, A. Effect of Monochromatic Infrared Energy on Quality of Life and Intraepidermal Nerve Fiber Density in Painful Diabetic Neuropathy: A Randomized, Sham Control Study. *Neurol. India* **69**, 1331–1337 (2021).
19. Gobbi, A., de Carvalho, G., Sapalo, A. T. & de Jesus Guirro, R. R. Acute application of photobiomodulation does not bring important gains for the muscular performance and functionality of diabetic individuals. *Lasers Med. Sci.* **36**, 995–1002 (2021).



20. Huang, H.-H., Stillman, T. J., Branham, L. A. & Williams, S. C. The Effects of Photobiomodulation Therapy on Porcine Islet Insulin Secretion. *Photobiomodulation Photomed. Laser Surg.* **40**, 395–401 (2022).
21. Irani, S. *et al.* Effect of low-level laser irradiation on in vitro function of pancreatic islets. *Transplant. Proc.* **41**, 4313–4315 (2009).
22. Liebman, C., Loya, S., Lawrence, M., Bashoo, N. & Cho, M. Stimulatory responses in  $\alpha$ - and  $\beta$ -cells by near-infrared (810 nm) photobiomodulation. *J. Biophotonics* **15**, e202100257 (2022).
23. Perrier Q, Cottet-Roussel C, Lamarche F, Tubbs E, Tellier C *et al.* Longue exposure of beta cell line and pancreatic rat islet to photobiomodulation: does it exist a risk? [Not yet published, under review].
24. Laporte, C. *et al.* Human mesenchymal stem cells improve rat islet functionality under cytokine stress with combined upregulation of heme oxygenase-1 and ferritin. *Stem Cell Res. Ther.* **10**, 85 (2019).
25. Thévenet, J., Gmyr, V., Delalleau, N., Pattou, F. & Kerr-Conte, J. Pancreatic islet transplantation under the kidney capsule of mice: model of refinement for molecular and ex-vivo graft analysis. *Lab. Anim.* **55**, 408–416 (2021).
26. Lablanche, S. *et al.* Respective effects of oxygen and energy substrate deprivation on beta cell viability. *Biochim. Biophys. Acta* **1847**, 629–639 (2015).
27. Chaudary, S. *et al.* In vitro effects of 635 nm photobiomodulation under hypoxia/reoxygenation culture conditions. *J. Photochem. Photobiol. B* **209**, 111935 (2020).
28. Cottet-Dumoulin, D. *et al.* Intercellular contacts affect secretion and biosynthesis of pancreatic islet cells. *J. Endocrinol.* **258**, (2023).
29. Pastore, D., Greco, M. & Passarella, S. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. *Int. J. Radiat. Biol.* **76**, 863–870 (2000).
30. Ball, K. A., Castello, P. R. & Poyton, R. O. Low intensity light stimulates nitrite-dependent nitric oxide synthesis but not oxygen consumption by cytochrome c oxidase: Implications for phototherapy. *J. Photochem. Photobiol. B* **102**, 182–191 (2011).
31. Quirk, B. & Whelan, H. T. Effect of Red-to-Near Infrared Light and a Nitric Oxide Donor on the Oxygen Consumption of Isolated Cytochrome c Oxidase. *Photobiomodulation Photomed. Laser Surg.* **39**, 463–470 (2021).
32. Quirk, B. J. & Whelan, H. T. Effect of Red-to-Near Infrared Light on the Reaction of Isolated Cytochrome c Oxidase with Cytochrome c. *Photomed. Laser Surg.* **34**, 631–637 (2016).
33. Clerc, P., Rigoulet, M., Lerverve, X. & Fontaine, E. Nitric oxide increases oxidative phosphorylation efficiency. *J. Bioenerg. Biomembr.* **39**, 158–166 (2007).
34. Lane, N. Cell biology: power games. *Nature* **443**, 901–903 (2006).
35. Rabinovitch, A. *et al.* Human pancreatic islet beta-cell destruction by cytokines is independent of nitric oxide production. *J. Clin. Endocrinol. Metab.* **79**, 1058–1062 (1994).
36. Chen, A. C.-H. *et al.* Low-level laser therapy activates NF- $\kappa$ B via generation of reactive oxygen species in mouse embryonic fibroblasts. *PLoS One* **6**, e22453 (2011).
37. Hamblin, M. R. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys.* **4**, 337–361 (2017).
38. Huang, Y.-Y., Sharma, S. K., Carroll, J. & Hamblin, M. R. Biphasic dose response in low level light therapy - an update. *Dose-Response Publ. Int. Hormesis Soc.* **9**, 602–618 (2011).
39. Stokes, R. A. *et al.* Transplantation sites for human and murine islets. *Diabetologia* **60**, 1961–1971 (2017).
40. Carlsson, P. O., Palm, F., Andersson, A. & Liss, P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* **50**, 489–495 (2001).
41. Jindal, R. M., Sidner, R. A., McDaniel, H. B., Johnson, M. S. & Fineberg, S. E. Intraportal vs kidney subcapsular site for human pancreatic islet transplantation. *Transplant. Proc.* **30**, 398–399 (1998).

## Article n° 3

# Photobiomodulation promotes the functionality and viability of human pancreatic islets in basal conditions and under cytokine stress conditions

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**Key Words:** Diabetes, Photobiomodulation, Human islets, Insulin secretion, Viability, Functionality

## **Introduction**

While several studies have demonstrated the metabolic benefits of islet transplantation in unstable diabetes<sup>1</sup>, there are still major challenges to overcome. For example, over 50% of islets are destroyed at the time of transplantation due to the instant blood mediated inflammation reaction (IBMIR) and cytokine stress<sup>2</sup>. The islets' resting period after isolation (24-72h) appears to be an opportune time to improve islet viability and possibly protect them from the consequences of IBMIR. Photobiomodulation (PBM) corresponds low level laser therapy with selective absorption of wavelengths by chromophores, mainly at the mitochondria level<sup>3</sup>, with demonstrated effects in neurologic stimulation and wound healing. Few data are available on insulin secreting cells: Liebman showed a significant increase in insulin secretion of BT6 cells exposed to PBM<sup>4</sup>, and Huang reported weak increase of insulin secretion in low glucose after PBM on porcine pancreatic islets<sup>5</sup>. The aim of this study was to evaluate the effects of PBM on viability and insulin secretion in human islets.

## **Materials and methods**

Human islets were obtained from isolation centers (Montpellier and Paris, France). The illumination (PBM) was made by a PBM device (EFFI-BL-IP69K, Effilux, Les Ulis, France), wavelength of 670 nm, continuous output power, 2.8 mW/cm<sup>2</sup> during 2h (20.2 J/cm<sup>2</sup>) or 24h (241.9 J/cm<sup>2</sup>). Cytokine stress was applied on human islets incubated in Miami medium with human IL1- $\beta$  (600 UI/mL), human TNF- $\alpha$  (6,000 UI/mL), mouse IFN- $\gamma$  (6,000 UI/mL). Islets were illuminated for 1) 2h or 24h without stress, 2) 24h during cytokine stress or, 3) 24h before cytokine stress. Analyses were performed immediately after the illumination. Viability was performed by confocal microscopy and cells were labelled with Syto13 and propidium iodide (at least 50 IEQ per condition). Glucose stimulated insulin secretion (GSIS) was performed by static incubation in Krebs-Ringer bicarbonate buffer medium and 2.8mM or 16.7mM glucose (at least 100 IEQ per condition). Total insulin content was extracted by overnight incubation at -20°C in acid ethanol. The result, obtained by ELISA, was expressed as percentage of secreted insulin in relation to total insulin and stimulation index (SI) was 100\*(insulin secreted in high glucose / insulin secreted in low glucose).

## Results

After 24h of PBM, increased viability was observed compared with control ( $91.9 \pm 2.07$  % after PBM vs.  $77.8 \pm 8.70$  % for control,  $p=0.037$ ), no effect is observed if PBM only lasted 2h ( $79.9 \pm 7.37$  %,  $p=0.90$ ) (Fig. 1A and B). Insulin SI was higher after 24h PBM compared with control ( $3.15 \pm 0.42$  vs.  $1.54 \pm 0.28$ ,  $p=0.002$ ), no effect is observed if PBM only lasted 2h ( $1.85 \pm 0.57$ ,  $p=0.600$ ) (Fig. 1C and D).

Cytokine stress decreased human islet viability compared with control ( $66.0 \pm 3.73$  % vs.  $79.3 \pm 2.73$  %,  $p=0.002$ ) (Fig. 1E and F), as well as insulin SI ( $0.52 \pm 0.17$  vs.  $1.70 \pm 0.40$ ,  $p=0.023$ ) (Fig. 1G and H). PBM before or during stress restored viability (respectively  $78.7 \pm 1.44$  %,  $p=0.002$  vs. cytokines, and  $79.1 \pm 2.51$  %,  $p<0.001$  vs. cytokines) and insulin SI (respectively  $2.22 \pm 1.00$ ,  $p=0.045$  vs. cytokines, and  $1.96 \pm 0.16$ ,  $p=0.002$  vs. cytokines).

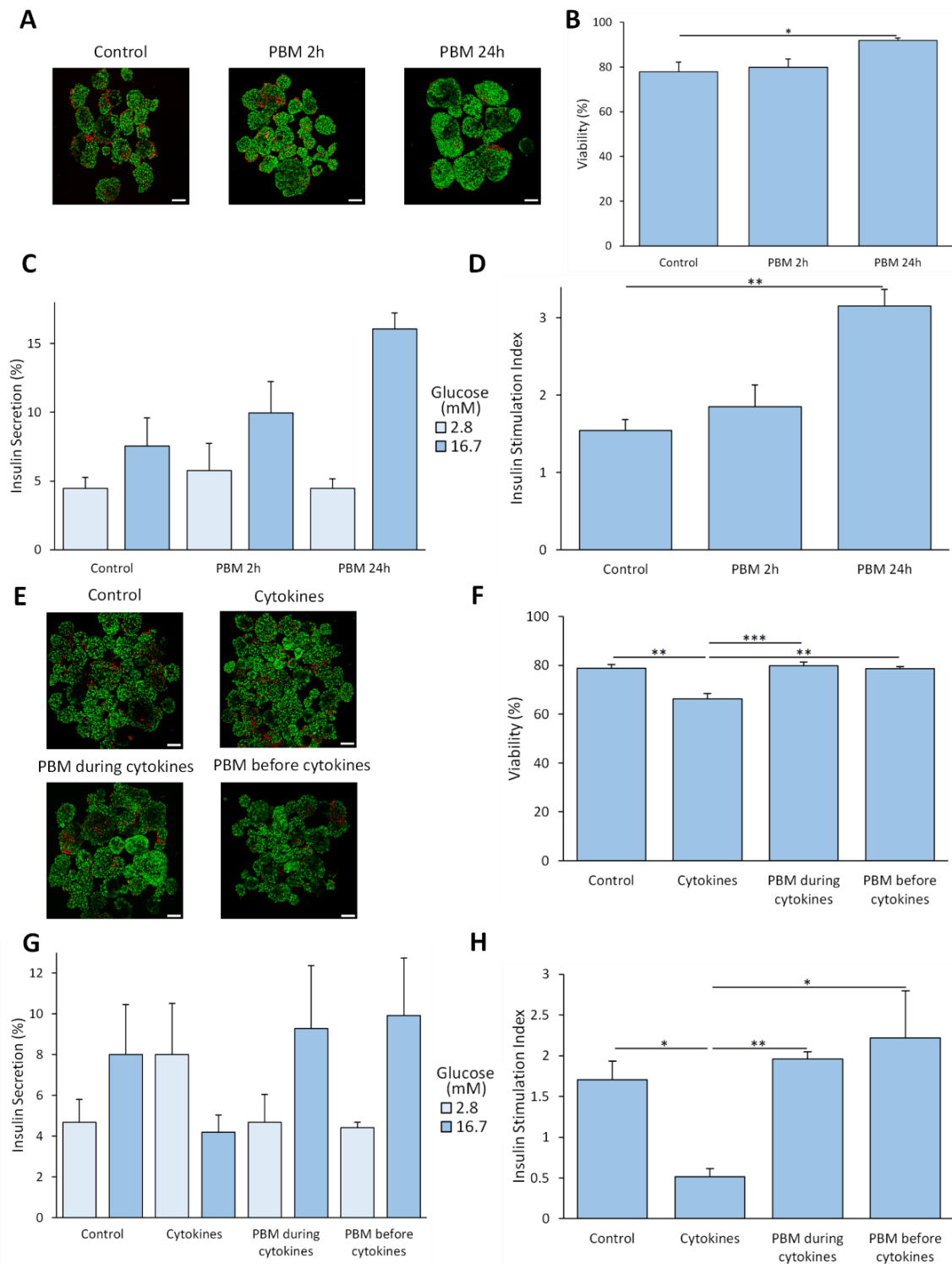
## Conclusion

Illuminating human islets during their resting period after isolation (24-72h) improves basal islet viability and functionality and prevents consequences of cytokine stress. Further studies on the mechanism of action of PBM on human islets are required.

## Acknowledgement

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**Figure 1.** Effect of PBM on the viability and insulin secretion of human pancreatic islets



Human islets are exposed to 2.8 mW/cm<sup>2</sup> PBM illumination. **A.** Representative images of islet viability experiment by confocal microscopy after PBM. Live cells are labelled with Syto13 (green), dead cells are labelled with PI (red). **B.** Effect of 2h and 24h of PBM on the viability of human islets (n = 4). **C.** Effect of PBM on human islets' insulin secretion in response to glucose. Results are normalized by total insulin content. **D.** Effect of PBM on insulin secretion index of human islets. Secretion index is 100\*(high glucose insulin secretion/low glucose insulin secretion) (n = 4). **E.** Representative images of islet viability experiment by confocal microscopy with cytokine stress ± PBM (before or during the cytokine stress). **F.** Effect of PBM on the viability of cytokine-stressed human islets (n = 3). **G.** Effect of PBM on insulin secretion in response to glucose from cytokine-stressed human islets. Results are normalized by total insulin content. **H.** Effect of PBM on insulin secretion index of cytokine-stressed human islets. Secretion index is 100\*(high glucose insulin secretion/low glucose insulin secretion) (n = 3). PBM: photobiomodulation, Results are expressed as mean ± SEM, Scale bars = 100 μm, One-way ANOVA Kruskal Wallis, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

## References

1. Lablanche, S. et al. Ten-year outcomes of islet transplantation in patients with type 1 diabetes: Data from the Swiss-French GRAGIL network. *Am J Transplant* 21, 3725–3733 (2021).
2. Nilsson, B., Ekdahl, K. N. & Korsgren, O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant* 16, 620–626 (2011).
3. Dompe, C. et al. Photobiomodulation-Underlying Mechanism and Clinical Applications. *J Clin Med* 9, 1724 (2020).
4. Liebman, C., Loya, S., Lawrence, M., Bashoo, N. & Cho, M. Stimulatory responses in  $\alpha$ - and  $\beta$ -cells by near-infrared (810 nm) photobiomodulation. *J Biophotonics* 15, e202100257 (2022).
5. Huang, H.-H., Stillman, T. J., Branham, L. A. & Williams, S. C. The Effects of Photobiomodulation Therapy on Porcine Islet Insulin Secretion. *Photobiomodul Photomed Laser Surg* 40, 395–401 (2022).

## Discussion

Dans ce travail, nous avons démontré l'intérêt croissant de l'utilisation de la PBM dans la prise en charge des complications du diabète (annexe 1). Cependant, peu de données sont disponibles concernant les cellules bêta (MIN6) et les îlots. Une exposition prolongée n'a eu aucun effet néfaste sur les cellules bêta et les îlots de rats. Sur les îlots humains, la PBM en situation basale améliore la viabilité et la fonctionnalité des îlots. De plus, nous montrons que la PBM peut prévenir la toxicité due à certains stress tels que la privation de substrats et le stress inflammatoire (cytokines), affectant ainsi les cellules bêta et les îlots chez les rats et les humains. Dans un modèle de rat diabétique, la greffe d'îlots préconditionnés par PBM n'a pas montré d'infériorité par rapport à une greffe d'îlots classique sur l'ensemble des paramètres étudiés. Néanmoins, aucun bénéfice incrémental n'a pu être observé.

Avec plus de 1 000 publications annuelles répertoriées sur MEDLINE depuis 2020, l'intérêt pour la PBM est manifeste. Dans le domaine du diabète, de nombreux bénéfices ont été démontrés ou sont en cours d'investigation. Les résultats positifs comprennent l'amélioration des paramètres de cicatrisation / ulcères, la réduction de la douleur et l'amélioration des perceptions sensorielles en cas de neuropathie. Cependant, les résultats concernant la récupération post-exercice ne sont à ce jour pas concluants. Les paramètres rapportés dans la littérature sont extrêmement variés et les résultats hétérogènes, ce qui rend difficile l'établissement de recommandations quant à l'utilisation de la PBM et à son rôle dans la prise en charge des parodontites et de la rétinopathie diabétique. Cette grande variabilité des paramètres peut également expliquer la disparité des résultats obtenus. De plus, un biais de publication majeur (16 % de résultats d'essais cliniques non publiés) est associé à un dépôt

aléatoire des protocoles d'essais cliniques sur la base mondiale ClinicalTrials.gov. Des effets prometteurs ont été identifiés dans le cas de la cicatrisation et du DT2 pour réduire l'intolérance au glucose et l'insulino-résistance. Quelques études se sont penchées sur l'effet direct de la PBM sur les cellules bêta et les îlots. La PBM a permis d'améliorer l'insulinosécrétion en bas glucose des cellules bêta (BTC6)<sup>214</sup> ainsi que l'index de stimulation de la sécrétion d'insuline des îlots de rats<sup>216</sup>, mais n'a pas eu d'effet sur la sécrétion d'insuline des îlots porcins (connus pour leur faible sécrétion d'insuline)<sup>215</sup>. La PBM semble donc avoir un impact sur les cellules bêta et les îlots, ce qui pourrait être intéressant dans la greffe d'îlots.

Il est important de rappeler que l'isolement des îlots pancréatiques humains constitue un processus stressant pour les îlots, qui sont déconnectés de leur micro-environnement. Ces îlots sont ensuite maintenus en culture ex vivo durant 24h à 72h afin de récupérer de l'isolement et d'établir l'induction immunosuppressive chez le receveur. Malgré l'optimisation des protocoles d'immunosuppression et l'ajout de traitements anti-inflammatoires (anti-TNF, pentoxifylline), une destruction significative des îlots survient immédiatement après la greffe. Cette perte d'îlots résulte de la combinaison de différents stress, à savoir un phénomène inflammatoire majeur (IBMIR)<sup>48-50</sup>, l'absence de substrat et d'oxygène (avant que la vascularisation des îlots ne soit efficace).

La PBM ayant montré des résultats intéressants pour améliorer la viabilité de différents types cellulaires<sup>118,137-142</sup> et réduire l'inflammation<sup>146</sup>, ajouté au fait que les îlots soient disponibles ex vivo pendant 24 à 72 heures, il semble opportun d'étudier un pré-conditionnement par PBM sur une longue durée. Bien que moins facile à mettre en



œuvre sur le plan clinique, une application simultanée de la PBM et de stress a également été étudiée. L'application continue de la PBM sur des périodes de plus d'une heure n'ayant jamais été étudiée auparavant, et étant donné le manque de données disponibles concernant les cellules bêta et les îlots, une étude de toxicité a été réalisée. Aucun effet délétère (ni positif) n'a été observé sur la viabilité, la fonctionnalité mitochondriale ou la sécrétion d'insuline des MIN6 et des îlots de rat non soumis à des stress. De plus, un effet bénéfique sur les îlots humains en condition basale (post isolement) a été observé sur leur viabilité et sécrétion d'insuline. Ainsi, l'étude avec des modèles de stress a été menée en utilisant les paramètres d'illumination précédemment définis (illumination par LED 670 nm, en continu, avec une densité de puissance de 2,8 mW/cm<sup>2</sup>).

La PBM a montré des effets protecteurs sur la viabilité et la fonctionnalité des cellules MIN6 et des îlots de rat soumis à une privation de substrat et un stress inflammatoire (cytokiniques). À noter que la privation de substrat n'a eu d'effet que sur la sécrétion d'insuline des cellules MIN6, mais pas sur les îlots. Étant donné que les îlots ne sont pas composés uniquement de cellules bêta, mais aussi d'autres types cellulaires (alpha qui sécrètent le glucagon, et delta qui sécrètent la somatostatine, notamment), les interactions paracrines et directes entre ces cellules assurent une sécrétion appropriée d'insuline<sup>217</sup> qui ne semble pas être altérée en cas de privation de substrat. Le modèle d'îlots de rongeurs est en effet plus complet, ce qui pourrait expliquer cette différence d'effet observée. En revanche, aucun effet de la PBM n'a été observé en cas de stress hypoxique. Cette différence d'effet de la PBM selon le stress semble être liée à l'effet pathologique du stress au niveau cellulaire. Les stress dus à la privation de substrat et aux cytokines altèrent le fonctionnement cellulaire en affectant les

mitochondries (augmentation des ROS et altération du potentiel de membrane mitochondriale), tandis qu'il a été démontré que le stress hypoxique modifie peu ces paramètres chez les cellules bêta. Étant donné que les effets supposés de la PBM repose sur son action au niveau des mitochondries<sup>81</sup>, il est cohérent de ne pas observer d'impact de la PBM si le fonctionnement des mitochondries n'est pas altéré (cas du stress hypoxique ou de l'étude de toxicité sans stress préalable).

Les stress étudiés in vitro surviennent de manière concomitante lors de la greffe d'îlots. Il aurait pu être intéressant d'étudier l'effet de la PBM sur les trois stress simultanément. Cependant, nous avons choisi de privilégier une approche plus globale en effectuant une étude in vivo avec un modèle de rat diabétique induit par la streptozotocine. Cette approche préalable à l'utilisation de la PBM sur des îlots humains (une ressource précieuse) nous a permis de démontrer l'absence de risques d'un pré-conditionnement d'îlots (par 24h de PBM) avant greffe. Les résultats obtenus sont rassurants, car les effets de la greffe sont comparables entre les deux groupes sur l'ensemble des paramètres étudiés : prise de poids, glycémies, sécrétion de c-peptide et intolérance au glucose évaluée par OGTT. Cependant, les effets à long terme de l'exposition n'ont pas été étudiés. Il est important de noter que la greffe a été réalisée chez un modèle de rat diabétique sous la capsule rénale (peu d'inflammation), tandis que chez l'humain, la greffe est réalisée au niveau portal (réaction inflammatoire type IBMIR très importante). La greffe d'îlots sur de petits animaux n'est pas réalisée au niveau portal en raison de saignements trop importants. Le modèle utilisé dans notre étude ne permettait donc pas de mimer de manière optimale les stress présents dans la greffe d'îlots chez l'humain. Il conviendrait d'étudier l'impact du pré-conditionnement dans un modèle plus proche de l'humain comme le porc où : la greffe

au niveau portal est techniquement possible, et la réaction inflammatoire (type IBMIR) est très prononcée. Ce modèle porcin pourrait également permettre d'étudier l'impact du pré-conditionnement par PBM au plus long cours.

Après avoir obtenu des résultats satisfaisants et encourageants, il était nécessaire d'étudier l'effet de la PBM sur les îlots humains. Ces îlots ont une viabilité après isolement plus faible que celle des îlots de rongeurs, et cette viabilité diminue relativement rapidement avec le temps. Le maintien prolongé en culture est actuellement difficile à réaliser et entraîne une altération de la viabilité et de la fonctionnalité des îlots. Nous avons pu observer qu'une exposition continue à la PBM pendant 24h sur les îlots humains (sans autre stress que celui inhérent à l'isolement) permettait d'améliorer leur viabilité et leur sécrétion d'insuline en réponse au glucose. Par conséquent, cela pourrait permettre d'améliorer la viabilité et la fonctionnalité des îlots avant la greffe. De plus, nous avons constaté qu'un pré-conditionnement des îlots humains par 24h de PBM, suivi d'un stress inflammatoire (cytokines), permettait d'obtenir une viabilité et une sécrétion d'insuline en réponse au glucose similaires à celles des îlots témoins (non stressés). Ce pré-conditionnement des îlots humains pourrait donc permettre de diminuer, en partie, la perte des îlots en post-greffe liée à la forte réaction anti-inflammatoire (IBMIR).

## **Conclusion et perspectives**

En conclusion, ce travail a mis en évidence l'intérêt de la PBM dans la prise en charge des complications du diabète, bien que son rôle et les paramètres d'illumination nécessitent encore d'être précisés. Nous avons pour la première fois démontré l'innocuité d'une exposition prolongée à la PBM sur les cellules bêta et les îlots de rongeurs. Le pré-conditionnement des cellules bêta et des îlots avant un stress permet d'améliorer leur viabilité, leur sécrétion d'insuline et leurs paramètres mitochondriaux. Dans un modèle de rat diabétique, le pré-conditionnement des îlots par PBM est rassurant et conduit à des effets métaboliques comparables à une greffe d'îlots classique. Il serait intéressant de pouvoir explorer plus largement les effets d'un pré-conditionnement dans un modèle plus proche de la greffe d'îlots humains (modèle porcin). Enfin, l'effet protecteur de la PBM sur la viabilité et la sécrétion d'insuline des îlots humains après isolement et avant un stress inflammatoire est un atout majeur. La PBM est une approche non invasive et relativement simple à mettre en œuvre. Elle pourrait contribuer à réduire la perte d'îlots après la greffe et donc les besoins en îlots pour atteindre un contrôle glycémique optimal dans les suites d'une greffe d'îlots.

## Bibliographie de la thèse

1. WHO | Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. *WHO* [https://www.who.int/diabetes/publications/diagnosis\\_diabetes2006/en/](https://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/).
2. IDF - Diabetes Atlas 2021. [En ligne] Available at: [https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF\\_Atlas\\_10th\\_Edition\\_2021](https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021).
3. Santé Publique France - Diabète. <https://www.santepubliquefrance.fr/maladies-et-traumatismes/diabete>.
4. Atkinson, M. A. The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harb Perspect Med* **2**, (2012).
5. Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R. & Fletcher, A. A. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Can Med Assoc J* **12**, 141–146 (1922).
6. Benhamou, P.-Y. *et al.* The beneficial effects of closed-loop insulin delivery in patients with highly unstable type 1 diabetes eligible for islet transplantation are maintained over 6 months: An extension study of the DBLHU-WP10 trial. *Diabetes Obes Metab* **24**, 956–961 (2022).
7. Petruzelkova, L. *et al.* First Use of Open-Source Automated Insulin Delivery AndroidAPS in Full Closed-Loop Scenario: Pancreas4ALL Randomized Pilot Study. *Diabetes Technol Ther* **25**, 315–323 (2023).
8. Mizokami-Stout, K. R. *et al.* The Contemporary Prevalence of Diabetic Neuropathy in Type 1 Diabetes: Findings From the T1D Exchange. *Diabetes Care* **43**, 806–812 (2020).
9. Gubitosi-Klug, R. *et al.* Associations of Microvascular Complications With the Risk of Cardiovascular Disease in Type 1 Diabetes. *Diabetes Care* **44**, 1499–1505 (2021).
10. Genua, I. *et al.* Obesity and related comorbidities in a large population-based cohort of subjects with type 1 diabetes in Catalonia. *Front Endocrinol (Lausanne)* **13**, 1015614 (2022).
11. Tran, P. M. H. *et al.* T1DMicro: A Clinical Risk Calculator for Type 1 Diabetes Related Microvascular Complications. *Int J Environ Res Public Health* **18**, 11094 (2021).
12. Weinstock, R. S. *et al.* Type 1 diabetes in older adults: Comparing treatments and chronic complications in the United States T1D Exchange and the German/Austrian DPV registries. *Diabetes Res Clin Pract* **122**, 28–37 (2016).
13. Umpierrez, G. & Korytkowski, M. Diabetic emergencies - ketoacidosis, hyperglycaemic hyperosmolar state and hypoglycaemia. *Nat Rev Endocrinol* **12**, 222–232 (2016).
14. Gill, G. V., Lucas, S. & Kent, L. A. Prevalence and characteristics of brittle diabetes in Britain. *QJM* **89**, 839–843 (1996).
15. Kelly, W. D., Lillehei, R. C., Merkel, F. K., Idezuki, Y. & Goetz, F. C. Allograft transplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery* **61**, 827–837 (1967).
16. Kandaswamy, R. *et al.* OPTN/SRTR 2016 Annual Data Report: Pancreas. *American Journal of Transplantation* **18**, 114–171 (2018).
17. Gruessner, R. W. G. & Gruessner, A. C. The current state of pancreas transplantation. *Nat Rev Endocrinol* **9**, 555–562 (2013).
18. Niclauss, N., Meier, R., Bédard, B., Berishvili, E. & Berney, T. Beta-Cell Replacement: Pancreas and Islet Cell Transplantation. *Endocr Dev* **31**, 146–162 (2016).
19. Lablanche, S. *et al.* Islet transplantation versus insulin therapy in patients with type 1 diabetes with severe hypoglycaemia or poorly controlled glycaemia after kidney transplantation (TRIMECO): a multicentre, randomised controlled trial. *Lancet Diabetes Endocrinol* **6**, 527–537 (2018).
20. Rafael, E. *et al.* Changes in liver enzymes after clinical islet transplantation. *Transplantation* **76**, 1280–1284 (2003).
21. Bhargava, R. *et al.* Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. *Diabetes* **53**, 1311–1317 (2004).
22. Casey, J. J. *et al.* Portal venous pressure changes after sequential clinical islet transplantation. *Transplantation* **74**, 913–915 (2002).
23. Wojtuszczyński, A. *et al.* Indications for islet or pancreatic transplantation: Statement of the TREPID working group on behalf of the Société francophone du diabète (SFD), Société française d'endocrinologie (SFE), Société francophone de transplantation (SFT) and Société française de néphrologie - dialyse - transplantation (SFNDT). *Diabetes Metab* **45**, 224–237 (2019).
24. Ricordi, C., Lacy, P. E., Finke, E. H., Olack, B. J. & Scharp, D. W. Automated method for isolation of human pancreatic islets. *Diabetes* **37**, 413–420 (1988).
25. Najarian, J. S. *et al.* Human islet transplantation: a preliminary report. *Transplant Proc* **9**, 233–236 (1977).
26. Shapiro, A. M. *et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* **343**, 230–238 (2000).
27. Gołębiewska, J. E. *et al.* Validation of a New North American Islet Donor Score for Donor Pancreas Selection and Successful Islet Isolation in a Medium-Volume Islet Transplant Center. *Cell Transplant* **28**, 185–194 (2019).

28. Wassmer, C.-H. *et al.* Impact of ischemia time on islet isolation success and post transplantation outcomes: a retrospective study of 452 pancreas isolations. *Am. J. Transplant.* (2020) doi:10.1111/ajt.16320.
29. Andres, A. *et al.* Impact of adverse pancreatic injury at surgical procurement upon islet isolation outcome. *Transpl. Int.* **27**, 1135–1142 (2014).
30. Niclauss, N. *et al.* Comparative impact on islet isolation and transplant outcome of the preservation solutions Institut Georges Lopez-1, University of Wisconsin, and Celsior. *Transplantation* **93**, 703–708 (2012).
31. Brandhorst, H. *et al.* Comparison of Clostripain and Neutral Protease as Supplementary Enzymes for Human Islet Isolation. *Cell Transplant* **28**, 176–184 (2019).
32. Brandhorst, D., Brandhorst, H. & Johnson, P. R. V. Enzyme Development for Human Islet Isolation: Five Decades of Progress or Stagnation? *Rev Diabet Stud* **14**, 22–38 (2017).
33. Chadwick, D. R. *et al.* Human islet purification: a prospective comparison of Euro-Ficoll and bovine serum albumin density gradients. *Acta Diabetol* **30**, 57–59 (1993).
34. Shimoda, M. *et al.* An effective purification method using large bottles for human pancreatic islet isolation. *Islets* **4**, 398–404 (2012).
35. Noguchi, H. *et al.* Islet Culture/Preservation Before Islet Transplantation. *Cell Med* **8**, 25–29 (2015).
36. Berney, T. Islet culture and counter-culture. Commentary on: Effect of short-term culture on functional and stress-related parameters in isolated human islets by Ihm *et al.* *Transpl. Int.* **22**, 531–533 (2009).
37. Murdoch, T. B., McGhee-Wilson, D., Shapiro, A. M. J. & Lakey, J. R. T. Methods of Human Islet Culture for Transplantation. *Cell Transplant* **13**, 605–618 (2004).
38. Rickels, M. R. & Robertson, R. P. Pancreatic Islet Transplantation in Humans: Recent Progress and Future Directions. *Endocr. Rev.* **40**, 631–668 (2019).
39. Lablanche, S. *et al.* Five-Year Metabolic, Functional, and Safety Results of Patients With Type 1 Diabetes Transplanted With Allogenic Islets Within the Swiss-French GRAGIL Network. *Diabetes Care* **38**, 1714–1722 (2015).
40. Lablanche, S. *et al.* Ten-year outcomes of islet transplantation in patients with type 1 diabetes: Data from the Swiss-French GRAGIL network. *Am J Transplant* **21**, 3725–3733 (2021).
41. Rickels, M. R. *et al.* Long-term Outcomes With Islet-Alone and Islet-After-Kidney Transplantation for Type 1 Diabetes in the Clinical Islet Transplantation Consortium: The CIT-08 Study. *Diabetes Care* **45**, 2967–2975 (2022).
42. Lablanche, S. *et al.* Randomised, prospective, medico-economic nationwide French study of islet transplantation in patients with severely unstable type 1 diabetes: the STABILOT study protocol. *BMJ Open* **7**, e013434 (2017).
43. Perrier Q, Lavallard V, Pernin N, Wassmer C, Cottet-Dumoulin D, Lebreton F, *et al.* Failure mode and effect analysis in human islet isolation: from the theoretical to the practical risk. 2021. ‘sous presse’.
44. Nano, R. *et al.* Heterogeneity of Human Pancreatic Islet Isolation Around Europe: Results of a Survey Study. *Transplantation* **104**, 190–196 (2020).
45. Lau, J. & Carlsson, P.-O. Low revascularization of human islets when experimentally transplanted into the liver. *Transplantation* **87**, 322–325 (2009).
46. Giuliani, M. *et al.* Central necrosis in isolated hypoxic human pancreatic islets: evidence for postisolation ischemia. *Cell Transplant* **14**, 67–76 (2005).
47. Llacua, A., de Haan, B. J., Smink, S. A. & de Vos, P. Extracellular matrix components supporting human islet function in alginate-based immunoprotective microcapsules for treatment of diabetes. *J Biomed Mater Res A* **104**, 1788–1796 (2016).
48. Onaca, N., Takita, M., Levy, M. F. & Naziruddin, B. Anti-inflammatory Approach With Early Double Cytokine Blockade (IL-1 $\beta$  and TNF- $\alpha$ ) Is Safe and Facilitates Engraftment in Islet Allotransplantation. *Transplant Direct* **6**, e530 (2020).
49. Toso, C. *et al.* Positron-emission tomography imaging of early events after transplantation of islets of Langerhans. *Transplantation* **79**, 353–355 (2005).
50. Nilsson, B., Ekdahl, K. N. & Korsgren, O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant* **16**, 620–626 (2011).
51. Makhlof, L. *et al.* The role of autoimmunity in islet allograft destruction: major histocompatibility complex class II matching is necessary for autoimmune destruction of allogeneic islet transplants after T-cell costimulatory blockade. *Diabetes* **51**, 3202–3210 (2002).
52. Rigby, M. R. *et al.* Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol* **1**, 284–294 (2013).
53. Posselt, A. M. *et al.* Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade. *Transplantation* **90**, 1595–1601 (2010).

54. Budde, K. *et al.* Conversion from Calcineurin Inhibitor- to Belatacept-Based Maintenance Immunosuppression in Renal Transplant Recipients: A Randomized Phase 3b Trial. *J Am Soc Nephrol* **32**, 3252–3264 (2021).
55. Lombardi, Y. & François, H. Belatacept in Kidney Transplantation: What Are the True Benefits? A Systematic Review. *Front Med (Lausanne)* **9**, 942665 (2022).
56. Terrec, F. *et al.* Late Conversion From Calcineurin Inhibitors to Belatacept in Kidney-Transplant Recipients Has a Significant Beneficial Impact on Glycemic Parameters. *Transplant Direct* **6**, e517 (2020).
57. Farina, M., Alexander, J. F., Thekkedath, U., Ferrari, M. & Grattoni, A. Cell encapsulation: Overcoming barriers in cell transplantation in diabetes and beyond. *Adv Drug Deliv Rev* **139**, 92–115 (2019).
58. Gholipourmalekabadi, M., Zhao, S., Harrison, B. S., Mozafari, M. & Seifalian, A. M. Oxygen-Generating Biomaterials: A New, Viable Paradigm for Tissue Engineering? *Trends Biotechnol* **34**, 1010–1021 (2016).
59. White, J. C., Stoppel, W. L., Roberts, S. C. & Bhatia, S. R. Addition of perfluorocarbons to alginate hydrogels significantly impacts molecular transport and fracture stress. *J Biomed Mater Res A* **101**, 438–446 (2013).
60. Abdi, S. I. H., Ng, S. M. & Lim, J. O. An enzyme-modulated oxygen-producing micro-system for regenerative therapeutics. *Int J Pharm* **409**, 203–205 (2011).
61. Ernst, A. U. *et al.* Nanotechnology in cell replacement therapies for type 1 diabetes. *Adv Drug Deliv Rev* **139**, 116–138 (2019).
62. Cañibano-Hernández, A., Sáenz Del Burgo, L., Espona-Noguera, A., Ciriza, J. & Pedraz, J. L. Current advanced therapy cell-based medicinal products for type-1-diabetes treatment. *Int J Pharm* **543**, 107–120 (2018).
63. Desai, T. & Shea, L. D. Advances in islet encapsulation technologies. *Nat Rev Drug Discov* **16**, 338–350 (2017).
64. Carlsson, P.-O. *et al.* Transplantation of macroencapsulated human islets within the bioartificial pancreas βAir to patients with type 1 diabetes mellitus. *Am J Transplant* **18**, 1735–1744 (2018).
65. Pepper, A. R. *et al.* A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat Biotechnol* **33**, 518–523 (2015).
66. Terman, M. & Terman, J. S. Controlled trial of naturalistic dawn simulation and negative air ionization for seasonal affective disorder. *Am J Psychiatry* **163**, 2126–2133 (2006).
67. Alora, M. B., Fitzpatrick, T. B. & Taylor, C. R. Total body heliotherapy. *Photodermatol Photoimmunol Photomed* **13**, 178–180 (1997).
68. Bae, J. M. *et al.* Phototherapy for Vitiligo: A Systematic Review and Meta-analysis. *JAMA Dermatol* **153**, 666–674 (2017).
69. Woodgate, P. & Jardine, L. A. Neonatal jaundice: phototherapy. *BMJ Clin Evid* **2015**, 0319 (2015).
70. Dolmans, D. E. J. G. J., Fukumura, D. & Jain, R. K. Photodynamic therapy for cancer. *Nat Rev Cancer* **3**, 380–387 (2003).
71. Martino, M. *et al.* Extracorporeal photopheresis, a therapeutic option for cutaneous T-cell lymphoma and immunological diseases: state of the art. *Expert Opin Biol Ther* **12**, 1017–1030 (2012).
72. Asensi Cantó, P., Sanz Caballer, J., Solves Alcaña, P., de la Rubia Comos, J. & Gómez Seguí, I. Extracorporeal Photopheresis in Graft-versus-Host Disease. *Transplant Cell Ther* **29**, 556–566 (2023).
73. Benden, C., Haughton, M., Leonard, S. & Huber, L. C. Therapy options for chronic lung allograft dysfunction-bronchiolitis obliterans syndrome following first-line immunosuppressive strategies: A systematic review. *J Heart Lung Transplant* **36**, 921–933 (2017).
74. Evans, J. R., Michelessi, M. & Virgili, G. Laser photocoagulation for proliferative diabetic retinopathy. *Cochrane Database Syst Rev* CD011234 (2014) doi:10.1002/14651858.CD011234.pub2.
75. Castellani, D. *et al.* GreenLight Laser™ Photovaporization versus Transurethral Resection of the Prostate: A Systematic Review and Meta-Analysis. *Res Rep Urol* **13**, 263–271 (2021).
76. Maiman TH, Stimulated optical radiation in ruby. *Nature*, 1960 187: p. 493–494.
77. Heiskanen, V. & Hamblin, M. R. Photobiomodulation: lasers vs. light emitting diodes? *Photochem Photobiol Sci* **17**, 1003–1017 (2018).
78. Hode, L. The importance of the coherency. *Photomed Laser Surg* **23**, 431–434 (2005).
79. Laakso, L., Richardson, C. & Cramond, T. Quality of light - is laser necessary for effective photobiostimulation? *Aust J Physiother* **39**, 87–92 (1993).
80. Enwemeka, C. S. The place of coherence in light induced tissue repair and pain modulation. *Photomed Laser Surg* **24**, 457 (2006).
81. de Freitas, L. F. & Hamblin, M. R. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J Sel Top Quantum Electron* **22**, 7000417 (2016).
82. Zein, R., Selting, W. & Hamblin, M. R. Review of light parameters and photobiomodulation efficacy: dive into complexity. *J Biomed Opt* **23**, 1–17 (2018).
83. Karu, T. Photobiology of low-power laser effects. *Health Phys* **56**, 691–704 (1989).

84. Huang, Y.-Y., Sharma, S. K., Carroll, J. & Hamblin, M. R. Biphasic dose response in low level light therapy - an update. *Dose Response* **9**, 602–618 (2011).
85. Carroll, J. D., Milward, M. R., Cooper, P. R., Hadis, M. & Palin, W. M. Developments in low level light therapy (LLLTh) for dentistry. *Dent Mater* **30**, 465–475 (2014).
86. Wu, S. *et al.* Cancer phototherapy via selective photoinactivation of respiratory chain oxidase to trigger a fatal superoxide anion burst. *Antioxid Redox Signal* **20**, 733–746 (2014).
87. Lopes-Martins, R. A. B. *et al.* Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats. *J Appl Physiol (1985)* **101**, 283–288 (2006).
88. Alves, A. C. *et al.* Effect of low-level laser therapy on the expression of inflammatory mediators and on neutrophils and macrophages in acute joint inflammation. *Arthritis Res Ther* **15**, R116 (2013).
89. Chen, C.-H. *et al.* Effects of low-level laser therapy on M1-related cytokine expression in monocytes via histone modification. *Mediators Inflamm* **2014**, 625048 (2014).
90. Zein, R., Selting, W. & Hamblin, M. R. Review of light parameters and photobiomodulation efficacy: dive into complexity. *J Biomed Opt* **23**, 1–17 (2018).
91. Glass, G. E. Photobiomodulation: A review of the molecular evidence for low level light therapy. *J Plast Reconstr Aesthet Surg* **74**, 1050–1060 (2021).
92. Wong-Riley, M. T. T. *et al.* Photobiomodulation directly benefits primary neurons functionally inactivated by toxins: role of cytochrome c oxidase. *J Biol Chem* **280**, 4761–4771 (2005).
93. Dompe, C. *et al.* Photobiomodulation-Underlying Mechanism and Clinical Applications. *J Clin Med* **9**, 1724 (2020).
94. Pastore, D., Greco, M. & Passarella, S. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. *Int J Radiat Biol* **76**, 863–870 (2000).
95. Lane, N. Cell biology: power games. *Nature* **443**, 901–903 (2006).
96. Quirk, B. J. & Whelan, H. T. What Lies at the Heart of Photobiomodulation: Light, Cytochrome C Oxidase, and Nitric Oxide-Review of the Evidence. *Photobiomodul Photomed Laser Surg* **38**, 527–530 (2020).
97. Hamblin, M. R. & Liebert, A. Photobiomodulation Therapy Mechanisms Beyond Cytochrome c Oxidase. *Photobiomodul Photomed Laser Surg* **40**, 75–77 (2022).
98. Ball, K. A., Castello, P. R. & Poyton, R. O. Low intensity light stimulates nitrite-dependent nitric oxide synthesis but not oxygen consumption by cytochrome c oxidase: Implications for phototherapy. *J Photochem Photobiol B* **102**, 182–191 (2011).
99. Quirk, B. J. & Whelan, H. T. Effect of Red-to-Near Infrared Light on the Reaction of Isolated Cytochrome c Oxidase with Cytochrome c. *Photomed Laser Surg* **34**, 631–637 (2016).
100. Quirk, B. & Whelan, H. T. Effect of Red-to-Near Infrared Light and a Nitric Oxide Donor on the Oxygen Consumption of Isolated Cytochrome c Oxidase. *Photobiomodul Photomed Laser Surg* **39**, 463–470 (2021).
101. Karu, T., Pyatibrat, L. & Kalendo, G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. *J Photochem Photobiol B* **27**, 219–223 (1995).
102. Benedicenti, S., Pepe, I. M., Angiero, F. & Benedicenti, A. Intracellular ATP level increases in lymphocytes irradiated with infrared laser light of wavelength 904 nm. *Photomed Laser Surg* **26**, 451–453 (2008).
103. Passarella, S. *et al.* Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium-neon laser. *FEBS Lett* **175**, 95–99 (1984).
104. Hilf, R., Murant, R. S., Narayanan, U. & Gibson, S. L. Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyrin derivative-induced photosensitization in R3230AC mammary tumors. *Cancer Res* **46**, 211–217 (1986).
105. Zhang, R. *et al.* Near infrared light protects cardiomyocytes from hypoxia and reoxygenation injury by a nitric oxide dependent mechanism. *J Mol Cell Cardiol* **46**, 4–14 (2009).
106. Wu, J.-Y. *et al.* Low-power laser irradiation suppresses inflammatory response of human adipose-derived stem cells by modulating intracellular cyclic AMP level and NF- $\kappa$ B activity. *PLoS One* **8**, e54067 (2013).
107. de Lima, F. M. *et al.* Low-level laser therapy (LLLTh) acts as cAMP-elevating agent in acute respiratory distress syndrome. *Lasers Med Sci* **26**, 389–400 (2011).
108. Pan, L.-C. *et al.* Single Cell Effects of Photobiomodulation on Mitochondrial Membrane Potential and Reactive Oxygen Species Production in Human Adipose Mesenchymal Stem Cells. *Cells* **11**, 972 (2022).
109. Huang, Y.-Y., Nagata, K., Tedford, C. E., McCarthy, T. & Hamblin, M. R. Low-level laser therapy (LLLTh) reduces oxidative stress in primary cortical neurons in vitro. *J Biophotonics* **6**, 829–838 (2013).
110. Ristow, M. & Schmeisser, S. Extending life span by increasing oxidative stress. *Free Radic Biol Med* **51**, 327–336 (2011).
111. Peng, C., Ouyang, Y., Lu, N. & Li, N. The NF- $\kappa$ B Signaling Pathway, the Microbiota, and Gastrointestinal Tumorigenesis: Recent Advances. *Frontiers in Immunology* **11**, (2020).



112. Chen, A. C.-H. *et al.* Low-level laser therapy activates NF- $\kappa$ B via generation of reactive oxygen species in mouse embryonic fibroblasts. *PLoS One* **6**, e22453 (2011).
113. Patrocínio-Silva, T. L. *et al.* The effects of low-level laser irradiation on bone tissue in diabetic rats. *Lasers Med Sci* **29**, 1357–1364 (2014).
114. Cury, V. *et al.* Low level laser therapy increases angiogenesis in a model of ischemic skin flap in rats mediated by VEGF, HIF-1 $\alpha$  and MMP-2. *J Photochem Photobiol B* **125**, 164–170 (2013).
115. Khadra, M., Lyngstadaas, S. P., Haanaes, H. R. & Mustafa, K. Determining optimal dose of laser therapy for attachment and proliferation of human oral fibroblasts cultured on titanium implant material. *J Biomed Mater Res A* **73**, 55–62 (2005).
116. Khadra, M., Kasem, N., Lyngstadaas, S. P., Haanaes, H. R. & Mustafa, K. Laser therapy accelerates initial attachment and subsequent behaviour of human oral fibroblasts cultured on titanium implant material. A scanning electron microscope and histomorphometric analysis. *Clin Oral Implants Res* **16**, 168–175 (2005).
117. Almeida-Lopes, L., Rigau, J., Zângaro, R. A., Guidugli-Neto, J. & Jaeger, M. M. Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. *Lasers Surg Med* **29**, 179–184 (2001).
118. Tripodi, N. *et al.* The effects of polarized photobiomodulation on cellular viability, proliferation, mitochondrial membrane potential and apoptosis in human fibroblasts: Potential applications to wound healing. *J Photochem Photobiol B* **236**, 112574 (2022).
119. Otterço, A. N. *et al.* Photobiomodulation mechanisms in the kinetics of the wound healing process in rats. *J Photochem Photobiol B* **183**, 22–29 (2018).
120. Chen, C.-H. *et al.* Low-level laser irradiation promotes cell proliferation and mRNA expression of type I collagen and decorin in porcine Achilles tendon fibroblasts in vitro. *J Orthop Res* **27**, 646–650 (2009).
121. Migliario, M., Pittarella, P., Fanuli, M., Rizzi, M. & Renò, F. Laser-induced osteoblast proliferation is mediated by ROS production. *Lasers Med Sci* **29**, 1463–1467 (2014).
122. Whelan, H. T. *et al.* Effect of NASA light-emitting diode irradiation on wound healing. *J Clin Laser Med Surg* **19**, 305–314 (2001).
123. Amid, R., Kadkhodazadeh, M., Ahsaie, M. G. & Hakakzadeh, A. Effect of low level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration. *J Lasers Med Sci* **5**, 163–170 (2014).
124. Wang, Y., Huang, Y.-Y., Wang, Y., Lyu, P. & Hamblin, M. R. Photobiomodulation of human adipose-derived stem cells using 810nm and 980nm lasers operates via different mechanisms of action. *Biochim Biophys Acta Gen Subj* **1861**, 441–449 (2017).
125. Yin, K., Zhu, R., Wang, S. & Zhao, R. C. Low-Level Laser Effect on Proliferation, Migration, and Antiapoptosis of Mesenchymal Stem Cells. *Stem Cells Dev* **26**, 762–775 (2017).
126. Tuby, H., Maltz, L. & Oron, U. Low-level laser irradiation (LLLI) promotes proliferation of mesenchymal and cardiac stem cells in culture. *Lasers Surg Med* **39**, 373–378 (2007).
127. Wu, Y. *et al.* Effects of low-level laser irradiation on mesenchymal stem cell proliferation: a microarray analysis. *Lasers Med Sci* **27**, 509–519 (2012).
128. Soleimani, M. *et al.* The effects of low-level laser irradiation on differentiation and proliferation of human bone marrow mesenchymal stem cells into neurons and osteoblasts--an in vitro study. *Lasers Med Sci* **27**, 423–430 (2012).
129. Chen, H. *et al.* Effect of photobiomodulation on neural differentiation of human umbilical cord mesenchymal stem cells. *Lasers Med Sci* **34**, 667–675 (2019).
130. Bai, J. *et al.* Low level laser therapy promotes bone regeneration by coupling angiogenesis and osteogenesis. *Stem Cell Res Ther* **12**, 432 (2021).
131. Leonida, A. *et al.* Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: in vitro pilot study. *Lasers Med Sci* **28**, 125–132 (2013).
132. Mvula, B. & Abrahamse, H. Differentiation Potential of Adipose-Derived Stem Cells When Cocultured with Smooth Muscle Cells, and the Role of Low-Intensity Laser Irradiation. *Photomed Laser Surg* **34**, 509–515 (2016).
133. Tsai, W.-C. *et al.* Low-level laser irradiation stimulates tenocyte migration with up-regulation of dynamin II expression. *PLoS One* **7**, e38235 (2012).
134. AlGhamdi, K. M., Kumar, A., Ashour, A. E. & AlGhamdi, A. A. A comparative study of the effects of different low-level lasers on the proliferation, viability, and migration of human melanocytes in vitro. *Lasers Med Sci* **30**, 1541–1551 (2015).
135. Liao, X. *et al.* Helium-neon laser irradiation promotes the proliferation and migration of human epidermal stem cells in vitro: proposed mechanism for enhanced wound re-epithelialization. *Photomed Laser Surg* **32**, 219–225 (2014).

136. Rochkind, S., El-Ani, D., Nevo, Z. & Shahar, A. Increase of neuronal sprouting and migration using 780 nm laser phototherapy as procedure for cell therapy. *Lasers Surg Med* **41**, 277–281 (2009).
137. Oyeboode, O. A. & Houreld, N. N. Photobiomodulation at 830 nm Stimulates Migration, Survival and Proliferation of Fibroblast Cells. *Diabetes Metab Syndr Obes* **15**, 2885–2900 (2022).
138. Pasternak-Mnich, K. *et al.* Effect of Photobiomodulation Therapy on the Increase of Viability and Proliferation of Human Mesenchymal Stem Cells. *Lasers Surg Med* **51**, 824–833 (2019).
139. Terena, S. M. L., Mesquita-Ferrari, R. A., de Siqueira Araújo, A. M., Fernandes, K. P. S. & Fernandes, M. H. Photobiomodulation alters the viability of HUVECs cells. *Lasers Med Sci* **36**, 83–90 (2021).
140. Safian, F. *et al.* Photobiomodulation with 810 nm Wavelengths Improves Human Sperms' Motility and Viability In Vitro. *Photobiomodul Photomed Laser Surg* **38**, 222–231 (2020).
141. Zare, F. *et al.* Photobiomodulation with 630 plus 810 nm wavelengths induce more in vitro cell viability of human adipose stem cells than human bone marrow-derived stem cells. *J Photochem Photobiol B* **201**, 111658 (2019).
142. Tsunoi, Y. *et al.* Viability Improvement of Three-Dimensional Human Skin Substitutes by Photobiomodulation during Cultivation. *Photochem Photobiol* **98**, 1464–1470 (2022).
143. Lim, W. *et al.* Modulation of lipopolysaccharide-induced NF- $\kappa$ B signaling pathway by 635 nm irradiation via heat shock protein 27 in human gingival fibroblast cells. *Photochem Photobiol* **89**, 199–207 (2013).
144. Martins, D. F. *et al.* Light-emitting diode therapy reduces persistent inflammatory pain: Role of interleukin 10 and antioxidant enzymes. *Neuroscience* **324**, 485–495 (2016).
145. Silva, V. R. *et al.* Low-level laser therapy inhibits bronchoconstriction, Th2 inflammation and airway remodeling in allergic asthma. *Respir Physiol Neurobiol* **194**, 37–48 (2014).
146. Hamblin, M. R. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys* **4**, 337–361 (2017).
147. Peres e Serra, A. & Ashmawi, H. A. Influence of naloxone and methysergide on the analgesic effects of low-level laser in an experimental pain model. *Rev Bras Anesthesiol* **60**, 302–310 (2010).
148. Lanzafame, R. J. *et al.* Reciprocity of exposure time and irradiance on energy density during photoradiation on wound healing in a murine pressure ulcer model. *Lasers Surg Med* **39**, 534–542 (2007).
149. Kana, J. S., Hutschenreiter, G., Haina, D. & Waidelich, W. Effect of low-power density laser radiation on healing of open skin wounds in rats. *Arch Surg* **116**, 293–296 (1981).
150. Bibikova, A. & Oron, U. Regeneration in denervated toad (*Bufo viridis*) gastrocnemius muscle and the promotion of the process by low energy laser irradiation. *Anat Rec* **241**, 123–128 (1995).
151. Gupta, A. *et al.* Superpulsed (Ga-As, 904 nm) low-level laser therapy (LLLT) attenuates inflammatory response and enhances healing of burn wounds. *J Biophotonics* **8**, 489–501 (2015).
152. Rathnakar, B. *et al.* Photo-biomodulatory response of low-power laser irradiation on burn tissue repair in mice. *Lasers Med Sci* **31**, 1741–1750 (2016).
153. Freire, M. do R. S. *et al.* LED and laser photobiomodulation in the prevention and treatment of oral mucositis: experimental study in hamsters. *Clin Oral Investig* **18**, 1005–1013 (2014).
154. Curra, M. *et al.* Photobiomodulation reduces oral mucositis by modulating NF- $\kappa$ B. *J Biomed Opt* **20**, 125008 (2015).
155. Castro, T. N. S. *et al.* Effects of photobiomodulation therapy in the integration of skin graft in rats. *Lasers Med Sci* **35**, 939–947 (2020).
156. Wikramanayake, T. C. *et al.* Effects of the Lexington LaserComb on hair regrowth in the C3H/HeJ mouse model of alopecia areata. *Lasers Med Sci* **27**, 431–436 (2012).
157. Wikramanayake, T. C. *et al.* Low-level laser treatment accelerated hair regrowth in a rat model of chemotherapy-induced alopecia (CIA). *Lasers Med Sci* **28**, 701–706 (2013).
158. Oron, U., Maltz, L., Tuby, H., Sorin, V. & Czerniak, A. Enhanced liver regeneration following acute hepatectomy by low-level laser therapy. *Photomed Laser Surg* **28**, 675–678 (2010).
159. Lin, Y.-S., Huang, M.-H. & Chai, C.-Y. Effects of helium-neon laser on the mucopolysaccharide induction in experimental osteoarthritic cartilage. *Osteoarthritis Cartilage* **14**, 377–383 (2006).
160. Ad, N. & Oron, U. Impact of low level laser irradiation on infarct size in the rat following myocardial infarction. *Int J Cardiol* **80**, 109–116 (2001).
161. Azbel', D. I. *et al.* [The effect of the blood serum from patients subjected to intravenous laser therapy on the parameters of synaptic transmission]. *Biull Eksp Biol Med* **116**, 149–151 (1993).
162. O'Brien, J. A. & Austin, P. J. Effect of Photobiomodulation in Rescuing Lipopolysaccharide-Induced Dopaminergic Cell Loss in the Male Sprague-Dawley Rat. *Biomolecules* **9**, 381 (2019).
163. Peoples, C. *et al.* Photobiomodulation enhances nigral dopaminergic cell survival in a chronic MPTP mouse model of Parkinson's disease. *Parkinsonism Relat Disord* **18**, 469–476 (2012).
164. Moro, C. *et al.* Photobiomodulation preserves behaviour and midbrain dopaminergic cells from MPTP toxicity: evidence from two mouse strains. *BMC Neurosci* **14**, 40 (2013).

165. Xuan, W., Agrawal, T., Huang, L., Gupta, G. K. & Hamblin, M. R. Low-level laser therapy for traumatic brain injury in mice increases brain derived neurotrophic factor (BDNF) and synaptogenesis. *J Biophotonics* **8**, 502–511 (2015).
166. Xuan, W., Vatansever, F., Huang, L. & Hamblin, M. R. Transcranial low-level laser therapy enhances learning, memory, and neuroprogenitor cells after traumatic brain injury in mice. *J Biomed Opt* **19**, 108003 (2014).
167. Wu, Q. *et al.* Low-level laser therapy for closed-head traumatic brain injury in mice: effect of different wavelengths. *Lasers Surg Med* **44**, 218–226 (2012).
168. Veronez, S. *et al.* Effects of different fluences of low-level laser therapy in an experimental model of spinal cord injury in rats. *Lasers Med Sci* **32**, 343–349 (2017).
169. Ayar, Z. *et al.* The effect of low-level laser therapy on pathophysiology and locomotor recovery after traumatic spinal cord injuries: a systematic review and meta-analysis. *Lasers Med Sci* **37**, 61–75 (2022).
170. de Oliveira, M. E., Da Silva, J. T., Brioschi, M. L. & Chacur, M. Effects of photobiomodulation therapy on neuropathic pain in rats: evaluation of nociceptive mediators and infrared thermography. *Lasers Med Sci* **36**, 1461–1467 (2021).
171. Janzadeh, A. *et al.* Time-dependent photobiomodulation management of neuropathic pain induced by spinal cord injury in male rats. *Lasers Med Sci* **38**, 120 (2023).
172. Eshaghi, E., Sadigh-Eteghad, S., Mohaddes, G. & Rasta, S. H. Transcranial photobiomodulation prevents anxiety and depression via changing serotonin and nitric oxide levels in brain of depression model mice: A study of three different doses of 810 nm laser. *Lasers Surg Med* **51**, 634–642 (2019).
173. Salehpour, F., Rasta, S. H., Mohaddes, G., Sadigh-Eteghad, S. & Salarirad, S. Therapeutic effects of 10-Hz Pulsed wave lasers in rat depression model: A comparison between near-infrared and red wavelengths. *Lasers Surg Med* **48**, 695–705 (2016).
174. Eells, J. T. *et al.* Therapeutic photobiomodulation for methanol-induced retinal toxicity. *Proc Natl Acad Sci U S A* **100**, 3439–3444 (2003).
175. de Almeida, P. *et al.* Low-level laser therapy improves skeletal muscle performance, decreases skeletal muscle damage and modulates mRNA expression of COX-1 and COX-2 in a dose-dependent manner. *Photochem Photobiol* **87**, 1159–1163 (2011).
176. Ferraresi, C. Use of Photobiomodulation Therapy in Exercise Performance Enhancement and Postexercise Recovery: True or Myth? *Photobiomodul Photomed Laser Surg* **38**, 705–707 (2020).
177. Castano, A. P. *et al.* Low-level laser therapy for zymosan-induced arthritis in rats: Importance of illumination time. *Lasers Surg Med* **39**, 543–550 (2007).
178. Rubio, C. R. *et al.* Helium-neon laser reduces the inflammatory process of arthritis. *Photomed Laser Surg* **28**, 125–129 (2010).
179. Rigonato-Oliveira, N. C. *et al.* Effect of Low-Level Laser Therapy (LLLT) in Pulmonary Inflammation in Asthma Induced by House Dust Mite (HDM): Dosimetry Study. *Int J Inflam* **2019**, 3945496 (2019).
180. Muili, K. A., Gopalakrishnan, S., Eells, J. T. & Lyons, J.-A. Photobiomodulation induced by 670 nm light ameliorates MOG35-55 induced EAE in female C57BL/6 mice: a role for remediation of nitrosative stress. *PLoS One* **8**, e67358 (2013).
181. Alsharnoubi, J., Mohamed, O. & Fawzy, M. Photobiomodulation effect on children's scars. *Lasers Med Sci* **33**, 497–501 (2018).
182. Kazemikhoo, N. *et al.* Effects of Photobiomodulation and Split-Thickness Skin Grafting in the Prognosis of Wound Healing in Children with Deep Burn Ulcers. *Photobiomodul Photomed Laser Surg* **40**, 148–154 (2022).
183. Barolet, D. & Boucher, A. Prophylactic low-level light therapy for the treatment of hypertrophic scars and keloids: a case series. *Lasers Surg Med* **42**, 597–601 (2010).
184. Al-Maweri, S. A. *et al.* Efficacy of low-level laser therapy in management of symptomatic oral lichen planus: a systematic review. *Lasers Med Sci* **32**, 1429–1437 (2017).
185. He, M., Zhang, B., Shen, N., Wu, N. & Sun, J. A systematic review and meta-analysis of the effect of low-level laser therapy (LLLT) on chemotherapy-induced oral mucositis in pediatric and young patients. *Eur J Pediatr* **177**, 7–17 (2018).
186. Gobbo, M. *et al.* Multicenter randomized, double-blind controlled trial to evaluate the efficacy of laser therapy for the treatment of severe oral mucositis induced by chemotherapy in children: laMPO RCT. *Pediatr Blood Cancer* **65**, e27098 (2018).
187. Chan, A., Armati, P. & Moorthy, A. P. Pulsed Nd: YAG laser induces pulpal analgesia: a randomized clinical trial. *J Dent Res* **91**, 79S-84S (2012).
188. Artés-Ribas, M., Arnabat-Dominguez, J. & Puigdollers, A. Analgesic effect of a low-level laser therapy (830 nm) in early orthodontic treatment. *Lasers Med Sci* **28**, 335–341 (2013).

189. Barolet, D., Roberge, C. J., Auger, F. A., Boucher, A. & Germain, L. Regulation of skin collagen metabolism in vitro using a pulsed 660 nm LED light source: clinical correlation with a single-blinded study. *J Invest Dermatol* **129**, 2751–2759 (2009).
190. Gentile, P. & Garcovich, S. The Effectiveness of Low-Level Light/Laser Therapy on Hair Loss. *Facial Plast Surg Aesthet Med* (2021) doi:10.1089/fpsam.2021.0151.
191. Thunshelle, C. & Hamblin, M. R. Transcranial Low-Level Laser (Light) Therapy for Brain Injury. *Photomed Laser Surg* **34**, 587–598 (2016).
192. Naeser, M. A. *et al.* Significant improvements in cognitive performance post-transcranial, red/near-infrared light-emitting diode treatments in chronic, mild traumatic brain injury: open-protocol study. *J Neurotrauma* **31**, 1008–1017 (2014).
193. Huang, Y.-Y. *et al.* Transcranial low level laser (light) therapy for traumatic brain injury. *J Biophotonics* **5**, 827–837 (2012).
194. Naeser, M. A., Saltmarche, A., Krengel, M. H., Hamblin, M. R. & Knight, J. A. Improved cognitive function after transcranial, light-emitting diode treatments in chronic, traumatic brain injury: two case reports. *Photomed Laser Surg* **29**, 351–358 (2011).
195. Shooshtari, S. M. J. *et al.* The effects of low level laser in clinical outcome and neurophysiological results of carpal tunnel syndrome. *Electromyogr Clin Neurophysiol* **48**, 229–231 (2008).
196. Chang, W.-D., Wu, J.-H., Jiang, J.-A., Yeh, C.-Y. & Tsai, C.-T. Carpal tunnel syndrome treated with a diode laser: a controlled treatment of the transverse carpal ligament. *Photomed Laser Surg* **26**, 551–557 (2008).
197. Cheung, W. K. W. *et al.* Low-level laser therapy for carpal tunnel syndrome: systematic review and network meta-analysis. *Physiotherapy* **106**, 24–35 (2020).
198. Schiffer, F. *et al.* Psychological benefits 2 and 4 weeks after a single treatment with near infrared light to the forehead: a pilot study of 10 patients with major depression and anxiety. *Behav Brain Funct* **5**, 46 (2009).
199. Yeh, S.-W. *et al.* Low-Level Laser Therapy for Fibromyalgia: A Systematic Review and Meta-Analysis. *Pain Physician* **22**, 241–254 (2019).
200. Zyciński, P. *et al.* Laser biostimulation in end-stage multivessel coronary artery disease--a preliminary observational study. *Kardiol Pol* **65**, 13–21; discussion 22-23 (2007).
201. Ferraresi, C. *et al.* Muscular pre-conditioning using light-emitting diode therapy (LEDT) for high-intensity exercise: a randomized double-blind placebo-controlled trial with a single elite runner. *Physiother Theory Pract* **31**, 354–361 (2015).
202. Pinto, H. D. *et al.* Photobiomodulation Therapy Improves Performance and Accelerates Recovery of High-Level Rugby Players in Field Test: A Randomized, Crossover, Double-Blind, Placebo-Controlled Clinical Study. *J Strength Cond Res* **30**, 3329–3338 (2016).
203. Tomazoni, S. S. *et al.* Infrared Low-Level Laser Therapy (Photobiomodulation Therapy) before Intense Progressive Running Test of High-Level Soccer Players: Effects on Functional, Muscle Damage, Inflammatory, and Oxidative Stress Markers-A Randomized Controlled Trial. *Oxid Med Cell Longev* **2019**, 6239058 (2019).
204. De Marchi, T. *et al.* Photobiomodulation therapy before futsal matches improves the staying time of athletes in the court and accelerates post-exercise recovery. *Lasers Med Sci* **34**, 139–148 (2019).
205. Martimbianco, A. L. C. *et al.* Photobiomodulation with low-level laser therapy for treating Achilles tendinopathy: a systematic review and meta-analysis. *Clin Rehabil* **34**, 713–722 (2020).
206. Brosseau, L. *et al.* Low level laser therapy (Classes I, II and III) for treating rheumatoid arthritis. *Cochrane Database Syst Rev* **2005**, CD002049 (2005).
207. Stausholm, M. B. *et al.* Efficacy of low-level laser therapy on pain and disability in knee osteoarthritis: systematic review and meta-analysis of randomised placebo-controlled trials. *BMJ Open* **9**, e031142 (2019).
208. Moskvina, S. V. & Khadartsev, A. A. Methods of effective low-level laser therapy in the treatment of patients with bronchial asthma (literature review). *Biomedicine (Taipei)* **10**, 1–20 (2020).
209. Höfling, D. B. *et al.* Low-level laser therapy in chronic autoimmune thyroiditis: a pilot study. *Lasers Surg Med* **42**, 589–596 (2010).
210. Höfling, D. B. *et al.* Low-level laser in the treatment of patients with hypothyroidism induced by chronic autoimmune thyroiditis: a randomized, placebo-controlled clinical trial. *Lasers Med Sci* **28**, 743–753 (2013).
211. Höfling, D. B. *et al.* Assessment of the effects of low-level laser therapy on the thyroid vascularization of patients with autoimmune hypothyroidism by color Doppler ultrasound. *ISRN Endocrinol* **2012**, 126720 (2012).
212. Höfling, D. B. *et al.* Effects of low-level laser therapy on the serum TGF- $\beta$ 1 concentrations in individuals with autoimmune thyroiditis. *Photomed Laser Surg* **32**, 444–449 (2014).

213. Höfling, D. B. *et al.* Safety and Efficacy of Low-Level Laser Therapy in Autoimmune Thyroiditis: Long-Term Follow-Up Study. *Int J Endocrinol* **2018**, 8387530 (2018).
214. Liebman, C., Loya, S., Lawrence, M., Bashoo, N. & Cho, M. Stimulatory responses in  $\alpha$ - and  $\beta$ -cells by near-infrared (810 nm) photobiomodulation. *J Biophotonics* **15**, e202100257 (2022).
215. Huang, H.-H., Stillman, T. J., Branham, L. A. & Williams, S. C. The Effects of Photobiomodulation Therapy on Porcine Islet Insulin Secretion. *Photobiomodul Photomed Laser Surg* **40**, 395–401 (2022).
216. Irani, S. *et al.* Effect of low-level laser irradiation on in vitro function of pancreatic islets. *Transplant Proc* **41**, 4313–4315 (2009).
217. Cottet-Dumoulin, D. *et al.* Intercellular contacts affect secretion and biosynthesis of pancreatic islet cells. *Journal of Endocrinology* **258**, (2023).

# Annexe 1

The article has been submitted to  
Frontiers in Endocrinology

## Article n° 4

### Diabetes in spotlight: Current knowledge and perspectives of photobiomodulation utilisation

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**Abbreviation**

AUC: Area under the curve

GTT: Glucose tolerance test

HOMA-IR: Homeostatic model assessment of insulin resistance

ITT: Insulin tolerance test

LED: Light emitting diode

PBM: Photobiomodulation

Pre-Post ITV: Pre-post interventional trial

QoL: Quality of life

RCT: Randomized controlled trial

T1D: Type 1 diabetes

T2D: Type 2 diabetes

## Abstract

**Introduction.** Diabetes is a global health concern characterized by chronic hyperglycemia resulting from insulinopenia and/or insulin resistance. The rising prevalence of diabetes and its associated complications (ulcers, periodontitis, bone healing, neuropathy, retinopathy, cardiopathy and nephropathy) necessitate innovative therapeutic approaches. Photobiomodulation (PBM), involves exposing tissues and cells to low-energy light radiation, leading to biological effects, largely via mitochondrial activation.

**Aims.** This review evaluates preclinical and clinical studies exploring the potential of PBM in diabetes and its complications, as well all clinical trials, both planned and completed, available on ClinicalTrials database.

**Results.** This review highlights the variability in PBM parameters across studies, hindering consensus on optimal protocols. Standardization of treatment parameters and rigorous clinical trials are needed to unlock PBM's full therapeutic potential. 87 clinical trials were identified that investigated PBM in diabetes mellitus (with 5,837 patients planned to be treated with PBM). Clinical trials assessing PBM effects on diabetic neuropathy revealed pain reduction and potential quality of life improvement. Studies focusing on wound healing indicated encouraging results, with PBM enhancing angiogenesis, fibroblast proliferation, and collagen density. PBM's impact on diabetic retinopathy remains inconclusive however, requiring further investigation. In glycaemic control, PBM exhibits positive effects on metabolic parameters, including glucose tolerance and insulin resistance.

**Conclusion.** Clinical studies have reported PBM-induced reductions in fasting and postprandial glycemia without an increased hypoglycaemic risk. This impact of PBM may be related to its effects on the beta cells and islets in the pancreas. Notwithstanding challenges, PBM emerges as a promising adjunctive therapy for managing diabetic neuropathy, wound healing, and glycaemic control. Further investigation into its impact on diabetic retinopathy and muscle recovery is warranted.

## Introduction

Diabetes is characterized by chronic hyperglycaemia due to insulinopenia (type 1 diabetes (T1D)) and/or insulin resistance (type 2 diabetes (T2D)). The International Diabetes Federation reported 537 million of potential cases of diabetes across the world in 2021 with an increment planned for 2045 at 783 million of potential cases<sup>1</sup>. As a result, diabetes caused 6.7 million of death in 2021<sup>1</sup> and led to USD 966 billion health expenditures<sup>1</sup> partially due to numerous complications related to diabetes disease<sup>2</sup> such as macrovascular complication (ischemic cardiomyopathy, stroke and arteriopathy)<sup>2-5</sup> and microvascular complications: 1) retinopathy, diabetes is the first cause of non-traumatic blindness<sup>6</sup>, 2) nephropathy, diabetes is the first cause of dialysis, 3) amputation and 4) neuropathy<sup>7,8</sup> leading to foot ulceration and exposing patient to a risk of lower limb amputation (diabetes is the first cause of non-traumatic amputation<sup>9</sup>). Finally, patients living with diabetes are also exposed to a risk of periodontitis<sup>10</sup>. Altogether, diabetic complications alter quality of life<sup>11,12</sup>. These complications can be prevented through optimal glycaemic control and could be managed with some medicines<sup>13,14</sup>. However, despite optimal medical management, prevention of diabetic complications remains a challenge and additional treatment remains mandatory.

Light was used as potential of treatment since the ancient Egypt. The biological reaction to light and its therapeutic applications are not new. For example, the beneficial effect of light on neonatal jaundice, discovered in the 1950s, made phototherapy (with blue light with a wavelength between 420-490 nm) the main modality for its treatment<sup>15</sup>. Another example, the effects of light on mood, demonstrated in the 80s, made it possible to propose light therapy as a treatment for seasonal affective disorders, and it has recently been shown to have an effect comparable to antidepressants in episodes major depression<sup>16</sup>.

Photobiomodulation therapy (PBM), formerly called "Low level laser therapy", is a phototherapy based on the exposure of tissues and cells to non-ionizing and very weak light radiation with a wavelength generally ranging from red (between 600-700 nm) to the near infrared (between 700-1400 nm) and resulting in biological effects following its absorption by endogenous chromophores. Historically, PBM



was first described by Endre Mester in 1968 who observed faster hair regrowth in rodents exposed to a low-energy laser with a wavelength of 694 nm<sup>17,18</sup>. In the same years, it was also developed by the National Aeronautics and Spatial Administration (NASA) to accelerate the healing and regeneration of muscle cells in astronauts<sup>19</sup>. For the past decades, biomedical research relating to PBM has been constantly increasing, indicating a growing interest in its therapeutic potential. Over the past three years (2020-2022), approximately 850 articles per year have been published and referenced in MEDLINE.

The main mechanism of action involves the mitochondria<sup>20</sup>, possessing photo-acceptors sensitive to the lengths used with PBM. Briefly, PBM activates the mitochondrial respiratory chain resulting in increased ATP production and activation of a set of secondary messengers involved in modulating inflammatory cytokines, growth factors, oxidative stress and cell survival. The main biological effects highlighted in preclinical and clinical studies are an anti-inflammatory, analgesic action, an increase in blood circulation, angiogenesis, and a healing/regeneration and tissue proliferation action<sup>21-23</sup>. Given these effects, the potential therapeutic applications are numerous. PBM is already used in certain medical disciplines. It is part, for example, of the recommendations for the prevention of mucositis in patients treated by radiotherapy.

The use of PBM in the context of neurodegenerative diseases, and in particular Parkinson's disease, is currently being studied and is the subject of clinical trials. PBM could represent an innovative therapeutic solution, to slow down the neurodegenerative process. The preclinical results in this direction are very encouraging, and clinical data should be published soon due to ongoing clinical trials. The metabolic syndrome in neurodegenerative diseases, and in particular Parkinson's disease, are well established<sup>24,25</sup>. The observed effect of PBM on cellular metabolism, inflammatory and scarring processes is a lead that may indicate an interest of PBM in the regulation of phenomena related to metabolic syndromes, such as diabetes.

In the present work, the purpose is to review the clinical studies using PBM and conducted in the field of the treatment of diabetes and diabetic complications.

## Materials and methods

Identification was made regarding:

- Pre-clinical and clinical data available until 23 May 2023
- Clinical trials available on [clinicaltrials.gov](https://clinicaltrials.gov)<sup>26</sup> until 23 May 2023
- Searching strategy and selection criteria of papers

A MEDLINE research was conducted via PubMed using the search terms: ((Photobiomodulation) or (Low-level laser therapy) or (Near-infrared therapy)) AND (diabetes or diabetic or T1D or T2D) AND (1900/01/01:2023/05/23[edat]). In addition, references from cited papers were investigated. For each paper, the following parameters were recorded: 1st author, year of publication, model used (including the number of patients in clinical trials), wavelength, light source (LED or laser), mode of administration (continuous or pulsed), PBM parameters (power density in  $\text{mW}/\text{cm}^2$ , time per exposure, energy density in  $\text{J}/\text{cm}^2$ , frequency, sites), and the study's conclusion. The data were analysed both collectively and individually, considering different diabetes conditions such as retinopathy, ulcers, and periodontitis. In the case of clinical trial papers, it was specified whether they were randomized clinical trials (RCTs), pre-post interventional trials (Pre-Post ITV) or observational studies.

- Searching strategy for clinical trials and classification

The screening of the ClinicalTrials database<sup>26</sup> was conducted using three terms: photobiomodulation, low-level laser therapy and near-infrared therapy. Only trials related to diabetes were considered eligible, and studies employing methods other than PBM were excluded (Fig. 1). For each included trial, the following parameters were recorded: starting date, country of the sponsor, expected number of enrolled patients, and enrolment status (not yet recruiting, recruiting, enrolling by invitation, active, suspended, terminated, completed, withdrawn, or unknown). Regarding clinical trials published in the Medline database, the following parameters were recorded: country of the sponsor and number of enrolled patients. All these trials were classified as completed.

## Results

Following Medline research, 124 articles met the search criteria. 170 different exposure times were studied and varied widely, with 35% of the studies (60/168) having exposure times (for one session) of less than a minute and 35% (59/168) exceeding 5 min (Fig. 2A). Laser was the predominant light source investigated in 80% of the studies (Fig. 2B), and continuous exposure was the primary mode of administration (84% of studies, Fig. 2C). The power density values ranged from 1 mW/cm<sup>2</sup> to 8.32 W/cm<sup>2</sup>, whereas the energy density ranged from 0.03 to 420 J/cm<sup>2</sup>. These studies investigated various wavelengths ranging from 425 to 1064 nm, with 82% of studies between 600 and 900 nm, and six studies exploring multiple wavelengths applying simultaneously (Fig. 2D). It should be noted that these parameters were not fully described or available in 37 studies (29%).

- Clinical trials in humans

42 trials were identified in ClinicalTrial database<sup>26</sup>, and 56 articles were found in Medline. After removing duplicate trials, 87 were conducted or planned (Fig. 2). These trials included 5,837 patients (see Table 1 for details). The top three countries planning to conduct trials on PBM and diabetes were the United States (n = 19), Brazil (n = 18), and India (n = 10) (Fig. 3).

- Impact of PBM on diabetic neuropathy

A total of 23 studies were conducted, including 5 on preclinical models<sup>27-31</sup> and 18 in humans<sup>32-49</sup> (Table 2). Among 14 studies investigating pain, 11 reported a decrease in pain after PBM, with 4 studies conducted on preclinical models and 7 on clinical subjects. In clinical trials, pain was evaluated using a visual analogic scale (VAS), either alone or in combination with other pain assessment scores with a follow-up period ranging from 4 to 90 days. For pre-post interventional trials (n = 3), the VAS scores decreased by an average of 2.3 to 5.2 points after PBM. In RCTs (n = 4), the VAS scores decreased more in the PBM group than in the control group (0 to 3 points vs. 3 to 5 points after 10 days)<sup>32,34</sup>, and the VAS scores at the end of the follow-up period were lower in the PBM group than in the control group

(6.9-7.9 vs. 5.9-5.3)<sup>37,38</sup>. Four studies investigated the effect of PBM on QoL. Two studies reported a positive impact of PBM on QoL associated with a decrease in pain<sup>32,34</sup>. Conversely, 2 studies did not report an impact of PBM on QoL but in these 2 studies PBM did not induced a decrease in pain<sup>40,41</sup>. The first study<sup>40</sup> used the highest power (350W), and the second study<sup>41</sup> used the longest exposure time (40 min) and application frequency (once a day for 90 days).

- Impact of PBM on diabetic retinopathy

A total of 6 studies were conducted, including 4 studies on preclinical models<sup>50-53</sup> and 2 studies in humans<sup>54,55</sup> (Table 3). Preclinical studies consistently reported a positive effect of PBM on retinal structure associated with a decrease in oxidative stress. In clinical studies focusing on macular oedema, findings were divergent regarding improvement of visual acuity between the two identified studies<sup>54,55</sup>. However, no side effects were reported in either study.

- Impact of PBM on glucose metabolism in T2D

A total of 9 studies were conducted, with 8 studies conducted on preclinical models<sup>56-64</sup> and one study in humans<sup>65</sup> (Table 4). The preclinical studies consistently reported positive effects of PBM on metabolic parameters with an improvement in glucose tolerance (with a glucose tolerance test area under the curve (GTT AUC) decreasing by 12–28%), a decrease in insulin resistance (with a 22% decrease in homeostatic model assessment of insulin resistance (HOMA-IR<sup>56</sup>) and a 10% decrease in insulin tolerance test (ITT) AUC<sup>58</sup>) and a decrease in fasting glycemia. PBM also showed positive effects on lipid profiles with a reduction in free fatty acid, triglycerides and cholesterol levels. In the clinical study<sup>65</sup>, PBM applied on eight muscles in patients with T2D resulted in a decrease in glycemia (fasting and 1h post-prandial glucose) observed 30 min to 12 h after PBM treatment. This decrease was evidenced by a reduction in the GTT AUC by 37% and a reduction of 1h postprandial glucose by 16%. Importantly, no additive effect of hypoglycaemic treatment was observed and no hypoglycaemia were reported.

- Impact of PBM on exercises and muscles in T2D

A total of 7 studies were conducted, including 3 studies on preclinical models<sup>66–68</sup> and 4 studies in humans<sup>69–72</sup> (Table 5). In preclinical models, PBM has been demonstrated to have a positive impact on biochemical parameters, such as a decrease in oxidative stress and an increase in antioxidant activity. However, in clinical studies focusing on muscular performance and functionality during or after exercise, PBM has failed to show any improvement. No significant effects on the muscular performance were observed.

- Impact of PBM on cicatrisation

A total of 44 studies were conducted to evaluate impact of PBM on wound healing, including 31 studies on preclinical models<sup>73–103</sup> and 13 studies on humans<sup>104–116</sup> (Table 6). In preclinical models, PBM had a predominantly positive effect on cicatrisation and healing in 94% of studies. PBM improved various aspects of wound healing, including collagen density, fibroblast proliferation, angiogenesis, granulation tissue formation, and epithelialization. These effects were often accompanied by a decrease inflammatory markers. Only one study, which poorly described PBM parameters, did not report a positive effect of PBM<sup>93</sup>. Most studies utilized wavelengths in the red to near-infrared spectrum, whereas studies investigating green wavelengths did not report positive effects of PBM<sup>73,83</sup>. In clinical studies, the majority (92%) reported a positive effect of PBM on chronic ulcers cicatrisation. Among the 12 RCTs, there was an increase in wound closure ranging from 15% to 47.3% in the control group compared to 37% to 90.8% in the PBM group. Consequently, the PBM groups had smaller wound areas compared to the control groups, with measurements of 2.39 cm<sup>2</sup> vs. 8.43 cm<sup>2</sup><sup>110</sup>, indicating a decrease in wound area of approximately 3.2 cm<sup>2</sup> vs. 10.4 cm<sup>2</sup><sup>112</sup>. The only study that did not report a positive effect of PBM utilized the shortest exposure time (less than one second)<sup>111</sup>. Two studies specified that PBM did not have any reported side effects<sup>104,107</sup> while other studies did not explicitly mention it.

A total of 16 studies were conducted to evaluate impact of PBM on bone cicatrisation, with 15 studies conducted on preclinical models<sup>117-131</sup> and one study in humans<sup>132</sup> (Table 7). Among these studies, only one preclinical study did not report a positive impact of PBM on bone repair. This study used the highest power among all studies, with a dosage of 369.4J/cm<sup>2</sup>. In addition to the effect of PBM on bone repair, several studies reported an increase in bone vascularization and a decrease in inflammation.

A total of 16 studies were conducted to evaluate the impact of PBM on chronic periodontitis in humans<sup>133-148</sup> (Table 8). The effects of PBM were evaluated using the following measures: 1) gingival index represents inflammation of the gingival tissue<sup>149</sup>, 2) the plaque index, which represents the presence of supragingival plaque on all four tooth surfaces<sup>150</sup>. Among the clinical studies, 11 were RCTs, 3 were RCTs specifically focused on pockets treated with PBM, and two were Pre-Post ITV. The results were heterogeneous, with a decrease in the plaque index observed in 50% of the studies, a decrease in the gingival index in 80% of the studies, a reduction in bleeding in 66% of the studies, a decrease in probing depth in 50% of the studies, and improvements in clinical attachment levels in 29% of the studies. Two studies investigated the effect of PBM on reducing the bacterial population at periodontitis sites, but the results were contradictory.

- Others utilisation described in diabetes mellitus (Table 9)

In a preclinical study, Yang et al.<sup>151</sup> reported a positive impact of PBM on erectile function two weeks after PBM exposure. This suggests a potential therapeutic effect of PBM on improving erectile function.

Asghari et al.<sup>152</sup> conducted a preclinical study and demonstrated a protective effect of PBM against ischemia/reperfusion injury in the diabetic kidney. They observed a decrease in tubular epithelial necrosis, polymorphonuclear cells in the outer medulla, cellular oedema, tubular dilatation, hyaline casts, and medullary congestion. These findings indicate the potential of PBM in mitigating kidney injury associated with ischemia/reperfusion.

Aghemohamdi et al.<sup>153</sup> demonstrated the positive impact of PBM in patients with T2D who experienced facial nerve palsy. After 12 sessions of PBM, 60% of the patients showed recovery on electromyogram without any reported side effects. However, the investigation of QoL outcomes were not investigated in this study.

## **Discussion**

This review shows a clear interest in the use of PBM in diabetes, both at preclinical (70 studies) and clinical level (56 studies out of 88 clinical trials identified by [clinicaltrials.gov](https://clinicaltrials.gov)<sup>26</sup>). However, the therapeutic effect of PBM is variable, with inconsistent illumination parameters that are not standardized across studies.

Regarding clinical trials, PBM has generated interest across various fields, with 2,573 clinical trials identified on [clinicaltrials.gov](https://clinicaltrials.gov)<sup>26</sup>. Although 42 clinical trials related to PBM in diabetes were found on [clinicaltrials.gov](https://clinicaltrials.gov)<sup>26</sup>, an additional 46 studies were identified through the Medline bibliographic search. Since PBM is not considered as a drug, the reporting of trials in the global clinical trials database is not consistent. Moreover, in some cases, trials may be reported directly to national registries, as seen in many studies conducted in Brazil. Another important point to note is that among the 67 completed trials, only 56 were published, indicating a significant publication bias (16%), which is likely underestimated. Moreover, quality of clinical trials must be upgraded, as there is heterogeneity in the number of patients included, in the presence of a control group and in the parameters used.

Currently, there is no consensus on the optimal PBM parameters to achieve biological or clinical effects. In terms of light sources, some studies have reported superior effects with coherent laser light<sup>154,155</sup>. However, a recent review found no difference in efficacy between LEDs and laser sources, with LEDs being more cost-effective. In the context of diabetes and wound healing, two studies compared lasers and LEDs. Al-Watban et al.<sup>96</sup> reported a better efficacy of a 633 nm laser compared

to LEDs with polychromatic light for ulcer healing, may be due to dilution of the effect as the irradiance was comparable for both. Vitoriano et al.<sup>105</sup> reported a greater reduction in ulcer size with an 830 nm laser compared to 850 nm LEDs (with comparable irradiance). Despite these two studies favouring lasers, numerous studies in the field of diabetes have reported positive effects of LED-based PBM. However, the choice between laser and LED was a technologic choice and could be led by the accuracy of wavelength search, the availability of the device and energetic consumption. It is important to note that the principle of PBM is based on the Arndt-Schultz law<sup>156</sup>, which describes a biphasic response. A dose that is too low will produce no effect while a dose that is too high can be toxic and induce mitochondrial permeabilization and apoptosis through activation of caspases<sup>157</sup>. This biphasic response to PBM was reflected in two studies on ulcer healing in diabetes. Hedge et al.<sup>90</sup> tested a 632.8 nm laser with different irradiances ranging from 1 to 5 J/cm<sup>2</sup>. While irradiances of 1 and 5 J/cm<sup>2</sup> resulted in poorer and slower wound healing, an irradiance of 3 J/cm<sup>2</sup> appeared to be optimal for improving and accelerating wound healing. Maiya et al.<sup>98</sup> tested a 632.8 nm laser with different irradiances ranging from 3 to 9 J/cm<sup>2</sup>. Irradiances between 3 and 7 J/cm<sup>2</sup> had a positive effect on healing, including increased epithelialization, tissue granulation, and accelerated wound healing; whereas irradiances of 8 and 9 J/cm<sup>2</sup> hindered the healing process. To date, there is no consensus on the power or optimal irradiance to be applied, and the wavelength applied is another parameter of interest that lacks consensus and may depend on the target tissue. Red and near-infrared light correspond to the absorption wavelengths of cytochrome c oxidase in the mitochondria<sup>20</sup>. Green light, on the other hand, is rarely used and not very effective in inducing biological changes as it was not in the spectre of absorption of cytochrome c oxidase. Two studies compared blue, green, and red wavelengths in diabetes. Dungal et al.<sup>73</sup> reported that blue light (470 nm) had no effect on wound healing, whereas green (540 nm) and red (629 nm) light accelerated wound healing. Fekrazad et al.<sup>83</sup> reported no effect of blue (425 nm) and green (532 nm) light, whereas red light (630 nm) promoted wound healing. Another study<sup>82</sup> reported that a 632.8 nm laser is more effective than an 830 nm laser



for wound healing. Given these findings, it is logical that the most commonly used wavelengths (80 studies) fall within the red and near-infrared range (600–810 nm).

The first experiment to investigate the effect of PBM on healing showed promising results<sup>17,18</sup>. Extensive research has been conducted in this field, with several preclinical and clinical reviews reporting positive effects of PBM on wound healing<sup>158</sup>, bone healing<sup>159</sup>, and periodontitis<sup>160</sup>. In a specific population of patients with diabetes, the results regarding these healing processes were encouraging. Numerous preclinical studies have focused on ulcer and wound healing, demonstrating the beneficial effects of PBM. These effects include improved angiogenesis and associated trophic factors, increased fibroblasts, reduced inflammation, increased collagen quantity, and even a reduction in colony-forming units (CFU). Clinically, these results were supported by a significant reduction in wound area (by a factor of 3.5 to 8.2) and increased wound closure (ranging from 22% to 47.9%), which may be associated with reduced pain. Wound healing issues in patients with diabetes significantly impact their QoL<sup>161</sup>. However, only one study<sup>111</sup> has examined the impact of PBM on the QoL of patients with diabetes and ulcers and did not demonstrate any beneficial effects of PBM on QoL. Overall, the data on wound healing are encouraging. The ideal parameters may involve repeated exposure over several weeks, at multiple sites as close as possible to the lesion, with a fluence between 1 and 10 J/cm<sup>2</sup>, continuous illumination using LED or laser, and a wavelength ranging from 660 to 830 nm.

Regarding bone healing, most preclinical studies (94% of studies) showed positive results. PBM has been found to have a beneficial effect, leading to improved vascularization, increased osteoblasts and osteocytes, reduced inflammation, increased bone volumes, and enhanced bone density. Clinically, only one study<sup>132</sup> has been conducted, demonstrating improved bone repair, density, and structure after dental implant insertion with one session of PBM before and after implantation. However, conclusions cannot be drawn from a single clinical study, but the promising results from preclinical and clinical studies should motivate further clinical trials to determine the optimal parameters for PBM.

To date, only clinical studies have investigated the effects of PBM on periodontitis, yielding heterogeneous results due to variations in the applied parameters. The exposure periods ranged from a single session to several days or even weeks. Among studies that examined the gingival index, plaque index, and clinical attachment level, 75% reported improvements in at least one of these parameters. Four studies reported negative results: one had the highest fluence<sup>139</sup>, one had the highest exposure frequency (once a day for 8 weeks<sup>134</sup>), one had poorly described illumination parameters and a wavelength beyond the infrared range<sup>136</sup>, and one had four PBM exposure sites<sup>143</sup>, whereas most studies reported one to two exposure sites. Finally, the ideal parameters could involve a single exposure or exposure over 2 to 3 days, on one to two sites in direct contact with gingival tissue or intra-pocket, with a fluence of 1 to a few J/cm<sup>2</sup>, continuous or pulsed laser illumination, and a wavelength range of 650 to 1064 nm. These data have been supported by previous results on in vitro model<sup>162</sup> reporting positive response of fibroblasts to the PBM in diabetic hypoxic wounded models. However, due to the wide variability in illumination parameters and obtained results, definitive conclusions regarding the therapeutic effects of PBM on periodontitis cannot be formally drawn. Further clinical trials are required to establish clearer conclusions.

Regarding neuropathy, five preclinical studies reported a positive effect of PBM on pain, leading to a decrease in cytokines and improvement in mitochondrial parameters. Clinically, in 77% of the studies, a 2-5 points reduction on the VAS was observed for pain. Out of the studies that considered the impact of PBM on QoL (24%), two studies reported no effect<sup>40,41</sup> (similarly, no effect on pain was observed), while two studies reported a 4-point improvement in Norfolk Quality of Life-Diabetic Neuropathy<sup>32</sup> and SF-36 scores on all these parameters<sup>34</sup>. No adverse effects of PBM were reported in any of the studies. Therefore, for neuropathy, the optimal parameters for PBM may involve repeated exposure over several weeks, on one or more sites in contact with the painful areas, a fluence of 1 to a few J/cm<sup>2</sup>, and wavelengths between 630 and 900 nm.

For diabetic retinopathy, only a few studies have been conducted in this field. Four preclinical studies reported promising results on the effect of PBM, showing histological improvements in the retina. However, clinically, only two studies reported a reduction in central macular thickness, with<sup>55</sup> or without<sup>54</sup> an improvement in vision. Therefore, it is not possible to conclude whether PBM is beneficial or safe for this indication based on the available data.

PBM is also gaining popularity among its potential benefits for post-physical activity recovery, this fact was still a source of debate<sup>163,164</sup>. In the context of T2D, a limited number of studies (n = 7) have been conducted. The results of 3 preclinical studies reported positive effects of PBM on oxidative stress, antioxidant activity, and muscular parameters. However, clinically (n = 4), no study reported improvements in performance or muscle functionality. Only one study<sup>69</sup> reported a benefit in terms of lactate concentration and cardiac parameters. Based on these findings, it can be concluded that the current parameters used for PBM do not provide benefits for post-exercise muscle recovery in patients with T2D.

Lastly, since diabetes is a metabolic disease characterized by an imbalance in glycaemic control, PBM has also been investigated in this field. Preclinical results have shown promising results, with 83% of the studies that examined glucose tolerance possibly due to a direct impact of PBM on islet insulin secretion capability and insulin resistance reporting an improvement in these parameters. The only negative study<sup>57</sup> applied the highest power (3.57 W/cm<sup>2</sup>) for the shortest period (8 seconds). Preclinical studies have also demonstrated beneficial effects of PBM on lipid profiles, including reduced ectopic fat in muscle, triglycerides, and free fatty acids. Additionally, a small-scale clinical study<sup>65</sup> (n = 10) reported beneficial effects of PBM, including a 37% reduction in post-meal AUC for glucose and approximately 16% faster postprandial glucose decay. No adverse effects were reported with PBM, and there was no increased risk of hypoglycaemia when PBM was combined with hypoglycaemic treatments. Overall, these findings support the potential of PBM in improving glycaemic control in

patients with type 2 diabetes. Further clinical trials with larger sample sizes are warranted to determine the optimal parameters for PBM. Moreover, the effect of PBM on glucose intolerance could be lead to the action of PBM on beta cells and islets. Liebman et al.<sup>165</sup> reported improvement of insulin secretion of beta cells and glucagon of alpha cells associated with a rise of calcium activity. Irani et al.<sup>166</sup> demonstrated that PBM could improve insulin secretion of rat pancreatic islets with poor insulin secretion. Huang et al.<sup>167</sup> investigated the effects of PBM on pig islets, which are being explored as a potential source of islets for xenotransplantation. However, they did not observe any significant positive or negative effects on glucose-stimulated insulin secretion. Further research is needed to explore the potential of PBM to enhance islet function for transplantation purposes, even if Asghari et al.<sup>152</sup> reported protector effect of PBM on ischemia-reperfusion injury in diabetic kidney of rats.

Overall, this review highlights the growing interest in PBM as a potential therapeutic approach for various aspects of diabetes. This study emphasizes the potential of PBM as a valuable approach for managing wound healing issues and neuropathic pain in diabetic patients in both preclinical and clinical studies. The potential benefits of PBM in bone cicatrisation and glycaemic control show promise. In retinopathy, the small number of studies make it impossible to draw any conclusion. In periodontitis, more extensive clinical trials are warranted to establish the optimal parameters and protocols for PBM. Likewise, the current evidence does not support the use of PBM for muscle recovery after physical exercise.

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## References

1. IDF - Diabetes Atlas 2021. [En ligne] Available at: [https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF\\_Atlas\\_10th\\_Edition\\_2021](https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021).
2. Marcovecchio, M. L., Lucantoni, M. & Chiarelli, F. Role of chronic and acute hyperglycemia in the development of diabetes complications. *Diabetes Technol. Ther.* **13**, 389–394 (2011).
3. Yamazaki, D., Hitomi, H. & Nishiyama, A. Hypertension with diabetes mellitus complications. *Hypertens. Res. Off. J. Jpn. Soc. Hypertens.* **41**, 147–156 (2018).
4. Henning, R. J. Type-2 diabetes mellitus and cardiovascular disease. *Future Cardiol.* **14**, 491–509 (2018).
5. Palau, V., Riera, M. & Soler, M. J. The reno-cardiovascular connection in the patient with Diabetes mellitus: What's new? *Endocrinol. Diabetes Nutr.* **64**, 237–240 (2017).
6. Sabanayagam, C. *et al.* Incidence and progression of diabetic retinopathy: a systematic review. *Lancet Diabetes Endocrinol.* **7**, 140–149 (2019).
7. Zakin, E., Abrams, R. & Simpson, D. M. Diabetic Neuropathy. *Semin. Neurol.* **39**, 560–569 (2019).
8. Zhang, P. *et al.* Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. *Ann. Med.* **49**, 106–116 (2017).
9. Barnes, J. A., Eid, M. A., Creager, M. A. & Goodney, P. P. Epidemiology and Risk of Amputation in Patients With Diabetes Mellitus and Peripheral Artery Disease. *Arterioscler. Thromb. Vasc. Biol.* **40**, 1808–1817 (2020).
10. Preshaw, P. M. *et al.* Periodontitis and diabetes: a two-way relationship. *Diabetologia* **55**, 21–31 (2012).
11. Navarro-Flores, E. & Cauli, O. Quality of Life in Individuals with Diabetic Foot Syndrome. *Endocr. Metab. Immune Disord. Drug Targets* **20**, 1365–1372 (2020).
12. Simpson, T. C. *et al.* Treatment of periodontitis for glycaemic control in people with diabetes mellitus. *Cochrane Database Syst. Rev.* **4**, CD004714 (2022).
13. Joseph, J. J. *et al.* Comprehensive Management of Cardiovascular Risk Factors for Adults With Type 2 Diabetes: A Scientific Statement From the American Heart Association. *Circulation* **145**, e722–e759 (2022).
14. Teo, E., Hassan, N., Tam, W. & Koh, S. Effectiveness of continuous glucose monitoring in maintaining glycaemic control among people with type 1 diabetes mellitus: a systematic review of randomised controlled trials and meta-analysis. *Diabetologia* **65**, 604–619 (2022).
15. Cortey, A. *et al.* [Management of jaundice in the newborn  $\geq 35$  GW: From screening to follow-up after discharge. Guidelines for clinical practice]. *Arch. Pediatr. Organe Off. Soc. Francaise Pediatr.* **24**, 192–203 (2017).
16. Geoffroy, P. A., Schroder, C. M., Reynaud, E. & Bourgin, P. Efficacy of light therapy versus antidepressant drugs, and of the combination versus monotherapy, in major depressive episodes: A systematic review and meta-analysis. *Sleep Med. Rev.* **48**, 101213 (2019).
17. Mester, E., Szende, B., Spiry, T. & Scher, A. Stimulation of wound healing by laser rays. *Acta Chir. Acad. Sci. Hung.* **13**, 315–324 (1972).
18. Mester, E., Szende, B. & Gärtner, P. [The effect of laser beams on the growth of hair in mice]. *Radiobiol. Radiother. (Berl.)* **9**, 621–626 (1968).
19. Cotler, H. B. A NASA discovery has current applications in orthopaedics. *Curr. Orthop. Pract.* **26**, 72 (2015).
20. Pastore, D., Greco, M. & Passarella, S. Specific helium-neon laser sensitivity of the purified cytochrome c oxidase. *Int. J. Radiat. Biol.* **76**, 863–870 (2000).
21. Hamblin, M. R. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys.* **4**, 337–361 (2017).
22. Dompe, C. *et al.* Photobiomodulation-Underlying Mechanism and Clinical Applications. *J. Clin. Med.* **9**, 1724 (2020).
23. Zein, R., Selting, W. & Hamblin, M. R. Review of light parameters and photobiomodulation efficacy: dive into complexity. *J. Biomed. Opt.* **23**, 1–17 (2018).
24. Glass, G. E. Photobiomodulation: A review of the molecular evidence for low level light therapy. *J. Plast. Reconstr. Aesthetic Surg. JPRAS* **74**, 1050–1060 (2021).
25. Desmet, K. D. *et al.* Clinical and experimental applications of NIR-LED photobiomodulation. *Photomed. Laser Surg.* **24**, 121–128 (2006).
26. Home - ClinicalTrials.gov. <https://clinicaltrials.gov/>.
27. Vieira, W. F. *et al.* Anti-hyperalgesic effects of photobiomodulation therapy (904 nm) on streptozotocin-induced diabetic neuropathy imply MAPK pathway and calcium dynamics modulation. *Sci. Rep.* **12**, 16730 (2022).

28. Rocha, I. R. C., Perez-Reyes, E. & Chacur, M. Effect of photobiomodulation on mitochondrial dynamics in peripheral nervous system in streptozotocin-induced type 1 diabetes in rats. *Photochem. Photobiol. Sci. Off. J. Eur. Photochem. Assoc. Eur. Soc. Photobiol.* **20**, 293–301 (2021).
29. Vieira, W. F., de Magalhães, S. F., Farias, F. H., de Thomaz, A. A. & Parada, C. A. Raman spectroscopy of dorsal root ganglia from streptozotocin-induced diabetic neuropathic rats submitted to photobiomodulation therapy. *J. Biophotonics* **12**, e201900135 (2019).
30. Abdel-Wahhab, K. G. *et al.* Efficiencies of Low-Level Laser Therapy (LLLT) and Gabapentin in the Management of Peripheral Neuropathy: Diabetic Neuropathy. *Appl. Biochem. Biotechnol.* **186**, 161–173 (2018).
31. da Silva Oliveira, V. R. *et al.* Photobiomodulation induces antinociception, recovers structural aspects and regulates mitochondrial homeostasis in peripheral nerve of diabetic mice. *J. Biophotonics* **11**, e201800110 (2018).
32. Rastogi, A., Uppula, P., Saikia, U. & Bhansali, A. Effect of Monochromatic Infrared Energy on Quality of Life and Intraepidermal Nerve Fiber Density in Painful Diabetic Neuropathy: A Randomized, Sham Control Study. *Neurol. India* **69**, 1331–1337 (2021).
33. M, A., Ummer V, S., Maiya, A. G., Hande, M. & V S, B. Effect of photobiomodulation on serum neuron specific enolase (NSE) among patients with diabetic peripheral neuropathy - A pilot study. *Diabetes Metab. Syndr.* **14**, 1061–1063 (2020).
34. da Silva Leal, M. V. *et al.* Effect of Modified Laser Transcutaneous Irradiation on Pain and Quality of Life in Patients with Diabetic Neuropathy. *Photobiomodulation Photomed. Laser Surg.* **38**, 138–144 (2020).
35. Anju, M., Chacko, L., Chettupalli, Y., Maiya, A. G. & Saleena Ummer, V. Effect of Low Level Laser Therapy on serum vitamin D and magnesium levels in patients with diabetic peripheral neuropathy - A pilot study. *Diabetes Metab. Syndr.* **13**, 1087–1091 (2019).
36. Cg, S. K. *et al.* Efficacy of low level laser therapy on painful diabetic peripheral neuropathy. *Laser Ther.* **24**, 195–200 (2015).
37. Bashiri, H. Evaluation of low level laser therapy in reducing diabetic polyneuropathy related pain and sensorimotor disorders. *Acta Med. Iran.* **51**, 543–547 (2013).
38. Yamany, A. A. & Sayed, H. M. Effect of low level laser therapy on neurovascular function of diabetic peripheral neuropathy. *J. Adv. Res.* **3**, 21–28 (2012).
39. Khamseh, M. E. *et al.* Diabetic distal symmetric polyneuropathy: effect of low-intensity laser therapy. *Lasers Med. Sci.* **26**, 831–835 (2011).
40. Swislocki, A. *et al.* A randomized clinical trial of the effectiveness of photon stimulation on pain, sensation, and quality of life in patients with diabetic peripheral neuropathy. *J. Pain Symptom Manage.* **39**, 88–99 (2010).
41. Lavery, L. A., Murdoch, D. P., Williams, J. & Lavery, D. C. Does anodyne light therapy improve peripheral neuropathy in diabetes? A double-blind, sham-controlled, randomized trial to evaluate monochromatic infrared photoenergy. *Diabetes Care* **31**, 316–321 (2008).
42. Arnall, D. A. *et al.* The restorative effects of pulsed infrared light therapy on significant loss of peripheral protective sensation in patients with long-term type 1 and type 2 diabetes mellitus. *Acta Diabetol.* **43**, 26–33 (2006).
43. Harkless, L. B., DeLellis, S., Carnegie, D. H. & Burke, T. J. Improved foot sensitivity and pain reduction in patients with peripheral neuropathy after treatment with monochromatic infrared photo energy--MIRE. *J. Diabetes Complications* **20**, 81–87 (2006).
44. Clifft, J. K., Kasser, R. J., Newton, T. S. & Bush, A. J. The effect of monochromatic infrared energy on sensation in patients with diabetic peripheral neuropathy: a double-blind, placebo-controlled study. *Diabetes Care* **28**, 2896–2900 (2005).
45. DeLellis, S. L., Carnegie, D. H. & Burke, T. J. Improved sensitivity in patients with peripheral neuropathy: effects of monochromatic infrared photo energy. *J. Am. Podiatr. Med. Assoc.* **95**, 143–147 (2005).
46. Leonard, D. R., Farooqi, M. H. & Myers, S. Restoration of sensation, reduced pain, and improved balance in subjects with diabetic peripheral neuropathy: a double-blind, randomized, placebo-controlled study with monochromatic near-infrared treatment. *Diabetes Care* **27**, 168–172 (2004).
47. Zinman, L. H. *et al.* Low-intensity laser therapy for painful symptoms of diabetic sensorimotor polyneuropathy: a controlled trial. *Diabetes Care* **27**, 921–924 (2004).
48. Kochman, A. B., Carnegie, D. H. & Burke, T. J. Symptomatic reversal of peripheral neuropathy in patients with diabetes. *J. Am. Podiatr. Med. Assoc.* **92**, 125–130 (2002).
49. Schindl, A., Heinze, G., Schindl, M., Pernerstorfer-Schön, H. & Schindl, L. Systemic effects of low-intensity laser irradiation on skin microcirculation in patients with diabetic microangiopathy. *Microvasc. Res.* **64**, 240–246 (2002).

50. Ahmed, S. A., Ghoneim, D. F., Morsy, M. E., Hassan, A. A. & Mahmoud, A. R. H. Low-Level Laser Therapy with 670 nm Alleviates Diabetic Retinopathy in an Experimental Model. *J. Curr. Ophthalmol.* **33**, 143–151 (2021).
51. Cheng, Y. *et al.* Photobiomodulation Inhibits Long-term Structural and Functional Lesions of Diabetic Retinopathy. *Diabetes* **67**, 291–298 (2018).
52. Saliba, A. *et al.* Photobiomodulation Mitigates Diabetes-Induced Retinopathy by Direct and Indirect Mechanisms: Evidence from Intervention Studies in Pigmented Mice. *PLoS One* **10**, e0139003 (2015).
53. Tang, J. *et al.* Low-intensity far-red light inhibits early lesions that contribute to diabetic retinopathy: in vivo and in vitro. *Invest. Ophthalmol. Vis. Sci.* **54**, 3681–3690 (2013).
54. Shen, W. *et al.* Preclinical and clinical studies of photobiomodulation therapy for macular oedema. *Diabetologia* **63**, 1900–1915 (2020).
55. Eells, J. T. *et al.* 670 nm Photobiomodulation as a Therapy for Diabetic Macular Edema: A Pilot Study. *Invest. Ophthalmol. Vis. Sci.* **58**, 932 (2017).
56. Min, S. H. *et al.* Duodenal Dual-Wavelength Photobiomodulation Improves Hyperglycemia and Hepatic Parameters with Alteration of Gut Microbiome in Type 2 Diabetes Animal Model. *Cells* **11**, 3490 (2022).
57. Bonifacio, M. *et al.* Effects of photobiomodulation on glucose homeostasis and morphometric parameters in pancreatic islets of diabetic mice. *Lasers Med. Sci.* **37**, 1799–1809 (2022).
58. Gong, L., Zou, Z., Liu, L., Guo, S. & Xing, D. Photobiomodulation therapy ameliorates hyperglycemia and insulin resistance by activating cytochrome c oxidase-mediated protein kinase B in muscle. *Aging* **13**, 10015–10033 (2021).
59. Gong, L., Zou, Z., Huang, L., Guo, S. & Xing, D. Photobiomodulation therapy decreases free fatty acid generation and release in adipocytes to ameliorate insulin resistance in type 2 diabetes. *Cell. Signal.* **67**, 109491 (2020).
60. Guo, S., Gong, L., Shen, Q. & Xing, D. Photobiomodulation reduces hepatic lipogenesis and enhances insulin sensitivity through activation of CaMKK $\beta$ /AMPK signaling pathway. *J. Photochem. Photobiol. B* **213**, 112075 (2020).
61. Hsu, Y.-H. *et al.* Far-infrared radiation prevents decline in  $\beta$ -cell mass and function in diabetic mice via the mitochondria-mediated Sirtuin1 pathway. *Metabolism*. **104**, 154143 (2020).
62. Silva, G. *et al.* Insulin resistance is improved in high-fat fed mice by photobiomodulation therapy at 630 nm. *J. Biophotonics* **13**, e201960140 (2020).
63. Silva, G. *et al.* Infrared photobiomodulation (PBM) therapy improves glucose metabolism and intracellular insulin pathway in adipose tissue of high-fat fed mice. *Lasers Med. Sci.* **33**, 559–571 (2018).
64. Yoshimura, T. M., Sabino, C. P. & Ribeiro, M. S. Photobiomodulation reduces abdominal adipose tissue inflammatory infiltrate of diet-induced obese and hyperglycemic mice. *J. Biophotonics* **9**, 1255–1262 (2016).
65. Scontri, C. M. C. B. *et al.* Dose and time-response effect of photobiomodulation therapy on glycemic control in type 2 diabetic patients combined or not with hypoglycemic medicine: a randomized, crossover, double-blind, sham controlled trial. *J. Biophotonics* e202300083 (2023) doi:10.1002/jbio.202300083.
66. da Silva Tonetto, L. *et al.* Effects of photobiomodulation on oxidative stress in rats with type 2 diabetes mellitus. *Lasers Med. Sci.* **38**, 90 (2023).
67. de Oliveira, A. R. *et al.* Effect of photobiomodulation and exercise on early remodeling of the Achilles tendon in streptozotocin-induced diabetic rats. *PLoS One* **14**, e0211643 (2019).
68. Frigero, M. *et al.* Effect of photobiomodulation therapy on oxidative stress markers of gastrocnemius muscle of diabetic rats subjected to high-intensity exercise. *Lasers Med. Sci.* **33**, 1781–1790 (2018).
69. Linares, S. N. *et al.* Dose Response Effect of Photobiomodulation on Hemodynamic Responses and Glucose Levels in Men with Type 2 Diabetes: A Randomized, Crossover, Double-Blind, Sham-Controlled Trial. *Photonics* **9**, 481 (2022).
70. Gobbi, A., de Carvalho, G., Sapalo, A. T. & de Jesus Guirro, R. R. Acute application of photobiomodulation does not bring important gains for the muscular performance and functionality of diabetic individuals. *Lasers Med. Sci.* **36**, 995–1002 (2021).
71. Milan-Mattos, J. C. *et al.* Acute effect of photobiomodulation using light-emitting diodes (LEDs) on baroreflex sensitivity during and after constant loading exercise in patients with type 2 diabetes mellitus. *Lasers Med. Sci.* **35**, 329–336 (2020).
72. Francisco, C. de O. *et al.* Effects of light-emitting diode therapy (LEDT) on cardiopulmonary and hemodynamic adjustments during aerobic exercise and glucose levels in patients with diabetes mellitus: A randomized, crossover, double-blind and placebo-controlled clinical trial. *Complement. Ther. Med.* **42**, 178–183 (2019).
73. Dungal, P. *et al.* Wavelength-Dependent Effects of Photobiomodulation for Wound Care in Diabetic Wounds. *Int. J. Mol. Sci.* **24**, 5895 (2023).

74. Ebrahimpour-Malekshah, R. *et al.* The stereological, immunohistological, and gene expression studies in an infected ischemic wound in diabetic rats treated by human adipose-derived stem cells and photobiomodulation. *Arch. Dermatol. Res.* (2023) doi:10.1007/s00403-023-02563-z.
75. Mehrvar, S. *et al.* 670 nm photobiomodulation improves the mitochondrial redox state of diabetic wounds. *Quant. Imaging Med. Surg.* **11**, 107–118 (2021).
76. Ahmadi, H. *et al.* Transplantation of photobiomodulation-preconditioned diabetic stem cells accelerates ischemic wound healing in diabetic rats. *Stem Cell Res. Ther.* **11**, 494 (2020).
77. Bagheri, M. *et al.* Combined effects of metformin and photobiomodulation improve the proliferation phase of wound healing in type 2 diabetic rats. *Biomed. Pharmacother. Biomedecine Pharmacother.* **123**, 109776 (2020).
78. Kouhkeheil, R. *et al.* Impact of Photobiomodulation and Condition Medium on Mast Cell Counts, Degranulation, and Wound Strength in Infected Skin Wound Healing of Diabetic Rats. *Photobiomodulation Photomed. Laser Surg.* **37**, 706–714 (2019).
79. Fekrazad, R. *et al.* Improved Wound Remodeling Correlates with Modulated TGF-beta Expression in Skin Diabetic Wounds Following Combined Red and Infrared Photobiomodulation Treatments. *Photochem. Photobiol.* **94**, 775–779 (2018).
80. Asghari, M. *et al.* The effect of combined photobiomodulation and metformin on open skin wound healing in a non-genetic model of type II diabetes. *J. Photochem. Photobiol. B* **169**, 63–69 (2017).
81. Leite, G. de P. M. F. *et al.* Photobiomodulation laser and pulsed electrical field increase the viability of the musculocutaneous flap in diabetic rats. *Lasers Med. Sci.* **32**, 641–648 (2017).
82. Fahimipour, F. *et al.* The effect of He-Ne and Ga-Al-As lasers on the healing of oral mucosa in diabetic mice. *J. Photochem. Photobiol. B* **159**, 149–154 (2016).
83. Fekrazad, R., Mirmoezzi, A., Kalhori, K. A. & Arany, P. The effect of red, green and blue lasers on healing of oral wounds in diabetic rats. *J. Photochem. Photobiol. B* **148**, 242–245 (2015).
84. Dancáková, L. *et al.* Low-level laser therapy with 810 nm wavelength improves skin wound healing in rats with streptozotocin-induced diabetes. *Photomed. Laser Surg.* **32**, 198–204 (2014).
85. Aparecida Da Silva, A. *et al.* Wound-healing effects of low-level laser therapy in diabetic rats involve the modulation of MMP-2 and MMP-9 and the redistribution of collagen types I and III. *J. Cosmet. Laser Ther. Off. Publ. Eur. Soc. Laser Dermatol.* **15**, 210–216 (2013).
86. Fathabadie, F. F., Bayat, M., Amini, A., Bayat, M. & Rezaie, F. Effects of pulsed infra-red low level-laser irradiation on mast cells number and degranulation in open skin wound healing of healthy and streptozotocin-induced diabetic rats. *J. Cosmet. Laser Ther. Off. Publ. Eur. Soc. Laser Dermatol.* **15**, 294–304 (2013).
87. Firat, E. T. *et al.* The effects of low-level laser therapy on palatal mucoperiosteal wound healing and oxidative stress status in experimental diabetic rats. *Photomed. Laser Surg.* **31**, 315–321 (2013).
88. Dadpay, M., Sharifian, Z., Bayat, M., Bayat, M. & Dabbagh, A. Effects of pulsed infra-red low level-laser irradiation on open skin wound healing of healthy and streptozotocin-induced diabetic rats by biomechanical evaluation. *J. Photochem. Photobiol. B* **111**, 1–8 (2012).
89. Park, J. J. & Kang, K. L. Effect of 980-nm GaAlAs diode laser irradiation on healing of extraction sockets in streptozotocin-induced diabetic rats: a pilot study. *Lasers Med. Sci.* **27**, 223–230 (2012).
90. Hegde, V. N. *et al.* Effect of laser dose and treatment schedule on excision wound healing in diabetic mice. *Photochem. Photobiol.* **87**, 1433–1441 (2011).
91. Peplow, P. V., Chung, T.-Y., Ryan, B. & Baxter, G. D. Laser photobiostimulation of wound healing: reciprocity of irradiance and exposure time on energy density for splinted wounds in diabetic mice. *Lasers Surg. Med.* **43**, 843–850 (2011).
92. Carvalho, P. de T. C. de, Silva, I. S. da, Reis, F. A. dos, Perreira, D. M. & Aydos, R. D. Influence of ingaalp laser (660nm) on the healing of skin wounds in diabetic rats. *Acta Cir. Bras.* **25**, 71–79 (2010).
93. Akyol, U. & Güngörmüş, M. The effect of low-level laser therapy on healing of skin incisions made using a diode laser in diabetic rats. *Photomed. Laser Surg.* **28**, 51–55 (2010).
94. Chung, T.-Y., Peplow, P. V. & Baxter, G. D. Laser photobiostimulation of wound healing: defining a dose response for splinted wounds in diabetic mice. *Lasers Surg. Med.* **42**, 656–664 (2010).
95. Santos, N. R. S. *et al.* Influence of the use of laser phototherapy (lambda660 or 790 nm) on the survival of cutaneous flaps on diabetic rats. *Photomed. Laser Surg.* **28**, 483–488 (2010).
96. Al-Watban, F. A. H. Laser therapy converts diabetic wound healing to normal healing. *Photomed. Laser Surg.* **27**, 127–135 (2009).
97. Güngörmüş, M. & Akyol, U. K. Effect of biostimulation on wound healing in diabetic rats. *Photomed. Laser Surg.* **27**, 607–610 (2009).
98. Maiya, A. G., Kumar, P. & Nayak, S. Photo-stimulatory effect of low energy helium-neon laser irradiation on excisional diabetic wound healing dynamics in Wistar rats. *Indian J. Dermatol.* **54**, 323–329 (2009).



99. Carvalho, P. de T. C. de, Mazzer, N., dos Reis, F. A., Belchior, A. C. G. & Silva, I. S. Analysis of the influence of low-power HeNe laser on the healing of skin wounds in diabetic and non-diabetic rats. *Acta Cir. Bras.* **21**, 177–183 (2006).
100. Rabelo, S. B. *et al.* Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. *Photomed. Laser Surg.* **24**, 474–479 (2006).
101. Maiya, G. A., Kumar, P. & Rao, L. Effect of low intensity helium-neon (He-Ne) laser irradiation on diabetic wound healing dynamics. *Photomed. Laser Surg.* **23**, 187–190 (2005).
102. Byrnes, K. R. *et al.* Photobiomodulation improves cutaneous wound healing in an animal model of type II diabetes. *Photomed. Laser Surg.* **22**, 281–290 (2004).
103. Reddy, G. K., Stehno-Bittel, L. & Enwemeka, C. S. Laser photostimulation accelerates wound healing in diabetic rats. *Wound Repair Regen. Off. Publ. Wound Heal. Soc. Eur. Tissue Repair Soc.* **9**, 248–255 (2001).
104. Haze, A. *et al.* Treatment of diabetic foot ulcers in a frail population with severe co-morbidities using at-home photobiomodulation laser therapy: a double-blind, randomized, sham-controlled pilot clinical study. *Lasers Med. Sci.* **37**, 919–928 (2022).
105. Vitoriano, N. A. M. *et al.* Comparative study on laser and LED influence on tissue repair and improvement of neuropathic symptoms during the treatment of diabetic ulcers. *Lasers Med. Sci.* **34**, 1365–1371 (2019).
106. de Alencar Fonseca Santos, J. *et al.* Effects of Low-Power Light Therapy on the Tissue Repair Process of Chronic Wounds in Diabetic Feet. *Photomed. Laser Surg.* **36**, 298–304 (2018).
107. Frangež, I., Nizič-Kos, T. & Frangež, H. B. Phototherapy with LED Shows Promising Results in Healing Chronic Wounds in Diabetes Mellitus Patients: A Prospective Randomized Double-Blind Study. *Photomed. Laser Surg.* **36**, 377–382 (2018).
108. Ruh, A. C. *et al.* Laser photobiomodulation in pressure ulcer healing of human diabetic patients: gene expression analysis of inflammatory biochemical markers. *Lasers Med. Sci.* **33**, 165–171 (2018).
109. Mathur, R. K. *et al.* Low-level laser therapy as an adjunct to conventional therapy in the treatment of diabetic foot ulcers. *Lasers Med. Sci.* **32**, 275–282 (2017).
110. Carvalho, A. F. M. de *et al.* Low-level laser therapy and *Calendula officinalis* in repairing diabetic foot ulcers. *Rev. Esc. Enferm. U P* **50**, 628–634 (2016).
111. Sandoval Ortíz, M. C., Herrera Villabona, E., Camargo Lemos, D. M. & Castellanos, R. Effects of low level laser therapy and high voltage stimulation on diabetic wound healing. *Rev Univ Ind Santander Salud* 107–117 (2014).
112. Kajagar, B. M., Godhi, A. S., Pandit, A. & Khatri, S. Efficacy of low level laser therapy on wound healing in patients with chronic diabetic foot ulcers—a randomised control trial. *Indian J. Surg.* **74**, 359–363 (2012).
113. Kaviani, A. *et al.* A randomized clinical trial on the effect of low-level laser therapy on chronic diabetic foot wound healing: a preliminary report. *Photomed. Laser Surg.* **29**, 109–114 (2011).
114. Minatel, D. G., Frade, M. A. C., França, S. C. & Enwemeka, C. S. Phototherapy promotes healing of chronic diabetic leg ulcers that failed to respond to other therapies. *Lasers Surg. Med.* **41**, 433–441 (2009).
115. Schindl, M. *et al.* Induction of complete wound healing in recalcitrant ulcers by low-intensity laser irradiation depends on ulcer cause and size. *Photodermatol. Photoimmunol. Photomed.* **15**, 18–21 (1999).
116. Schindl, A. *et al.* Low-intensity laser irradiation improves skin circulation in patients with diabetic microangiopathy. *Diabetes Care* **21**, 580–584 (1998).
117. Dalirsani, Z. *et al.* Effects of diode low-level laser therapy on healing of tooth extraction sockets: a histopathological study in diabetic rats. *Lasers Med. Sci.* **36**, 1527–1534 (2021).
118. Lee, J.-H. *et al.* The Effects of Photobiomodulation on Bone Defect Repairing in a Diabetic Rat Model. *Int. J. Mol. Sci.* **22**, 11026 (2021).
119. Diker, N., Aytac, D., Helvacioğlu, F., Dagdelen, C. & Oguz, Y. Evaluation of the Effects of Low-Level Laser Therapy on Diabetic Bone Healing. *J. Craniofac. Surg.* **30**, 1994–1998 (2019).
120. Gomes, M. F. *et al.* Effects of the photobiomodulation using different energy densities on the periodontal tissues under orthodontic force in rats with type 2 diabetes mellitus. *Braz. Oral Res.* **32**, e61 (2018).
121. Mostafavinia, A. *et al.* Evaluation of the Effects of Photobiomodulation on Partial Osteotomy in Streptozotocin-Induced Diabetes in Rats. *Photomed. Laser Surg.* **36**, 406–414 (2018).
122. Mostafavinia, A. *et al.* Evaluation of the Effects of Photobiomodulation on Bone Healing in Healthy and Streptozotocin-Induced Diabetes in Rats. *Photomed. Laser Surg.* **35**, 537–545 (2017).
123. Yildirimturk, S., Sirin, Y., Soluk Tekkesin, M., Gurler, G. & Firat, D. The effects of low-level laser therapy on the healing of bone defects in streptozotocin-induced diabetic rats: A histological and morphometric evaluation. *J. Cosmet. Laser Ther. Off. Publ. Eur. Soc. Laser Dermatol.* **19**, 397–403 (2017).
124. Patrocínio-Silva, T. L. *et al.* Low-level laser therapy associated to a resistance training protocol on bone tissue in diabetic rats. *Arch. Endocrinol. Metab.* **60**, 457–464 (2016).
125. Magri, A. M. P. *et al.* Photobiomodulation and bone healing in diabetic rats: evaluation of bone response using a tibial defect experimental model. *Lasers Med. Sci.* **30**, 1949–1957 (2015).

126. Nascimento, M. F. do *et al.* Improvement of bone repair in diabetic rats subjected to  $\lambda$ 780 nm low-level laser therapy. *Acta Cir. Bras.* **30**, 660–667 (2015).
127. Patrocínio-Silva, T. L. *et al.* The effects of low-level laser irradiation on bone tissue in diabetic rats. *Lasers Med. Sci.* **29**, 1357–1364 (2014).
128. Akyol, U. K. & Güngörmüş, M. Effect of biostimulation on healing of bone defects in diabetic rats. *Photomed. Laser Surg.* **28**, 411–416 (2010).
129. Abdi, S. *et al.* The effects of helium-neon light therapy on healing of partial osteotomy of the tibia in streptozotocin induced diabetic rats. *Photomed. Laser Surg.* **27**, 907–912 (2009).
130. Bayat, M., Abdi, S., Javadieh, F., Mohsenifar, Z. & Rashid, M. R. The effects of low-level laser therapy on bone in diabetic and nondiabetic rats. *Photomed. Laser Surg.* **27**, 703–708 (2009).
131. Javadieh, F., Bayat, M., Abdi, S., Mohsenifar, Z. & Razi, S. The effects of infrared low-level laser therapy on healing of partial osteotomy of tibia in streptozotocin-induced diabetic rats. *Photomed. Laser Surg.* **27**, 641–646 (2009).
132. Attia, M. S. *et al.* The Influence of Low-Level Laser Therapy on CBCT Radiographic and Biochemical Profiles of Type II Controlled Diabetic Patients After Dental Implant Insertion: A Randomized Case-Control Study. *Cureus* **15**, e36559 (2023).
133. Kamatham, S. A. & Chava, V. K. Comparison of salivary calprotectin levels in periodontitis associated with diabetes mellitus after low-level laser therapy as an adjunct to scaling and root planing: A randomized clinical trial. *J. Indian Soc. Periodontol.* **26**, 143–150 (2022).
134. Pulivarthi, P., Chava, V. K. & Gunupati, S. Salivary tumor necrosis factor-alpha levels in periodontitis associated with diabetes mellitus after low level laser therapy as an adjunct to scaling and root planning: A randomized clinical trial. *J. Indian Soc. Periodontol.* **26**, 236–244 (2022).
135. Mrasori, S. *et al.* Effects of Low Level Laser Therapy (LLLT) on Serum Values of Interleukin 6 (IL-6) in Patients with Periodontitis and Type 2 Diabetes Mellitus (T2DM). *Acta Inform. Medica AIM J. Soc. Med. Inform. Bosnia Herzeg. Cas. Drustva Za Med. Inform. BiH* **29**, 59–64 (2021).
136. Soi, S., Bains, V. K., Srivastava, R. & Madan, R. Comparative evaluation of improvement in periodontal and glyceemic health status of type 2 diabetes mellitus patients after scaling and root planing with or without adjunctive use of diode laser. *Lasers Med. Sci.* **36**, 1307–1315 (2021).
137. Kocak, E. *et al.* Effect of diode laser application as an adjunct to nonsurgical periodontal therapy on the reduction of red complex microorganisms in type 2 diabetics with chronic periodontitis. *Lasers Med. Sci.* **35**, 1403–1410 (2020).
138. Özberk, S. S., Gündoğar, H., Özkaya, M., Taner, İ. L. & Erciyas, K. The effect of photobiomodulation therapy on nonsurgical periodontal treatment in patients with type 2 diabetes mellitus: a randomized controlled, single-blind, split-mouth clinical trial. *Lasers Med. Sci.* **35**, 497–504 (2020).
139. Castro Dos Santos, N. *et al.* Photobiomodulation for the treatment of periodontal pockets in patients with type 2 diabetes: 1-year results of a randomized clinical trial. *Lasers Med. Sci.* **34**, 1897–1904 (2019).
140. Chandra, S. & Shashikumar, P. Diode Laser - A Novel Therapeutic Approach in the Treatment of Chronic Periodontitis in Type 2 Diabetes Mellitus Patients: A Prospective Randomized Controlled Clinical Trial. *J. Lasers Med. Sci.* **10**, 56–63 (2019).
141. Dengizek Eltas, S., Gursel, M., Eltas, A., Alptekin, N. O. & Ataoglu, T. Evaluation of long-term effects of diode laser application in periodontal treatment of poorly controlled type 2 diabetic patients with chronic periodontitis. *Int. J. Dent. Hyg.* **17**, 292–299 (2019).
142. Li, F. & Xu, H.-S. [Effects of low level laser combined with basic periodontal therapy on cytokines and LPS, leptin in gingival crevicular fluid of diabetes mellitus complicated with chronic periodontitis patients]. *Shanghai Kou Qiang Yi Xue Shanghai J. Stomatol.* **27**, 637–640 (2018).
143. Demirturk-Gocgun, O. *et al.* Role of Low-Level Laser Therapy as an Adjunct to Initial Periodontal Treatment in Type 2 Diabetic Patients: A Split-Mouth, Randomized, Controlled Clinical Trial. *Photomed. Laser Surg.* **35**, 111–115 (2017).
144. Koçak, E. *et al.* Nonsurgical periodontal therapy with/without diode laser modulates metabolic control of type 2 diabetics with periodontitis: a randomized clinical trial. *Lasers Med. Sci.* **31**, 343–353 (2016).
145. Javed, F. *et al.* Efficacy of non-surgical periodontal therapy with adjunct Nd:YAG laser therapy in the treatment of periodontal inflammation among patients with and without type 2 diabetes mellitus: A short-term pilot study. *J. Photochem. Photobiol. B* **149**, 230–234 (2015).
146. Obradović, R. *et al.* A histological evaluation of a low-level laser therapy as an adjunct to periodontal therapy in patients with diabetes mellitus. *Lasers Med. Sci.* **28**, 19–24 (2013).
147. Obradović, R. *et al.* Low-level lasers as an adjunct in periodontal therapy in patients with diabetes mellitus. *Diabetes Technol. Ther.* **14**, 799–803 (2012).
148. Obradović, R. *et al.* [Low power laser efficacy in the therapy of inflamed gingive in diabetics with parodontopathy]. *Vojnosanit. Pregl.* **68**, 684–689 (2011).

149. Loe, H. & Silness, J. PERIODONTAL DISEASE IN PREGNANCY. I. PREVALENCE AND SEVERITY. *Acta Odontol. Scand.* **21**, 533–551 (1963).
150. Silness, J. & Loe, H. PERIODONTAL DISEASE IN PREGNANCY. II. CORRELATION BETWEEN ORAL HYGIENE AND PERIODONTAL CONDITON. *Acta Odontol. Scand.* **22**, 121–135 (1964).
151. Yang, L. *et al.* Effect of near-infrared laser treatment on improving erectile function in rats with diabetes mellitus. *Andrology* (2023) doi:10.1111/andr.13422.
152. Asghari, A., Takhtfooladi, M. A. & Hoseinzadeh, H. A. Effect of photobiomodulation on ischemia/reperfusion-induced renal damage in diabetic rats. *Lasers Med. Sci.* **31**, 1943–1948 (2016).
153. Aghamohamdi, D., Fakhari, S., Farhoudi, M. & Farzin, H. The Efficacy of Low-Level Laser Therapy in the Treatment of Bell's Palsy in Diabetic Patients. *J. Lasers Med. Sci.* **11**, 310–315 (2020).
154. Hode, L. The importance of the coherency. *Photomed. Laser Surg.* **23**, 431–434 (2005).
155. Zalevsky, Z. & Belkin, M. Coherence and speckle in photomedicine and photobiology. *Photomed. Laser Surg.* **29**, 655–656 (2011).
156. Heiskanen, V. & Hamblin, M. R. Photobiomodulation: lasers vs. light emitting diodes? *Photochem. Photobiol. Sci. Off. J. Eur. Photochem. Assoc. Eur. Soc. Photobiol.* **17**, 1003–1017 (2018).
157. de Freitas, L. F. & Hamblin, M. R. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J. Sel. Top. Quantum Electron. Publ. IEEE Lasers Electro-Opt. Soc.* **22**, 7000417 (2016).
158. Deana, N. F., Zaror, C., Del Sol, M., Bagnato, V. S. & Alves, N. Wound contraction rate in excised and unexcised burn wounds with laser photobiomodulation: Systematic review and meta-analysis of preclinical studies. *Burns J. Int. Soc. Burn Inj.* **49**, 261–274 (2023).
159. Lopes, C. de C. A. *et al.* Effectiveness of Photobiomodulation Therapy on Human Bone Healing in Dentistry: A Systematic Review. *Photobiomodulation Photomed. Laser Surg.* **40**, 440–453 (2022).
160. Ebrahimi, P. *et al.* Effect of photobiomodulation in secondary intention gingival wound healing—a systematic review and meta-analysis. *BMC Oral Health* **21**, 258 (2021).
161. Ribu, L., Birkeland, K., Hanestad, B. R., Moum, T. & Rustoen, T. A longitudinal study of patients with diabetes and foot ulcers and their health-related quality of life: wound healing and quality-of-life changes. *J. Diabetes Complications* **22**, 400–407 (2008).
162. Ayuk, S. M., Houreld, N. N. & Abrahamse, H. Effect of 660 nm visible red light on cell proliferation and viability in diabetic models in vitro under stressed conditions. *Lasers Med. Sci.* **33**, 1085–1093 (2018).
163. De Marchi, T., Ferlito, J. V., Ferlito, M. V., Salvador, M. & Leal-Junior, E. C. P. Can Photobiomodulation Therapy (PBMT) Minimize Exercise-Induced Oxidative Stress? A Systematic Review and Meta-Analysis. *Antioxid. Basel Switz.* **11**, 1671 (2022).
164. Ferraresi, C. Use of Photobiomodulation Therapy in Exercise Performance Enhancement and Postexercise Recovery: True or Myth? *Photobiomodulation Photomed. Laser Surg.* **38**, 705–707 (2020).
165. Liebman, C., Loya, S., Lawrence, M., Bashoo, N. & Cho, M. Stimulatory responses in  $\alpha$ - and  $\beta$ -cells by near-infrared (810 nm) photobiomodulation. *J. Biophotonics* **15**, e202100257 (2022).
166. Irani, S. *et al.* Effect of low-level laser irradiation on in vitro function of pancreatic islets. *Transplant. Proc.* **41**, 4313–4315 (2009).
167. Huang, H.-H., Stillman, T. J., Branham, L. A. & Williams, S. C. The Effects of Photobiomodulation Therapy on Porcine Islet Insulin Secretion. *Photobiomodulation Photomed. Laser Surg.* **40**, 395–401 (2022).

**Table 1.** Number of patients treated in clinical trials with PBM for diabetes condition

Status of studies	Number of studies	Expected number of participants
Active, not recruiting	1	80
Completed	67	4954
Not yet recruiting	2	160
Recruiting	5	300
Suspended	1	60
Terminated	4	75
Unknown status	6	208
Withdrawn	2	0
<b>Total</b>	<b>88</b>	<b>5837</b>

*These numbers of participant represent the expected enrolment for trial not already completed*

**Table 2.** Effects of PBM on diabetic neuropathy

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Vieira 2022 <sup>27</sup>	Lewis rat Streptozotocin induced	904	Continuous laser	7W/cm <sup>2</sup> , 29sec, 203J/cm <sup>2</sup> Frequency: once a day Site: 1, dorsal, direct contact	↓ pain ↓ cytokines (TNFα, IL-1β, IL-10) No effect on metabolic parameters
Rocha 2021 <sup>28</sup>	Wistar rat Streptozotocin induced	904	Continuous laser	340mW/cm <sup>2</sup> , 18sec, 6,23J/cm <sup>2</sup> Frequency: once a day, for 10 days Site: on sciatic nerve routes, direct contact	Restore fusion/fission mitochondria dynamic
Vieira 2019 <sup>29</sup>	Lewis rat Streptozotocin induced	904	Continuous laser	7W/cm <sup>2</sup> , 29sec, 203J/cm <sup>2</sup> Frequency: once a day for 8 days Sites: 4 points, direct contact	↓ pain
Abdel-Wahhab 2018 <sup>30</sup>	Albinos rat Streptozotocin induced	808	Continuous laser	30sec, 90J* Frequency: 3 times a week for 8 weeks Sites: 3 points	↓ pain ↓ PGE2, TNFα, IL1β, IL10
da Silva Oliveira 2018 <sup>31</sup>	C57BL6 mice Streptozotocin induced	660	Continuous laser	107mW/cm <sup>2</sup> , 15sec, 1.6J/cm <sup>2</sup> Frequency: once a day, for 21 days Site: 1, plantar hind paw, direct contact	↓ pain Prevent myelin degeneration ↓ loss of C fibre
Rastogi 2021 <sup>32</sup>	Human, n = 38 Diabetes RCT	890	LED	30min, 58.5J/cm <sup>2</sup> /min Frequency: 3 times a week, for 12 weeks Sites: 4, plantar foot x2, posterior & anterior distal leg	↓ pain (VAS decrease of 5.3 vs. 3 at 3 months) ↑ QoL (Norfolk-QoL DN 8 vs. 12 at 3 months) No effect on cutaneous reinnervation
Anju 2020 <sup>33</sup>	Human, n = 50 T2D Pre-Post ITV	632.8	Continuous laser	5.7mW/cm <sup>2</sup> , 9min, 3.1J/cm <sup>2</sup> Frequency: once a day, for 10 days Sites: 2, plantar and dorsal foot	↓ vibration perception threshold ↓ neurone specific enolase
da Silva Leal 2020 <sup>34</sup>	Human, n = 30 T2D RCT	660	Continuous laser	1.66mW/cm <sup>2</sup> , 30min, 3J/cm <sup>2</sup> Frequency: once a day, for 10 days, 20 days washout and start 2 times more Site: 1, radial artery region, direct contact	↓ pain VAS: no change vs. decrease of 3 points LANSS: no change vs. decrease of 3 points Pain detects questionnaire: no change vs. decrease of 5 points ↑ QoL (SF-36, all parameters increase)

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Anju 2019 <sup>35</sup>	Human, n = 40 T2D Pre-Post ITV	632.8	Continuous laser	5.7mW/cm <sup>2</sup> , 9min, 3.1J/cm <sup>2</sup> Frequency: once a day, for 10 days Sites: 2, plantar and dorsal foot	↑ Mg and Vitamin D
Kumar 2015 <sup>36</sup>	Human, n = 19 T2D Pre-Post ITV	660 + 850	Continuous laser	5.7mW/cm <sup>2</sup> , 9min, 3.1J/cm <sup>2</sup> Frequency: once a day, for 10 days Sites: 2, plantar and dorsal foot, direct contact	↓ pain (VAS: decrease of 5.2 points after 10 days) ↓ vibration perception threshold ↑ microcirculation
Bashiri 2013 <sup>37</sup>	Human, n = 60 T1D & T2D RCT	780	Continuous laser	8.3mW/cm <sup>2</sup> , 5min, 2.5J/cm <sup>2</sup> Frequency: 2 times a week, for 4 weeks Site: unknown	↓ pain (VAS: score of 7.9 vs. 5.9 for PBM after 4 weeks)
Yamany 2012 <sup>38</sup>	Human, n = 30 T2D RCT	850	Continuous laser	6.3mW/cm <sup>2</sup> , 15min, 5.7J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites: 2, plantar surface of foot, lombo-sacral area, 30cm above the area	↓ pain (VAS score of 6.9 vs. 5.3 for PBM after 4 weeks) ↑ microcirculation ↑ sural nerve conduction No effect on peroneal nerve
Khamesh 2011 <sup>39</sup>	Human, n = 27 T2D Pre-Post ITV	800	Continuous laser	1W* Frequency: 10 sessions Sites: 6 paravertebral points, 4 on sciatique nerve routes	↑ neuronal potential amplitude
		905	Pulsed laser	25W*, 10J/cm <sup>2</sup> Frequency: 10 sessions Sites: 6 paravertebral points and 4 on sciatique nerve routes	
Swisloki 2010 <sup>40</sup>	Human, n = 121 Diabetes RCT	870	Continuous laser	350W*, 7min, 1800J* Frequency: Once a day, 4 days Sites: 16 on each foot	Restoration sensation No effect on pain (VAS, PQAS) No effect on quality of life (SF-36)
Lavery 2008 <sup>41</sup>	Human, n = 60 Diabetes RCT	890	Continuous LED	40min, 1,3J/cm <sup>2</sup> /min Frequency: once a day, for 90 days Sites: 4, plantar foot x2, medial & lateral side of the calf	No effect on pain (VAS) No effect on QoL (Neuro QoL) No effect on peripheral sensation

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Arnall 2006 <sup>42</sup>	Human, n = 22 T1D & T2D RCT (for foot)	880 + 650	Pulsed LED	30min Frequency: 3 times a week, for 8 weeks Sites: 2, volar and dorsum of foot	↑ mechanical sensation ↓ perception threshold ↑ peripheral protective sensation
Harkless 2006 <sup>43</sup>	Human, n = 979 Diabetes Pre-Post ITV	890	Pulsed laser	Unknown	↓ pain (VAS: decrease of 4.8 points) ↑ foot sensation
Cliff 2005 <sup>44</sup>	Human, n = 70 Diabetes RCT	890	Continuous laser	30min, 58.5J/cm <sup>2</sup> /min Frequency: 3 times a week, for 4 weeks Sites: 4, plantar foot x2, posterior & anterior distal leg	No effect on sensation
DeLellis 2005 <sup>45</sup>	Human, n = 790 T2D Pre-Post ITV	890	Pulsed laser	No detail	↓ sensitivity impairment Restoration protective sensation
Leonard 2004 <sup>46</sup>	Human, n = 27 T1D & T2D Pre-Post ITV	890	Continuous laser	40min, 50J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites: 4, inferior member	↓ pain (VAS: decrease of 2.3 points after 4 weeks) ↓ place without sensation Restoration protective sensation
Zinman 2004 <sup>47</sup>	Human, n = 50 Diabetes RCT	905	Continuous laser	60mW*, 5min Frequency: 2 times a week, for 4 weeks Sites: 2 painfull sites, directe contact	No effect on pain (p = 0.07) (VAS)
Kochman 2002 <sup>48</sup>	Human, n = 49 T1D & T2D Pre-Post ITV	Unknown	Continuous laser	30min Sites: 4, Posterior & anterior tibia, dorsal & ventral foot	↓ sensitive impairment ↑ neural function (hot/cold discrimination)
Schindl 2002 <sup>49</sup>	Human, n = 30 Diabetes RCT	632.8	Continuous laser	30J/cm <sup>2</sup> Frequency: Once Site: 1	↑ microcirculation

IL: Interleukin, LANSS: Leeds assessment of neuropathy symptoms and signs, LED: Light emitting diode, PBM: photobiomodulation, PQAS: Pain qualities assessment scale, Pre-Post ITV: Pre-post interventional trial, QoL: Quality of life, RCT: randomized controlled trial, T1D: Type 1 diabetes, T2D: Type 2 diabetes, TNF: Tumor necrosis factor, VAS: Visual analogic scale, \*Surface of PBM not reported

**Table 3.** Effect of PBM on diabetic retinopathy

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Ahmed 2021 <sup>50</sup>	Wistar rat Streptozotocin induced	670	Continuous laser	5mW/cm <sup>2</sup> , 90sec, 0.9J/cm <sup>2</sup> Frequency: 2 times a week, for 6 weeks Sites: 2, each eye, directly in eye	Improve structure of inner nuclear layer and retinal pigmented epithelium ↓ anatomical abnormalities
Cheng 2018 <sup>51</sup>	C57BL/6J mice Streptozotocin induced	670	Continuous LED	25mW/cm <sup>2</sup> , 240sec, 6J/cm <sup>2</sup> Frequency: once a day, for 8 months Site: 1, back of the animal	↓ degenerescence of retinal capillaries ↓ albumin accumulation in the inner nuclear and in the outer plexiform layers of the retina Preservation of spatial frequency threshold contrast sensitivity
Saliba 2015 <sup>52</sup>	C57BL/6J mice Streptozotocin induced	670	Continuous LED	20mW/cm <sup>2</sup> , 240sec, 5J/cm <sup>2</sup> Frequency: once a day, for 10 weeks Site: Total body	↓ superoxyde in retina ↓ abnormalities induced in leukostasis No effect on contrast sensitivity
Tang 2013 <sup>53</sup>	Lewis rat Streptozotocin induced	670	Continuous LED	25mW/cm <sup>2</sup> , 240sec, 6J/cm <sup>2</sup> Frequency: once a day, for 10 weeks Site: total body	↓ diabetes induced abnormality of retinal function and abnormalities of electroretinograms ↓ retinal ganglion cell death Inhibited leukostasis
Shen 2020 <sup>54</sup>	Human, n = 21 Diabetes Pre-Post ITV	670	Continuous LED	25-100-200mW/cm <sup>2</sup> , 90sec, 2.25-9-18J/cm <sup>2</sup> Frequency: 12 sessions over 5 weeks Site: fundus contact lens	↓ central macular thickness (100 and 200>25) No effect on visual acuity No adverse event
Eells 2017 <sup>55</sup>	Human, n = 10 Diabetes RCT	670	Unknown	45mW/cm <sup>2</sup> , 100sec, 4.5J/cm <sup>2</sup> Frequency: 3 consecutive days per week, for 8 weeks Sites: 2, each eye, directly in eye	↓ central retinal thickness ↑ visual acuity (+ 6 letters)

LED: Light emitting diode, Pre-post ITV: Pre-post interventional trial, RCT: Randomized controlled trial



**Table 4.** Effect of PBM on glucose metabolism in T2D

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Min 2022 <sup>56</sup>	Diabetic Goto Kakizaki rat	630	Continous LED	3.7mW/cm <sup>2</sup> , 600sec, 2.22J/cm <sup>2</sup> Frequency: 1 session Site: intra-duodenal	No effect
		630 + 850	Continous LED	32.72mW/cm <sup>2</sup> , 100sec, 3.6J/cm <sup>2</sup> Frequency: 1 session Site: intra-duodenal	↓ glucose intolerance at 4 weeks (↓ 14.5% GTT AUC) ↓ insulin resistance only at 1 week (↓22% HOMA-IR) ↑ expression of insulin in beta cells
Bonifacio 2021 <sup>57</sup>	C57BL/6 mice High fat diet	808	Continuous laser	3.57W/cm <sup>2</sup> , 8sec, 30J/cm <sup>2</sup> Frequence: 3 times a week, for 4 weeks Site: 1, direct contact with skin in pancreas region	No effect on glucose tolerance (GTT) No effect on insulin resistance (ITT) No effect on fasting glycemia No effect on body weight No effect on pancreas morphology No effect on adiposity tissue No effect on pancreas morphology
Gong 2021 <sup>58</sup>	C57BL/6 mice High fat diet and mice C57BLK5 diabetic	635	Continuous laser	72.1mW/cm <sup>2</sup> , 10min, 43.3J/cm <sup>2</sup> Frequency: once a day, for 10 weeks Sites: 2, direct contact with skin	↓ glucose intolerance (↓ 12% GTT AUC) ↑ insulin sensitivity (↓ 10% ITT AUC) ↓ fed glycaemia (500 vs. 280mg/dL) ↓ fasted glycaemia (490 vs. 200mg/dL) ↑ glycogen in muscle ↓ ectopic fat in muscle ↓ triglycerides and free fatty acid
Gong 2020 <sup>59</sup>	C57BL/6 mice High fat diet	635	Continuous laser	72.1mW/cm <sup>2</sup> , 10min, 43.3J/cm <sup>2</sup> Frequency: once a day, for 10 weeks Site: abdomen, direct contact with skin	↓ triglycerids, plasmatique FFA ↑ relative oxygen species
Guo 2020 <sup>60</sup>	C57BL/6 mice High fat diet	635	Continuous laser	72.1mW/cm <sup>2</sup> , 10min, 43.3J/cm <sup>2</sup> Frequency: once a day, for 8 weeks Site: abdomen, direct contact with skin	↓ glucose intolerance (GTT) ↑ insulin sensitivity (ITT) Protect against obesity and hyperglycaemia (results similar to control) ↓ weight, glycaemia, triglycerides, cholesterol, insulinemia ↓ hepatic steatosis

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Silva 2020 <sup>62</sup>	Swiss albinos mice High fat diet	630	Continous LED	779.53mW/cm <sup>2</sup> , 40sec, 31.18J/cm <sup>2</sup> Frequency: 5 days per week, for 4 weeks Sites: 5, direct contact with skin	↓ glucose intolerance (↓ 28% GTT AUC) ↓ fasting hyperinsulinemia (↓insulin concentration by 3)
Silva 2018 <sup>63</sup>	Swiss albinos mice High fat diet	780	Continuous laser	259mW/cm <sup>2</sup> , 40sec, 10J/cm <sup>2</sup> Frequency: 5 days per week, for 4 weeks Sites: 5, direct contact with skin	↓ glucose intolerance (↓ 16% GTT AUC) No effect on insulinresistance (HOMA-IR) ↓ fatty mass epididymal ↓ total cholesterol ↑ insulin signalling pathway
Yoshimura 2016 <sup>64</sup>	C57BL/6 mice High fat diet	843	Continous LED	19mW/cm <sup>2</sup> , 300sec, 5.7J/cm <sup>2</sup> Frequency: day 1, 3, 7, 10, 14 and 21 Site: abdomen, direct contact with skin	↓ glycaemia (98 vs. 118 mg/dL for non-treated group) No effect on weight ↓ abdominal fatty infiltration
Scontri 2023 <sup>65</sup>	Human, n = 10 T2D RCT	830	Continuous LED	114.28mW/cm <sup>2</sup> , 50 or 120sec, 5.71 or 13.71J/cm <sup>2</sup> Frequency: One session and 7 days of washout Sites: 8, muscles, in contact with skin	Effect only with 5.71J/cm <sup>2</sup> ↓ post prandial glycaemia (30 min to 12h after PBM) Better effect on glycaemic control than hypoglycaemic treatments ↓ 37% GTT AUC Faster glucose decay post prandial (16%, -60 vs. -70mg/dL/h) No additive effect with hypoglycaemic treatments

AUC: Area under the curve, FFA: Free fatty acid, GTT: glucose tolerance test, HOMA-IR: Homeostasis model assessment insulin resistance, ITT: insulin tolerance test, LED: Light emitting diode, PBM: photobiomodulation, RCT: randomized controlled trial, T2D: Type 2 diabetes

**Table 5.** Effect of PBM on exercise and muscle in T2D

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
da Silva Tonetto 2023 <sup>66</sup>	Wistar rat Streptozotocin and diet induced	660	Continuous laser	571mW/cm <sup>2</sup> , 36.75sec, 21J/cm <sup>2</sup> Frequency: 5 days per week, for 6 weeks Sites: 2, medium and laterally of gastrocnemius	↓ oxidative activity ↑ antioxidative activity (↑ super oxide dismutase)
de Oliveira 2019 <sup>67</sup>	Wistar rat Streptozotocin induced	660	Continuous laser	250mW/cm <sup>2</sup> , 16sec, 2J/cm <sup>2</sup> Frequency: 3 times a week, for 3 weeks Site: 1, dorsal	No effect on glucose concentration No effect on muscle parameters if PBM was not associated with exercise
Frigerio 2018 <sup>68</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	107.1mW/cm <sup>2</sup> , 44sec, 4.71J/cm <sup>2</sup> Frequency: 1/session of exercise Sites: 3, gastrocnemius	↓ oxidative stress (↓ lactate, ↓ catalase, ↑ supe oxide dismutase) ↑ VO <sub>2</sub> max and speed of run
Linares 2022 <sup>69</sup>	Human, n = 13 DT2 RCT	850	Continuous LED	375mW/cm <sup>2</sup> , 140 to 1120 sec, 52.5-420J/cm <sup>2</sup> Frequency: 1/session of exercise Sites: 7, oblique and rectus abdomen, quadriceps femoris, triceps surae, hamstrings bilateral	↓ glycemia and lactate 15min after PBM Improvement of cardiac parameters
Gobbi 2021 <sup>70</sup>	Human, n = 17 DT2 RCT	620	Continuous LED	52.86mW/cm <sup>2</sup> , 96sec, 5.074J/cm <sup>2</sup> Frequency: once a day, for 3 days Sites: 4, ankle flexor and extensor bilaterally	No impact on muscular performance No impact on muscular functionality
		940	Continuous LED	33.7mW/cm <sup>2</sup> , 106sec, 3.572J/cm <sup>2</sup> Frequency: once a day, for 3 days Sites: 4, ankle flexor and extensor bilaterally	
		620 + 940	Continuous LED	Same parameters of 2 others	
Milan-Mattos 2020 <sup>71</sup>	Human, n = 7 T2D RCT	850	Continuous LED	375mW/cm <sup>2</sup> , 40sec, 15J/cm <sup>2</sup> ou 80sec, 30J/cm <sup>2</sup> Frequency: 1/session of exercise Sites: 2, quadriceps and triceps bilaterally	No impact on baroreflex during orafter exercise No impact of PBM on cardiovascular autonomic control
Francisco 2019 <sup>72</sup>	Human, n = 16 T2D RCT	850	Continuous LED	375mW/cm <sup>2</sup> , 40sec, 15J/cm <sup>2</sup> Frequency: 1/session of exercise Sites: 2, quadriceps and triceps bilaterally	No impact of PBM on lactate concentration No impact on cardiopulmonary and hemodynamic adjustments

LED: light emitting diode, PBM: photobiomodulation, RCT: Randomized controlled trial, T2D: Type 2 diabetes

**Table 6.** Effect of PBM on wound healing and ulcer

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusion
Dungel 2023 <sup>73</sup>	C57BL diabetic mice	629	Pulsed LED	40mW/cm <sup>2</sup> , 360sec, 14.4J/cm <sup>2</sup> Frequency: day 0 and 1 Site: near to the wound	↑ wound closure ↑ angiogenesis
		540	Pulsed LED	40mW/cm <sup>2</sup> , 360sec, 14.4J/cm <sup>2</sup> Frequency: day 0 and 1 Site: near to the wound	↑ wound closure ↑ angiogenesis
		470	Pulsed LED	40mW/cm <sup>2</sup> , 360sec, 14.4J/cm <sup>2</sup> Frequency: day 0 and 1 Site: near to the wound	No effect
Ebrahimipour-Malekshah 2023 <sup>74</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	20mW/cm <sup>2</sup> , 200sec, 1.08J/cm <sup>2</sup> Frequency: once a day, for 14 days Sites: 9, direct contact	↑ granulation tissue formation ↓ neutrophils, ↑ macrophages ↑ fibroblasts ↑ vascularisation (VEGF)
Mehrvar 2021 <sup>75</sup>	Diabetic mice	670	Continuous LED	60mW/cm <sup>2</sup> , 90sec, 4.5J/cm <sup>2</sup> Frequency: 5 days per week, for 2 weeks Site: 1, next to the wound	↓ wound area ↓ oxidative stress ↑ Red-Ox ratio
Ahmadi 2020 <sup>76</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	1mW/cm <sup>2</sup> , 200sec, 0.2J/cm <sup>2</sup> Frequency: once a day, for 14 sessions Sites: 9, next to the wound	↑ wound healing ↓ inflammation (neutrophils) ↑ fibroblasts ↑ vascular length
Bagheri 2020 <sup>77</sup>	Wistar rat Streptozotocin induced	890	Continuous laser	1mW/cm <sup>2</sup> , 300sec, 0.324J/cm <sup>2</sup> Frequency: once a day, for 7 days Site: 1, next to the wound	↓ inflammation (macrophages, neutrophils) ↑ fibroblast
Kouhkeil 2019 <sup>78</sup>	Rat Streptozotocin induced	890	Continuous laser	1.08mW/cm <sup>2</sup> , 200sec, 0.2J/cm <sup>2</sup> Frequency: 6 days per week, for 2 weeks Site: 1, next to the wound	↓ mast cells ↓ CFU ↑ wound strength

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusion
Fekrazad 2018 <sup>79</sup>	Wistar rat Streptozotocin induced	660	Continuous laser	30mW*, 33sec, 2J/cm <sup>2</sup> Frequency: every 2 days, for 10 days Site: near to the wound	No effect
		810	Continuous laser	200mW*, 5sec, 2J/cm <sup>2</sup> Frequency: every 2 days, for 10 days Site: near to the wound	No effect
		660 + 810	Continuous laser	Same parameters	↓ TGF-β1
Asghari 2017 <sup>80</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	0.324J/cm <sup>2</sup> Frequency: 6 days per week, for 2 weeks Site: 12, next to the wound	↑ wound healing ↓ CFU
Leite 2017 <sup>81</sup>	Wistar rat Alloxan induced	660	Continuous laser	1W/cm <sup>2</sup> , 9 or 130sec, 10 or 140J/cm <sup>2</sup> Frequency: once a day, for 3 days Site: 1, next to the wound	140J/cm <sup>2</sup> > 10J/cm <sup>2</sup> ↑ wound healing ↑ mast cells number, VEGF, FGF, neovascularization ↓ leukocytes number
Fahimipour 2016 <sup>82</sup>	Albinos mice Streptozotocin induced	632.8	Continuous laser	250mW/cm <sup>2</sup> , 16sec, 4J/cm <sup>2</sup> Frequency: once a day, for 14 days Sites: 2, next to the wound	632.8 > 830 to improve healing ↑ density of collagen fibres
		830	Continuous laser	250mW/cm <sup>2</sup> , 16sec, 4J/cm <sup>2</sup> Frequency: once a day, for 14 days Sites: 2, next to the wound	↑ number of fibroblasts ↑ neovascularisation
Fekrazad 2015 <sup>83</sup>	Wistar rat Streptozotocin induced	425	Continuous laser	55mW/cm <sup>2</sup> , 36sec, 2J/cm <sup>2</sup> Frequency: day 0, 1, 2, 4, 6, 8 Site: 1, next to the wound	Red > Blue & green ↑ wound healing
		532	Continuous laser	50mW/cm <sup>2</sup> , 40sec, 2J/cm <sup>2</sup> Frequency: day 0, 1, 2, 4, 6, 8 Site: 1, next to the wound	
		630	Continuous laser	50mW/cm <sup>2</sup> , 40sec, 2J/cm <sup>2</sup> Frequency: day 0, 1, 2, 4, 6, 8 Site: 1, next to the wound	

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusion
Dancáková 2014 <sup>84</sup>	SD rat Streptozotocin induced	810	Continuous laser	30mW/cm <sup>2</sup> , 30sec, 0.9J/cm <sup>2</sup> Frequency: once a day, for 7 days Site: 1, next to the wound	↑ wound healing ↑ wound tensile & strength ↑ granulation tissue
Aparecida da Silva 2013 <sup>85</sup>	Wistar rat Streptozotocin induced	660	Continuous laser	1.43W/cm <sup>2</sup> , 80sec, 4J/cm <sup>2</sup> Frequency: one session Site: 1, next to the wound	↑ collagen density ↓ MMP2 and MMP9
Fathabadie 2013 <sup>86</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	1.08mW/cm <sup>2</sup> , 200sec, 0.2J/cm <sup>2</sup> Frequency: once a day, for 6 days Sites: 18, next to the wound	↑ mast cells
Firat 2013 <sup>87</sup>	Wistar rat Streptozotocin induced	940	Continuous laser	1.1W/cm <sup>2</sup> , 9sec, 10J/cm <sup>2</sup> Frequency: every 2 days, for 7 days Site: 1, next to the wound	↓ inflammation ↑ collagen synthesis ↑ fibroblasts
Dadpay 2012 <sup>88</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	1.08mW/cm <sup>2</sup> , 30 or 300sec, 0.03 or 0.2J/cm <sup>2</sup> Frequency: 6 days per week, for 2 weeks Sites: 18, next to the wound	↑ Enhancing maximum stress and elastic modulus
Park 2012 <sup>89</sup>	SD rat Streptozotocin induced	980	Continuous laser	232.5mW/cm <sup>2</sup> , 60sec, 13.95J/cm <sup>2</sup> Frequency: once a day, for 14 days Site: 1, next to the wound	↓ inflammation cells infiltration ↑ number of fibroblasts ↑ wound healing
Hegde 2011 <sup>90</sup>	Swiss Albinos mice Streptozotocin induced	632.8	Continuous laser	4.02mW/cm <sup>2</sup> , 255 to 1277sec, 1 to 5J/cm <sup>2</sup> Frequency: once Site: 1, next to the wound	The best = 3J/cm <sup>2</sup> ↑ wound healing ↑ collagen synthesis
Peplow 2011 <sup>91</sup>	Diabetic mice	660	Continuous laser	233-313mW/cm <sup>2</sup> , 20sec, 2J* 116-156mW/cm <sup>2</sup> , 40 sec, 2J* 58-78mW/cm <sup>2</sup> , 80sec, 2J* Frequency: once a day, for 7 days Site: 1, next to the wound	Same effects between puissance ↑ wound healing ↑ epithelialization, granulation
Akyol 2010 <sup>93</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	100mW/cm <sup>2</sup> , 20sec, 2J/cm <sup>2</sup> Frequency: every 2 day, for 8 days Site: 1, next to the wound	↑ wound healing No effect on inflammation No effect on epithelialization

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusion
Carvalho 2010 <sup>92</sup>	Wistar rat Alloxan induced	660	Continuous laser	166mW/cm <sup>2</sup> , 24sec, 4J/cm <sup>2</sup> Frequency: unknown Site: 1, next to the wound	↑ fibre of collagen ↓ macrophages
Chung 2010 <sup>94</sup>	Diabetic mice	660	Continuous laser	10sec -> 1J*, 20sec -> 1.6J*, 40sec -> 3.2J* Frequency: once a day, for 7 days Site: 1, next to the wound	Best one = 1.6J/day ↑ wound healing
Santos 2010 <sup>95</sup>	Wistar rat Streptozotocin induced	660	Continuous laser	30mW* -> 2,5J/cm <sup>2</sup> Frequency: once a day, for 8 days Sites: 16, next to the wound	790 better ↑ angiogenesis
		790	Continuous laser	40mW* -> 2,5J/cm <sup>2</sup> Frequency: once a day, for 8 days Site: 16, next to the wound	
Al-Watban 2009 <sup>96</sup>	SD rat Streptozotocin induced	532	Continuous laser	20.4mW/cm <sup>2</sup> , 290 to 1470sec, 5 to 30J/cm <sup>2</sup> Frequency: 3 times per week	Best = Laser 633 ↑ wound healing
		633	Continuous laser	15.56mW/cm <sup>2</sup> , 322 to 1932sec, 5 to 30J/cm <sup>2</sup> Frequency: 3 times per week	
		810	Continuous laser	22.2mW/cm <sup>2</sup> , 225 to 1350sec, 5 to 30J/cm <sup>2</sup> Frequency: 3 times per week	
		980	Continuous laser	22.2mW/cm <sup>2</sup> , 225 to 1350sec, 5 to 30J/cm <sup>2</sup> Frequency: 3 times per week	
		1060	Continuous laser	66.37mW/cm <sup>2</sup> , 75 to 450sec, 5 to 30J/cm <sup>2</sup> Frequency: 3 times per week	
		510-872	Continuous LED	13.6mW/cm <sup>2</sup> , 367 to 2206sec, 5 to 30J/cm <sup>2</sup> Frequency: 3 times per week This was a polychromatous LED	
Güngörmüş 2009 <sup>97</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	10J/cm <sup>2</sup> Frequency: every 2 days for 8 days Site: unknown	↑ wound healing
Maiya 2009 <sup>98</sup>	Wistar rat Alloxan induced	632.8	Continuous laser	10mW/cm <sup>2</sup> , 3 to 27min, 3 to 9J/cm <sup>2</sup> Frequency: 5 days per week until cicatrisation Site: 1, next to the wound	3 to 7J/cm <sup>2</sup> : ↑ epithelialization, tissue granulation, ↑ wound healing 8-9J/cm <sup>2</sup> : ↓ reparative process

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusion
Carvalho 2006 <sup>99</sup>	Wistar rat Alloxan induced	632.8	Continuous laser	200mW/cm <sup>2</sup> , 60sec, 4J/cm <sup>2</sup> Frequency: once a day, for 14 days Site: 1, next to the wound	↑ fibre of collagen
Rabelo 2006 <sup>100</sup>	Wistar rat Streptozotocin induced	632.8	Continuous laser	588mW/cm <sup>2</sup> , 17sec, 10J/cm <sup>2</sup> Frequency: once a day, for 15 days Site: 1, next to the wound	↓ wound area ↓ local inflammation ↓ inflammatory cells
Maiya 2005 <sup>101</sup>	Wistar rat Alloxan induced	632.8	Continuous laser	4.8J/cm <sup>2</sup> Frequency: 5 days per week until cicatrisation Site: 1, next to the wound	↑ collagen ↑ fibroblastic and capillary proliferation ↑ granulation tissue formation, vascularisation, epithelialization
Byrnes 2004 <sup>102</sup>	Purina sand rat chow 5L09 DT2 model	632.8	Continuous laser	16mW/cm <sup>2</sup> , 250sec, 4J/cm <sup>2</sup> Frequency: once a day, for 3 days Site: 1, next to the wound	↑ wound closure ↑ collagen, bFGF ↑ neovascularization
Reddy 2001 <sup>103</sup>	SD rat Streptozotocin induced	632.8	Continuous laser	1J/cm <sup>2</sup> Frequency: once a day, for 5 days Site: 1, next to the wound	↑ collagen ↑ maximum strain ↑ toughness
Haze 2022 <sup>104</sup>	Human, n = 20 Diabetes RCT	808	Continuous laser	138mW/cm <sup>2</sup> , 8min, 1.1J/cm <sup>2</sup> Frequency: once a day, for 12 weeks Site: next to the wounds	↓ wound area (12.5 vs. 1.5cm <sup>2</sup> ) ↑ wound closure (49.4 vs. 97.3%) No side effects link to PBM
Vitoriano 2019 <sup>105</sup>	Human, n = 12 Diabetes RCT (for 2 sources of light)	850	Continuous LED	240mW/cm <sup>2</sup> , 22sec, 14.64J/cm <sup>2</sup> Frequency: 2 times a week, for 5 weeks Sites: 6, next to the wound	Laser seems better ↓ wound area (1.45 to 0.64 vs 1.76 to 0.36cm <sup>2</sup> )
		830	Laser	250mW/cm <sup>2</sup> , 28sec, 15.48J/cm <sup>2</sup> Frequency: 2 times a week, for 5 weeks Sites: 3, next to the wound	
de Alencar Fonseca Santos 2018 <sup>106</sup>	Human, n = 18 Diabetes RCT	660	Continuous laser	490mW/cm <sup>2</sup> , 13sec, 6J/cm <sup>2</sup> Frequency: every 2 days, for 4 weeks Site: 1, next to the wound	↑ wound healing index ↑ pressure ulcer scale for healing No effect on pain (VAS)
Franguez 2018 <sup>107</sup>	Human, n = 60 Diabetes RCT	625 (24%) 660 (71%) 850 (5%)	Pulsed LED	5min, 2.4J/cm <sup>2</sup> Frequency: 3 times a week, for 8 weeks Site: 1, next to the wound	↑ Falanga score (score of healing) No effect on size



Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusion
Ruh 2018 <sup>108</sup>	Human, n = 8 Diabetes Pre-Post ITV	660	Continuous laser	167mW/cm <sup>2</sup> , 12sec, 2J/cm <sup>2</sup> Frequency: once a day for 12 days Site: 1, next to the wound	↓ wound size (data not shown) ↓ TNFα, ↑ TGFβ, ↑ VEGF No effect on IL6
Mathur 2017 <sup>109</sup>	Human, n = 30 T2D RCT	660	Continuous laser	50mW/cm <sup>2</sup> , 60sec, 3J/cm <sup>2</sup> Frequency: once a day, for 15 days Sites: 5-8, above the wound	↑ wound closure (15% vs. 37%) No side effects
Carvalho 2016 <sup>110</sup>	Human, n = 32 DT2 RCT	658	Continuous laser	50mW/cm <sup>2</sup> , 80sec, 4J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Site: 1, next to the wound	↓ wound area (8.43 vs. 2.39cm <sup>2</sup> ) ↓ pain (VAS: 4.8 vs 1.9) ↑ neovascularization
Sandoval Ortiz 2014 <sup>111</sup>	Human, n = 9 Diabetes RCT	685	Continuous laser	~11mW/cm <sup>2</sup> , 0.14-0.18sec, 1.5-2J/cm <sup>2</sup> Frequency: Unknown Sites: multiple along the edges of the ulcer and in the wound bed, next to the wound	No effect on cicatrisation No effect on protective sensation No effect on QoL (EQ-5D)
Kajagar 2012 <sup>112</sup>	Human, n = 68 T2D RCT	660 + 850	Pulsed LED	60mW*, 2-4J/cm <sup>2</sup> Frequency: once a day, for 15 days Site: 1, above the wound	↓ ulcer area (decrease of 32 vs. 104cm <sup>2</sup> )
Kaviani 2011 <sup>113</sup>	Human, n = 23 T2D RCT	685	Continuous laser	50mW/cm <sup>2</sup> , 200sec, 10J/cm <sup>2</sup> Frequency: 6 days per week, at least 2 weeks Site: 1, next to the wound	↑ wound closure (47.3% vs 73.7% after 4 weeks) ↑ wound healing (non-ischemic wound)
Minatel 2009 <sup>114</sup>	Human, n = 14 Diabetes RCT	890 + 660	Continuous LED	100mW/cm <sup>2</sup> , 30sec, 3J/cm <sup>2</sup> Frequency: 2 times a week, for 3 months Site: 1, next to the wound	↑ granulation ↑ wound closure (43.3% vs. 90.8%)
Schindl 1999 <sup>115</sup>	Human, n = 8 Diabetes Descriptive	632.8	Continuous laser	30mW*, 30J/cm <sup>2</sup> Frequency: 3 times a week until cicatrisation Site: unknown	100% of closure of chronic ulcers after 32 to 130 sessions
Schindl 1998 <sup>116</sup>	Human, n = 30 Diabetes RCT	632.8	Continuous laser	10mW/cm <sup>2</sup> , 50min, 30J/cm <sup>2</sup> Frequency: 1 time Site: 1, skin surface	↑ skin temperature ↑ microcirculation in patient with microangiopathy

CFU: colony forming unit, FGF: fibroblast growth factor, LED: Light emitting diode, MMP: Matrix metalloproteinases, PBM: Photobiomodulation, Pre-Post ITV: Pre-post interventional trial, QoL: Quality of life, Rat SD: Rat Sprague Dawley, RCT: Randomized controlled trial, T2D: Type 2 diabetes, TGF: Transforming Growth factor, TNF: Tumor necrosis factor, VAS: Visual analogic scale, VEGF: Vascular endothelial growth factor, \*Surface of PBM not reported

**Table 7.** Effect of PBM on bone cicatrisation

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Dalirsani 2021 <sup>117</sup>	Wistar rat Streptozotocin induced	660	Continuous laser	76.4mW/cm <sup>2</sup> , 24sec, 7.2J/cm <sup>2</sup> Frequency: once a day, for 14 days Site: 1, direct contact	↑ bone formation ↓ inflammation ↑ vascularisation
		802	Continuous laser	127.32mW/cm <sup>2</sup> , 14sec, 7J/cm <sup>2</sup> Frequency: once a day, for 14 days Site: 1, direct contact	↓ inflammation ↑ vascularisation
Lee 2021 <sup>118</sup>	Wistar rat Streptozotocin induced	660	Continuous laser	2.42mW/cm <sup>2</sup> , 1652sec, 4J/cm <sup>2</sup> Frequency: once a day, for 12 weeks Site: 1, near to the bone defect place	↑ bone formation ↑ bone fracture healing No effect on osteogenique factor
Diker 2019 <sup>119</sup>	SD rat Streptozotocin induced	808	Continuous laser	3.5W/cm <sup>2</sup> , 22sec, 78.5J/cm <sup>2</sup> Frequency: once a day, for 3 days Site: 1, direct contact	↑ bone formation ↑ osteoblasts
Gomes 2018 <sup>120</sup>	Wistar rat Streptozotocin induced	780	Continuous laser	16W/cm <sup>2</sup> , 10, 20 or 40sec, 160, 320 or 640J/cm <sup>2</sup> Frequency: every 2 days, for 21 days Site: 1, direct contact	Only or 640J/cm <sup>2</sup> : Better maintenance of periodontal tissue subjected to a force
Mostafavinia 2018 <sup>121</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	1.5W/cm <sup>2</sup> , 1300sec, 1.5J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites: 3, direct contact	↑ bone formation ↑ bone cortical volume ↑ bone trabecular volume ↑ osteoblasts and osteocytes
Mostafavinia 2017 <sup>122</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	8.32W/cm <sup>2</sup> , 1300sec, 1.5J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites: 3, direct contact	↑ bone density
Yildirimturk 2017 <sup>123</sup>	SD rat Streptozotocin induced	820	Continuous laser	0.5W/cm <sup>2</sup> , 32sec, 16J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Site: 1, tibiae, direct contact	↑ bone formation ↑ vascularisation No effect of osteoblast quantity
Patrocínio Silva 2016 <sup>124</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	3.57W/cm <sup>2</sup> , 33sec, 120J/cm <sup>2</sup> Frequency: 3 times a week, for 8 weeks Site: 1, direct contact	↑ bone density ↑ bone mineral content stiffness ↑ cortical tibia area

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Magri 2015 <sup>125</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	3.57mW/cm <sup>2</sup> , 8 or 16 or 33sec, 30, 60 or 120J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites : 2, direct contact	↑ bone formation No histological effect
Nascimento 2015 <sup>126</sup>	Wistar rat Alloxan induced	780	Continuous laser	1.75W/cm <sup>2</sup> , 10sec, 17.5J/cm <sup>2</sup> Frequency: every 2 days, for 7 days Site: 1, direct contact	↑ bone formation ↓ inflammation ↑ alkaline phosphatase
Patrocínio Silva 2014 <sup>127</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	3.57W/cm <sup>2</sup> , 33sec, 120J/cm <sup>2</sup> Frequency: 3 times a week, for 6 weeks Sites: 4, direct contact	↑ bone density ↑ cortical area ↑ values of fracture force ↑ osteogenic potential
Akyol 2010 <sup>128</sup>	Wistar rat Streptozotocin induced	808	Continuous laser	100mW/cm <sup>2</sup> , 20sec, 2J/cm <sup>2</sup> Frequency: every 2 days, for 7 days Site: 1, right distal epiphysis	↑ bone repair ↑ substantia spongia formation No effect on union bone marrow
Abdi 2009 <sup>129</sup>	Wistar rat Alloxan induced	780	Continuous laser	318mW/cm <sup>2</sup> , 1166sec, 369,4J/cm <sup>2</sup> Frequency: 3 times a week, for 6 weeks Sites: 2, direct contact	No effect on bone repair
Bayat 2009 <sup>130</sup>	Wistar rat Streptozotocin induced	632.8	Continuous laser	3.17mW/cm <sup>2</sup> , 90 or 1200sec, 88.6 or 382.2J/cm <sup>2</sup> Frequency: once a day, for 14 days Sites: 4, direct contact	↑ bone density ↑ bone lamella meshwork ↑ maximum force and load at the break ↓ bend stiffness
Javadieh 2009 <sup>131</sup>	Wistar rat Streptozotocin induced	890	Pulsed laser	265 or 530sec, 5 or 10J/cm <sup>2</sup> Frequency: 3 times a week, for 6 weeks Sites : 2, direct contact	↑ bone repair ↑ bending stiffness ↑ maximum force
Attia 2023 <sup>132</sup>	Human, n = 40 T2D RCT	808	Continuous laser	125mW/cm <sup>2</sup> , 1.23min, 0,15J/cm <sup>2</sup> Frequency: 2 times, pre and post implantation Sites: 6, direct contact	↑ bone repair and density ↑ bone structure

Rat SD: Rat Sprague Dawley, RCT: Randomized controlled trial, T2D: Type 2 diabetes

**Table 8.** Effect of PBM on chronic diabetes periodontitis

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Kamatham 2022 <sup>133</sup>	Human, n = 60 T2D RCT	650	Continuous laser	0.4W* Frequency: 1 session Site: 1/tooth, in contact with gingival tissue	↓ inflammation ↓ calprotectin No effect on probing depth, clinical attachment level
Pulivarthi 2022 <sup>134</sup>	Human, n = 30 T2D RCT	650	Continuous laser	0.8W/cm <sup>2</sup> , 15sec, 12J/cm <sup>2</sup> Frequency: once a day, for 8 weeks Sites: 3, in contact with gingival tissue	No effect on TNFα No effect on bleeding index, probing depth, clinical attachment level
Mrasori 2021 <sup>135</sup>	Human, n = 80 T2D RCT	660	Continuous laser	10mW*, 8min Frequency: 5 days per week, for 3 months Sites: 5, in contact with gingival tissue	↓ IL6
Soi 2021 <sup>136</sup>	Human, n = 44 T2D RCT	940	Pulsed laser	0.8W*, 15sec, 24J* Frequency: unknown Sites: 2/tooth, into the periodontal pocket	No effect of adjunction of PBM to SRP (scaling and root planning)
Koçak 2020 <sup>137</sup>	Human, n = 60 T2D RCT	940	Pulsed laser	1.061W/cm <sup>2</sup> , 20sec Frequency: 1 session Sites: 2/tooth, intra periodontal pocket	No effect on bacteria level (P.gingivalis, T.forsythia, T.denticola)
Özberk 2020 <sup>138</sup>	Human, n = 22 T2D RCT	980	Continuous laser	33mW/cm <sup>2</sup> , 15sec, 0.5J/cm <sup>2</sup> Frequency: day 0, 1, 3 and 7 Sites: 2/tooth, in contact with maxilla and mandibula	↓ probing depth (2.9 vs. 2.6 mm) ↓ clinical attachment level (3.0 vs. 2.8 mm) No effect on plaque index and gingival index
Castro dos Santos 2019 <sup>139</sup>	Human, n = 24 T2D RCT (on pocket)	660	Continuous laser	1.1W/cm <sup>2</sup> , 20sec, 22J/cm <sup>2</sup> Frequency: 1 session Sites: 2, buccal and lingual	No effect on probing depth, clinical attachment level
Chandra 2019 <sup>140</sup>	Human, n = 40 T2D RCT	808	Continuous laser	1.5-1.8W/cm <sup>2</sup> , time in second Frequency: 1 session Site: 1, intra periodontal pocket	↓ plaque index (1.56 vs. 1.26) ↓ gingival index (1.56 vs. 1.04) ↓ probing depth (2.63 vs. 1.80) ↓ clinical attachment level (7.50 vs. 6.65) ↓ bacteria level (35% more reduction with PBM)

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Dengizek Eltas 2019 <sup>141</sup>	Human, n = 40 T2D RCT	810	Continuous laser	1W*, 15-20sec Frequency: once a day Sites: 3/tooth	↓ gingival index (0.91 vs. 0.58) ↓ bleeding on probing (31.7 vs. 24.7%) ↓ probing depth (2.99 vs. 2.77mm) No effect on plaque index, clinical attachment level and inflammation (CRP)
Li 2018 <sup>142</sup>	Human, n = 80 T2D RCT	Unknown	Unknown	Unknown	↓ level of TNF, IL-1, LPS Increase leptin
Demirturk- Gocgun 2017 <sup>143</sup>	Human, n = 22 T2D RCT (on pocket)	808	Continuous laser	0.89W/cm <sup>2</sup> , 5sec, 4.46J/cm <sup>2</sup> Frequency: Day 1, 2 and 7 Sites: 4, in contact with gingival tissue	No effect on bleeding of probing, probing depth, clinical attachment level, plaque index
Koçak 2016 <sup>144</sup>	Human, n = 60 T2D RCT	940	Pulsed laser	1.061W/cm <sup>2</sup> , 20sec Frequency: 1 session Sites: 2/tooth, intra periodontal pocket	↓ VCAM No effect on IL1/6/8/ICAM
Javed 2015 <sup>145</sup>	Human, n = 22 T2D RCT (on pocket)	1064	Pulsed laser	1430W/cm <sup>2</sup> , 60 to 120sec (depending of the accessibility of the pocket), 240-480J* Frequency: unknown Site: 1/tooth, into the periodontal pocket	↓ plaque index (6.4 vs. 1.5) at 1 month, not 3 months ↓ bleeding probing (5.5 vs. 2.1) at 1 month, not 3 months
Obradović 2013 <sup>146</sup>	Human, n = 300 T1D, T2D RCT	670	Continuous laser	2mW/cm <sup>2</sup> , 16min, 2J/cm <sup>2</sup> Frequency: once a day for 5 days Site: 1, in contact with gingival tissue	↓ alteration of periodontium (histologic description)
Obradović 2012 <sup>147</sup>	Human, n = 200 T1D, T2D RCT (on pocket)	670	Continuous laser	5mW*, 14min Frequency: once a day, for 5 days Site: 1, in contact with the jaws	↓ gingival index (0.31 vs. 0.16) ↓ inflammation ↑ cytomorphometric parameters
Obradović 2011 <sup>148</sup>	Human, n = 150 T1D, T2D Pre-Post ITV	Unknown	Unknown	5mW* Frequency: for 5 days Site: only right site of the jaw	↓ gingival index (data not available) ↓ nuclei areal

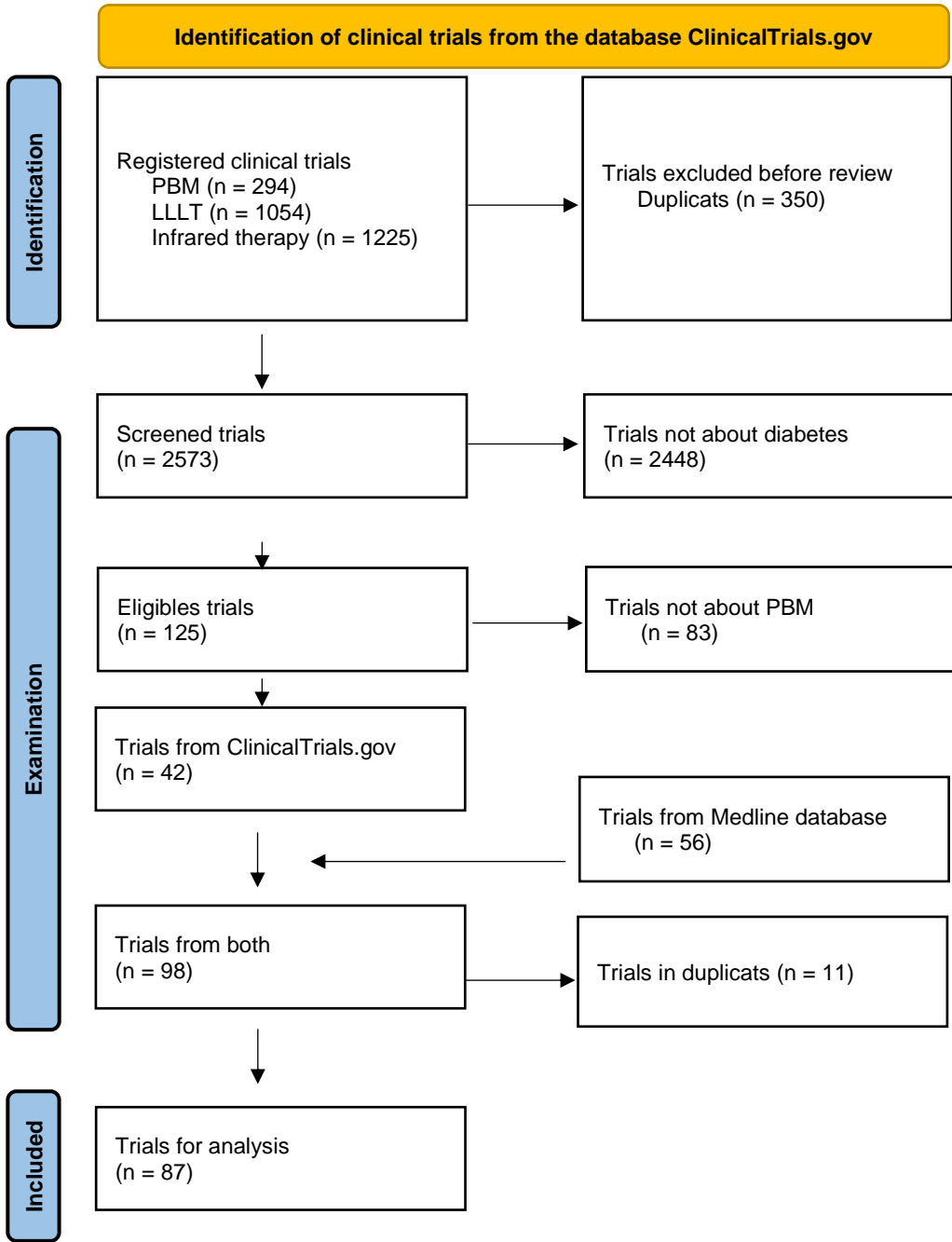
CRP: C reactive protein, ICAM: Intercellular adhesion molecule, IL: Interleukin, LPS: lipopolysaccharide, PBM: photobiomodulation, Pre-post ITV: Pre-post interventional trial, RCT: Randomized controlled trial, SRP: scaling and root planning, T1D: Type 1 diabetes, T2D: Type 2 diabetes, TNF: Tumor necrosis factor, VCAM: Vascular cell adhesion molecule, \*Surface of PBM not reported

**Table 9.** Effect of PBM on other complications

Author / Year	Model	Wave (nm)	Light	PBM parameters	Conclusions
Yang 2023 <sup>151</sup>	SD rat Streptozotocin induced	808	Laser	4J/cm <sup>2</sup> Frequency: for 2 weeks Site: Unknown	↑ erectile function ↑ mitochondrial function and morphology ↓ oxidative stress
Asghari 2016 <sup>152</sup>	Wistar rat Streptozotocin induced	685	Continuous laser	53.6mW/cm <sup>2</sup> , 60sec, 3.2J/cm <sup>2</sup> Frequency: H0, H1, H2 Sites: 6, direct contact with skin	↓ ischemia-reperfusion injury ↓ creatininemia ↓ tubular dilatation, glomerular atrophy ↑ glutathione, superoxide dismutase and catalase
Aghamohamdi 2020 <sup>153</sup>	Human, n = 30 Diabetes Pre-Post ITV	830	Pulsed laser	334mW*, 60sec, 16J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites: 9, pathway of facial nerve, direct skin contact	Recovery in electromyogram in diabetic patient with Bell's palsy
		980	Pulsed LED	9min, 5J/cm <sup>2</sup> Frequency: 3 times a week, for 4 weeks Sites: 9, pathway of facial nerve, direct skin contact	

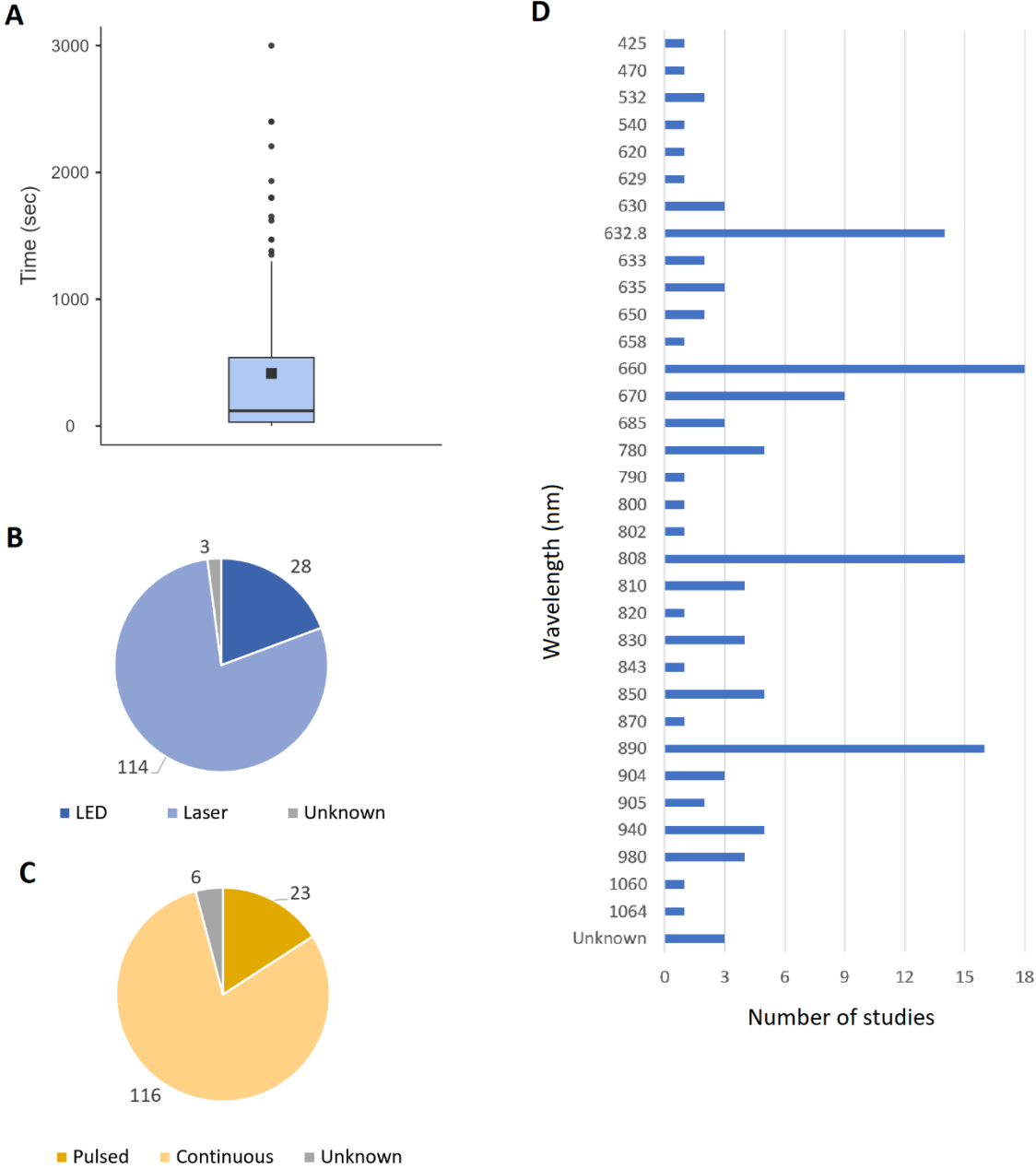
LED: Light emitting diode, Pre-Post ITV: Pre-post interventional trial, \*Surface of PBM not reported

Figure 1. Flow chart for selection of clinical trials



LLLT: Low level laser therapy, PBM: Photobiomodulation

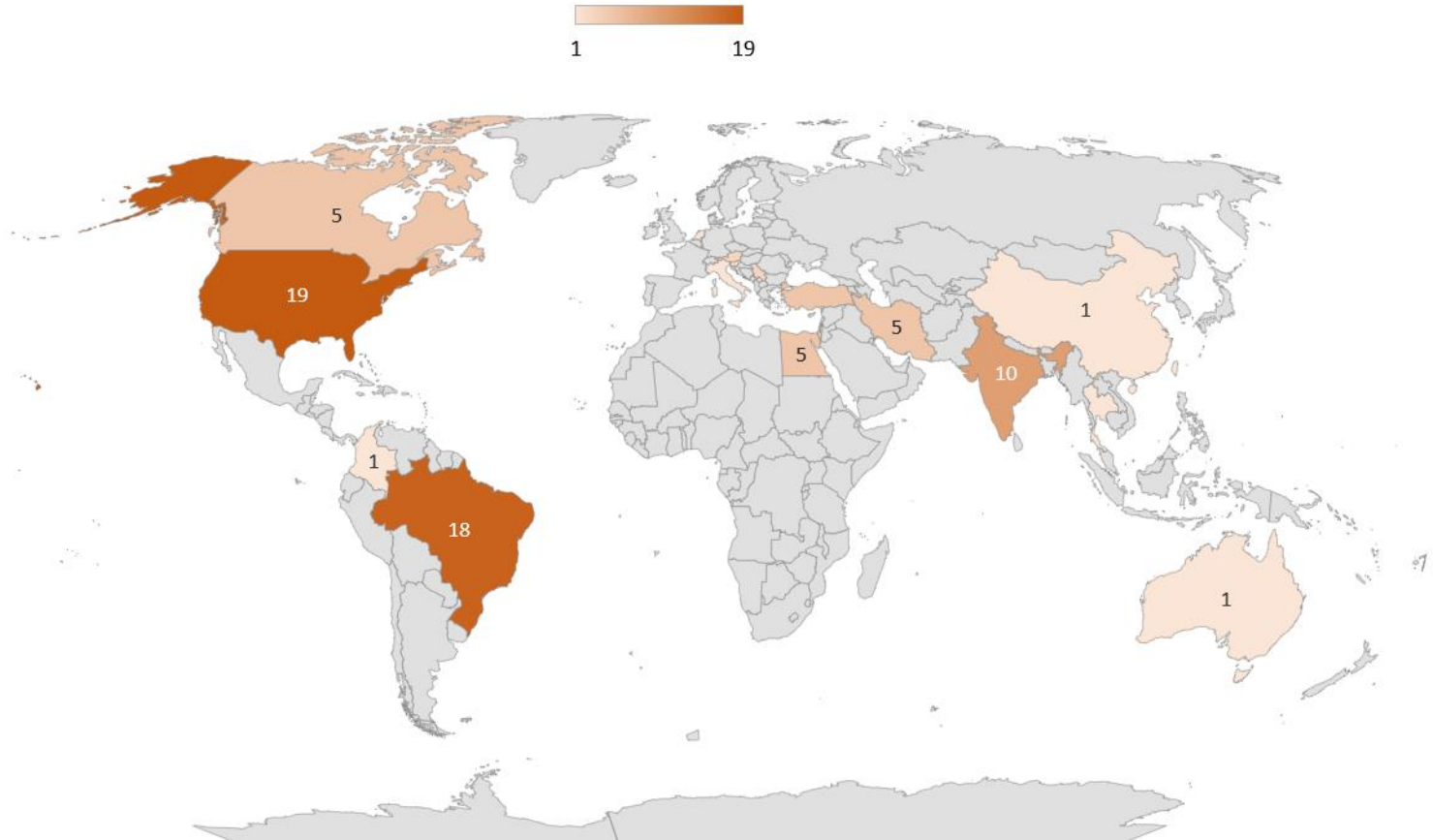
**Figure 2. Global PBM parameters**



The data from both preclinical and clinical studies are presented. **A.** Repartition of time exposure to PBM, black square represents the mean. **B.** Repartition of source of light uses. **C.** Repartition of the mode of illumination uses. **D.** Repartition of wavelength uses.



**Figure 3.** Repartition around the world of clinical trials about PBM and diabetes



*87 trials have been conducted or are planned, 43 in America, 26 in Asia, 12 in Europe, 5 in Africa, and 1 in Oceania*

## Résumé de la thèse

**Titre :** Evaluation de l'effet de la photobiomodulation sur la viabilité et la fonctionnalité des cellules bêta et îlots pancréatiques

**Introduction.** Malgré un traitement optimal par insuline, certains patients diabétiques présentent une variabilité glycémique importante associée à des événements métaboliques majeurs impactant leur qualité de vie, définissant le diabète instable. La greffe d'îlots pancréatiques est alors indiquée. Ces îlots sont isolés du pancréas d'un donneur décédé puis maintenus en culture pendant 24 à 72h pour permettre l'immunosuppression du receveur. La viabilité des îlots humains diminue après leur isolement, et environ la moitié seront détruits suite à la greffe en raison de divers stress (inflammation, privation de substrat, hypoxie), obligeant à multiplier les greffes (2 à 3) pour atteindre un objectif glycémique optimal. La photobiomodulation (PBM) implique l'absorption sélective de certaines longueurs d'onde par les mitochondries, entraînant des modifications biologiques telles qu'une amélioration de la viabilité cellulaire et une réduction de l'inflammation. Bien que la PBM ait démontré des effets positifs dans le traitement de complications du diabète telles que les ulcères, les douleurs neuropathiques et l'intolérance au glucose, son impact direct sur les cellules bêta et les îlots est peu documenté.

**Objectifs.** Ce travail visait à évaluer l'effet in vitro de la PBM à 670 nm et 2,8 mW/cm<sup>2</sup> sur les cellules bêta (MIN6), les îlots de rats et humains et son influence in vivo sur des rats diabétiques induits par la streptozotocine, greffés d'îlots préconditionnés par PBM.

**Résultats.** Les résultats ont démontré qu'une exposition de 5sec à 24h à la PBM n'altérait ni la viabilité ni la fonctionnalité des MIN6 et des îlots de rats. Le pré-conditionnement des cellules MIN6 et des îlots de rats avec 24h de PBM avant un stress inflammatoire (TNF- $\alpha$ , Interféron- $\gamma$ , IL-1 $\beta$ ) ou par privation de substrats (glucose et sérum) a réduit l'impact de ces stress sur : la viabilité, la sécrétion d'insuline en réponse au glucose et les fonctions mitochondriales. Cette protection était également observée lorsque la PBM était appliquée simultanément aux stress. En revanche, aucun effet n'était observé en cas de stress par hypoxie (1% d'O<sub>2</sub>), probablement en lien avec un impact plus faible de ce stress sur les fonctions mitochondriales des cellules bêta. Dans un modèle de rats diabétiques greffés d'îlots préconditionnés par PBM et comparés à des îlots non préconditionnés, les paramètres métaboliques tels que la prise de poids, la glycémie, la sécrétion de c-peptide et le test de tolérance au glucose étaient comparables, indiquant que le pré-conditionnement des îlots n'altérait pas leur fonctionnement in vivo à long terme. De plus, l'application de la PBM sur les îlots humains après isolement améliorait leur viabilité et leur sécrétion d'insuline en réponse au glucose. Le pré-conditionnement de ces îlots humains par la PBM avant un stress inflammatoire permettait de maintenir leur viabilité et leur fonctionnalité à un niveau similaire à celui d'îlots non stressés.

**Discussion.** En somme, la PBM appliquée aux cellules bêta et aux îlots de rats, non soumis à un stress préalable, n'affecte pas leur viabilité ni leur fonctionnalité in vitro et in vivo. Après isolement, les îlots humains présentent une viabilité réduite par rapport aux îlots de rats, principalement à cause du processus d'isolement des îlots humains. Ainsi, la PBM appliquée aux îlots humains après leur isolement améliore leur viabilité et leur fonctionnalité. De plus, l'application préalable de la PBM avant des stress in vitro permettait de limiter l'impact de ces stress sur les MIN6, les îlots de rats et les îlots humains. Ces résultats suggèrent que la PBM pourrait être envisagée comme une technique non invasive et relativement simple à mettre en œuvre, applicable pendant la période de culture des îlots humains (24-72h) pour les protéger du stress associé à la greffe. Ceci pourrait potentiellement réduire le nombre de greffes nécessaires par patient pour atteindre un contrôle glycémique satisfaisant.

## Abstract Thesis

**Title:** Impact of photobiomodulation on the survival and functionality of beta cells and pancreatic islets

**Introduction.** Despite optimal insulin treatment and appropriate therapeutic education, certain diabetic patients exhibit significant glycaemic variability, characteristic of unstable diabetes. Pancreatic islet transplantation is indicated in these cases. These islets were isolated from the pancreas of a deceased donor and cultured for 24-72h to enable recipient immune system preparation through immunosuppression. However, the viability of human islets diminishes after isolation, and around half of the islets are destroyed after transplantation due to various stresses (inflammation, substrate deprivation, hypoxia), necessitating multiple transplants (2 to 3) to achieve optimal glycaemic control. Photobiomodulation (PBM) involves the selective absorption of specific wavelengths by mitochondria, resulting in biological changes such as enhanced cell viability and reduced inflammation. While PBM has demonstrated positive effects in treating diabetes-related complications, such as ulcers, neuropathic pain, and glucose intolerance, its direct impact on beta cells and islets is relatively underexplored.

**Aims.** This study aimed to assess the effect of 670 nm, 2.8 mW/cm<sup>2</sup> PBM on beta cells (MIN6), rat islets, and human islets, and in vivo influence on streptozotocin-induced diabetic rat models transplanted with islets pre-conditioned with PBM.

**Results.** The results showed that exposure to PBM for 5s to 24h did not impair the viability or functionality of MIN6 cells or rat islets. Pre-conditioning MIN6 cells and rat islets with 24h of PBM before inflammatory stress (TNF- $\alpha$ , Interferon- $\gamma$ , IL-1 $\beta$ ) or substrate deprivation (glucose and serum) reduced the impact of these stresses on viability, glucose-responsive insulin secretion, and mitochondrial functions. This protective effect was also observed when PBM was concurrently applied with stress. However, no effect was observed under hypoxic stress conditions (1% O<sub>2</sub>), likely due to the weaker impact of this stress on beta cell mitochondrial function. In a model involving diabetic rats transplanted with PBM-preconditioned islets compared to non-preconditioned islets, metabolic parameters such as weight gain, glycaemia, C-peptide secretion, and glucose tolerance test were comparable, indicating that pre-conditioned islets did not compromise their long-term in vivo functionality. Moreover, application of PBM to human islets after isolation improved their viability and insulin secretion in response to glucose. Preconditioning of these human islets with PBM before inflammatory stress maintained their viability and functionality at levels similar to those in non-stressed islets.

**Discussion.** In summary, PBM applied to stress-free beta cells and rat islets does not affect their viability and functionality in vitro and in vivo. Isolation of human islets resulted in reduced viability compared to rat islets, primarily because of isolation process and transportation time (before isolation and after isolation to lab experiment). Thus, the application of PBM to isolated human islets significantly enhances their viability and functionality. Furthermore, pre-emptive PBM application before in vitro stress helps mitigate the impact of stress on MIN6 cells, rat islets, and human islets. These findings suggest that PBM could be considered as a non-invasive and relatively simple technique, applicable during the human islet culture period (24-72h) to protect them from the stress associated with transplantation. This could potentially reduce the number of transplants required per patient to achieve a satisfactory glycaemic control.