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# DISSERTATION

## PHOTOTHERAPY FOR THE TREATMENT OF WOUND INFECTIONS AND THE IMPROVEMENT OF WOUND HEALING

ausgeführt zum Zwecke der Erlangung des akademischen Grades einer Doktorin der technischen Wissenschaften unter der Leitung von

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Wien, Oktober 2020

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# ABSTRACT

1-2 % of all people suffer from chronic wounds once in a lifetime. Advanced age, chronic underlying diseases (e.g. diabetes mellitus, vascular diseases) and factors such as restricted mobility and infections are the most common causes of a disrupted healing process. The rapidly increasing number of antibiotic resistances and the associated lack of effective treatment strategies further aggravate the problem.

Biophysical therapeutic methods have gained popularity and trust in the past decades through numerous positive reports from preclinical and clinical studies. Enabling a combination of promoting wound healing and fighting infections, phototherapy plays a special role within this field. By irradiation with light, especially in the red and infrared range, photobiomodulation (PBM) stimulates many molecular mechanisms, which in turn stimulate processes relevant to wound healing, such as cell proliferation, cell migration and vascularization. However, by activating photoactive substances, so-called photosensitizers, light can also have a destructive effect on cells. Therefore, in addition to its use in tumor treatment, antimicrobial photodynamic therapy (aPDT) is more and more used for the fight against infections.

The first part of this work deals with the effects and the underlying mechanisms of PBM on wound-relevant cell lines, challenged by hypoxia/reoxygenation (H/R) and nutrient deprivation to simulate a wound environment. It was demonstrated that 10 min of pulsed red LED (light-emitting diode) light irradiation significantly increased the proliferation of fibroblasts and myoblasts. The effects were associated with elevated oxygen flux, ATP (adenosine triphosphate) levels and ROS (reactive oxygen species) concentrations.

To be able to test alternative therapeutic options for fighting infection under conditions as realistic as possible, the second part of this thesis was dedicated to the development of an infection wound model in mice that reflects the wound situation of immunocompromised patients. The precise dosing of immunosuppressant and polymicrobial, fecal suspension led to an established wound infection and wound healing was significantly impaired. The wounds were analyzed by the measurement of wound size, microbiological swabs and grading on a developed wound score scale.

The third part of this work focused on the extensive testing of aPDT. *In vitro,* bacteria were treated in suspension culture and in more wound-relevant assays on agar surface and in fibrin matrix. In a subsequent *in vivo* study, the prior established infection wound

model was used to test the effect of aPDT. *In vitro*, a strong dependency of aPDT on the treatment environment was shown and partially caused a markedly diminished efficacy. *In vivo,* a two-times application of aPDT showed significantly improved and faster wound healing compared to the untreated, infected wounds, graded by both, quantitative wound size and qualitative parameters based on clinical observation of wound parameters.

In summary, phototherapy and especially the use of red LED light showed a high potential in the stimulation of wound healing and in infection control. These results contribute to further knowledge important for the advancement of treatment protocols to reach the highest benefit for affected patients in the future.

# KURZFASSUNG

1-2 % der weltweiten Bevölkerung leiden zumindest einmal im Leben an einer chronischen Wunde. Die immer älter werdende Gesellschaft, chronische Grunderkrankungen (z.B. Diabetes mellitus, vaskuläre Erkrankungen) und Faktoren, wie eingeschränkte Mobilität und Infektionen, sind die häufigsten Ursachen für den gestörten Heilungsprozess. Die rasant ansteigende Zahl an Antibiotikaresistenzen und das damit verbundene Fehlen von wirksamen Behandlungsstrategien verstärkt die Problematik weiter.

Biophysikalische Behandlungsmethoden haben in den letzten Jahrzehnten durch zahlreiche positive Berichte aus präklinischen und klinischen Studien an Bekanntheit und Vertrauen gewonnen. Die Phototherapie sticht dabei besonders hervor, da sie eine gleichzeitige Förderung der Wundheilung und Bekämpfung von Infektionen ermöglicht. Photobiomodulation (PBM) erreicht durch die Bestrahlung mit Licht, vor allem im roten und infraroten Bereich, die Anregung vieler molekularer Mechanismen, die wiederum wundheilungsrelevante Prozesse, wie Zellproliferation, Zellmigration und Vaskularisierung stimulieren. Über die Aktivierung von photoaktiven Substanzen, sogenannten Photosensitizern, kann Licht jedoch auch eine destruktive Wirkung auf Zellen bewirken. Die antimikrobielle photodynamische Therapie (aPDT) wird daher neben ihrem Einsatz in der Tumorbehandlung auch immer mehr zur Bekämpfung von Infektionen herangezogen.

Der erste Teil dieser Arbeit beschäftigt sich mit der Wirkung und den zugrunde liegenden Mechanismen von PBM auf wundrelevante Zelllinien, denen zur Simulation der Wundumgebung sowohl Sauerstoff, als auch Nährstoffe entzogen wurden. Es wurde festgestellt, dass die Proliferation von Fibroblasten und Myoblasten durch 10-minütige Bestrahlung mit rotem LED (light-emitting diode)-Licht signifikant ansteigt. Dieser Effekt wurde mit erhöhtem Sauerstoffffluss, sowie erhöhten ATP (Adenosintriphosphat) und ROS (reactive oxygen species) Konzentrationen in Zusammenhang gebracht.

Um alternative Behandlungsmöglichkeiten zur Infektionsbekämpfung unter möglichst realistischen Bedingungen testen zu können, wurde im zweiten Teil dieser Arbeit ein Infektionswundmodell in Mäusen entwickelt, das die Wundsituation von immungeschwächten Patienten widerspiegeln soll. Die genaue Dosierung von Immunsuppressivum und polymikrobieller Fäkalsuspension führte zu einer etablierten Wundinfektion und einer signifikanten Beeinträchtigung der Wundheilung. Die Wunden

wurden durch Messung der Wundgröße, mikrobiologischer Abstriche und Einstufung auf einer entwickelten Wundbewertungsskala analysiert.

Der dritte Teil dieser Arbeit konzentrierte sich auf die ausführliche Testung der aPDT. *In vitro* wurden Bakterien in Suspensionskultur und in wundrelevanteren Assays auf Nähragar und in Fibrinmatrix behandelt. In einer anschließenden *in vivo* Studie wurde das zuvor etablierte Infektionswundmodell herangezogen, um die Wirkung von aPDT zu testen. Die *in vitro* Assays zeigten eine starke Abhängigkeit der aPDT von der Behandlungsumgebung, welche zum Teil zu einer deutlich verminderten Wirksamkeit führte. *In vivo* zeigte eine zweimalige Anwendung von aPDT eine signifikant verbesserte und schnellere Wundheilung im Vergleich zu unbehandelten, infizierten Wunden. Dies wurde sowohl durch die quantitative Analyse der Wundgröße, als auch anhand qualitativer Wundparameter bestätigt.

Insgesamt zeigte die Phototherapie und insbesondere die Verwendung von rotem LED-Licht ein hohes Potenzial im Einsatz zur Stimulierung der Wundheilung und Infektionskontrolle. Diese Ergebnisse tragen zum allgemeinen Wissen bei, das für die Weiterentwicklung von Behandlungsprotokollen benötigt wird, um in Zukunft den bestmöglichen Nutzen für betroffene Patienten zu erzielen.

# 1. Introductory Chapter

## 1.1. Background

### 1.1.1. Skin Anatomy and Wound Healing

Measured by the surface, skin is the largest organ in the human body. It shields from many dangers, like mechanical stress, radiation, microbial infection, and extreme temperatures. Healthy skin consists of three layers – epidermis, dermis and hypodermis (Figure 1). The *epidermis* forms an impermeable layer and is composed of keratinized, stratified, avascular epithelium. The most common cell type is the keratinocyte storing the proteins keratin and keratohyalin, which give the epidermis most of its properties. The underlying *dermis* anchors the epidermis and is rich in connective tissue, vasculature and mechanoreceptors. Connective tissue is composed of elastin and collagen, produced by fibroblasts and responsible for the elasticity and strength of the skin. This layer gives the skin immunity and provides nutrients and oxygen, also to the avascular structures. The *hypodermis* connects the dermis to the underlying fascia. It is composed of connective and adipose tissue functioning as energy storage and source of growth factors [1, 2].

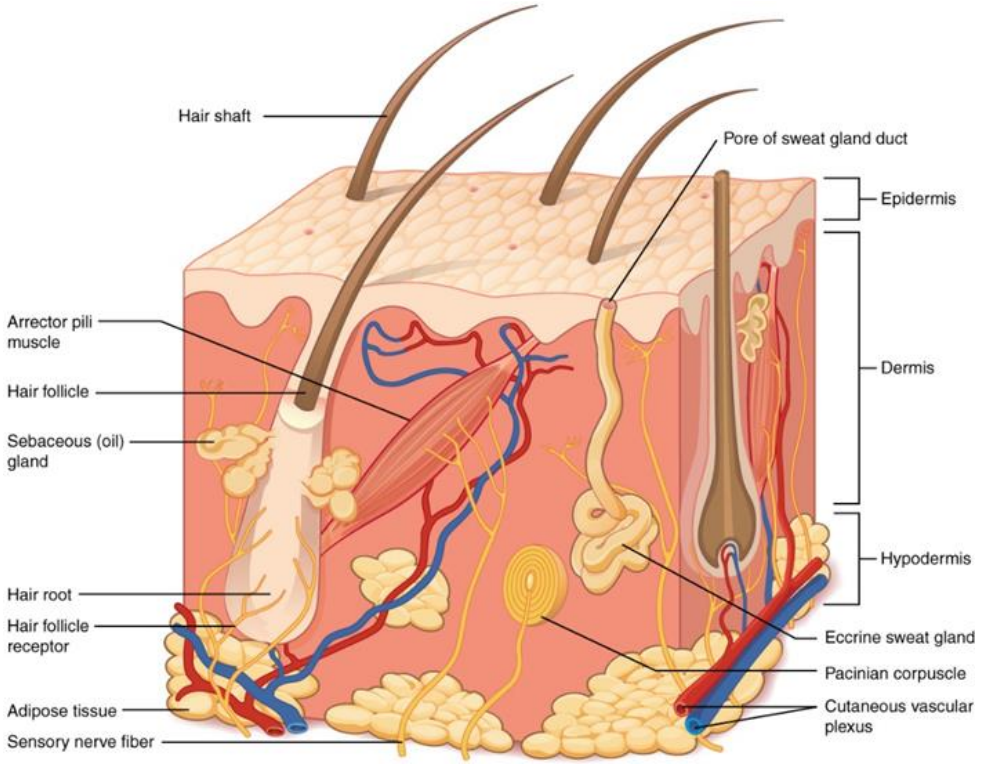


**Figure 1: Schematic illustration of the three skin layers – epidermis, dermis and hypodermis – and their components.** "Layers of Skin" by Lindsay M. Biga, Sierra Dawson, Amy Harwell, Robin Hopkins, Joel Kaufmann, Mike LeMaster, Philip Matern, Katie Morrison-Graham, Devon Quick & Jon Runyeon, from the book Anatomy & Physiology, chapter 5.1., licensed under a Creative Commons Attribution-ShareAlike 4.0 International License.

If the skin is injured, the rapid repair is dependent on a complex synchronization of various cell types and factors within and between the three different layers of the skin. The repair follows a four-staged model undergoing the phases homeostasis, inflammation, proliferation and remodeling (Figure 2).

As a first response *homeostasis* aims to control the bleeding by vasoconstriction and the formation of the thrombus, which serves as a provisional scaffold and releases important growth factors for the subsequent processes. The thrombus is formed by two consecutive processes, the platelet aggregation and formation of the platelet plug (primary hemostasis) and the coagulation cascade resulting in a fibrin mesh (secondary hemostasis). This whole process is elicited by the damage of the endothelium [3].

In the *inflammation* phase immune cells infiltrate the wound area and initiate debris clearance and pathogen control in an orchestrated process. As a first line defense against infections, neutrophils release an oxidative burst, produce toxic granules, perform phagocytosis and form neutrophil extracellular traps (NETs) [4, 5]. Within 48-96 hours

tissue-activated macrophages are transformed out of monocytes and replace the majority of neutrophils [6]. Early pro-inflammatory macrophages not only phagocyte pathogens and expended neutrophils, but also digest extracellular matrix and the thrombus to enable their migration. Later appearing, anti-inflammatory macrophages contribute to vessel formation and ECM deposition [2]. Further on, Langerhans cells, dermal dendritic cells and T cells are activated to support the clearance of autologous and foreign antigens. Dendritic epithelial T cells (DETCs) also promote keratinocyte proliferation [7, 8] and take over memory function [9].

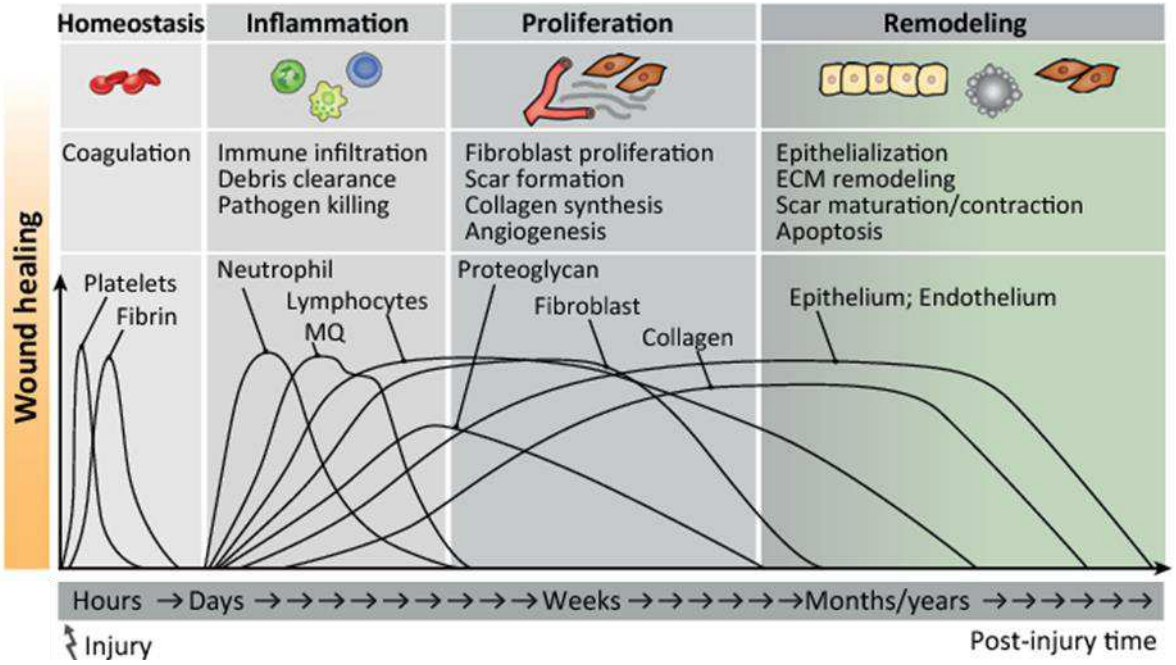


**Figure 2: Summary of the processes and components involved in the classical four-stage wound healing model.** Adapted from ref. [10] with permission from Elsevier.

Angiogenesis, collagen deposition, wound contraction and finally, the formation of granulation tissue are concurrently taking place in the *proliferation* phase. This phase starts by fibroblasts entering the wound site and overlaps with the inflammation phase. During angiogenesis, endothelial cells proliferate, migrate to the wound site and branch to form new blood vessels. These are needed for all the processes to happen, which demand huge amounts of nutrients and oxygen. At the same time, also fibroblasts proliferate and invade the clot to produce collagen matrix (mainly collagen III and fibronectin). Some of the fibroblasts differentiate into myofibroblasts, which contract the wound edges. The collagen matrix together with the myofibroblasts strengthen the wound

and combined with new blood cells, inflammatory cells, endothelial cells, myofibroblasts and other ECM components forms the granulation tissue [11, 12].

The last phase, *remodeling*, is discussed to begin as soon as collagen formation and degradation balance out and can last for even years [13]. During this phase, most of the macrophages, endothelial cells and myofibroblasts undergo apoptosis or leave the wound site and all the processes finally slow down [2]. The wound bed is re-epithelialized, and the granulation tissue is substituted by scar tissue. Keratinocytes at the wound edge differentiate and migrate from the basal lamina of the epidermis to the wound site to fill the gap [14], while basal keratinocytes proliferate to ensure replenishment [15, 16]. Collagen type III is replaced by collagen I and the disorganized fibers are rearranged, cross-linked and aligned along tension lines. This makes the wound stronger and reduces scar thickness [17]. The epidermis of the scar differs from uninjured skin and will only reach a maximum tensile strength of about 80 % [18, 19].

### 1.1.2. Challenges in Wound Healing

Wounds can be initiated by physical (pressure, burns, radiation), electrical, chemical, or immunological injury to the tissue, or by a break in the epithelial lining of the skin (e.g. surgery). While the majority of all these wounds heals without complications, 1-2 % of all people undergo chronic wound healing during their lifetime [20]. Per definition, a chronic wound does not proceed through the normal course of wound repair within three months [21]. Systemic (diabetes mellitus, vascular diseases, obesity and malnutrition, malignancies and advanced age [22]), as well as local factors (infection, pressure) can negatively influence a proper wound healing process and lead to the three main types of chronic wounds: venous and arterial ulcers, diabetic ulcers and pressure ulcers [23].

One prominent characteristic of chronic wounds is ischemia caused by diabetic microangiopathy, peripheral arterial disease, chronic venous insufficiency and chronic fibrosis. Recurring ischemia-reperfusion cycles in the wound area lead to high levels of inflammatory cytokines and neutrophil infiltration associated with reactive oxygen species (ROS), destructive enzymes and downregulated nitric oxide (NO) levels. These, in turn, fuel the inflammatory state and the wounds are finally locked in a prolonged, perpetuated inflammatory cycle. Repeated cycles of ischemia and reperfusion potentiate the pathogenicity of the cellular events and result in tissue necrosis [23, 24].

Furthermore, the defect epithelial layer, together with the moist, nutrient rich environment and a dysregulated inflammatory response constitutes a perfect environment for microbial colonialization [25]. As a defense mechanism, the human body reacts with inflammation, the release of proteases and oxidants. These agents attack the microorganisms, but also degrade cytokines and extracellular matrix (ECM) [26] and further worsen the wound situation besides ischemia-reperfusion cycles.

Depending on the wound, the patient's immune system and the species of bacteria, the number of bacteria necessary to impair wound healing, varies. As a rule of thumb, $10^5$ organisms/g tissue for closed wounds and $10^6$ organisms/g tissue for open wounds are considered critical [27]. Additionally, formation of biofilms was confirmed in 60-90 % of all chronic wounds. This three-dimensional, bacteria-produced ECM provides an extra barrier to antimicrobial treatment [28].

Besides the higher tolerance and poor response of the microorganisms towards antimicrobials caused by the biofilm matrix, the ischemic wound situation might also reduce the concentration of systemic antibiotics reaching the wound area. This, in turn, might contribute to the development of antibiotic resistances, which add to the complicated treatment of infected chronic wounds [29]. During the last decades, sequencing methods identified, that the microbial diversity in chronic wounds is huge and that the vast majority of all strains are strict or facultative anaerobes, that were not detected by standard culturing techniques [30]. These polymicrobial communities were found to build synergies that increase their virulence [31, 32]. Especially, ESKAPE pathogens, a group of six pathogens (***E****nterococcus faecium,* ***S****taphlococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa, and* ***E****nterobacter spp.*) which currently show an increase in multidrug resistances and virulence, are responsible for a large number of nosocomial infections [33].

These complex, multifactorial wound situations often exceed the potential of conventional treatment approaches, in particular in consideration of the emerging problem with multidrug resistances. The research community targets various approaches including phage therapy, growth factors, engineered skin, nano-based technologies, stem cell research and biophysical treatments [22, 34].

In both *in vitro* and *in vivo* studies biophysical treatments, like ultrasound therapy, extracorporeal shock wave therapy, negative pressure wound therapy and electrical field stimulation therapy show convincing evidence to have a positive impact on the healing of

chronic wounds [35]. However, only phototherapy, employing the effects of photobiomodulation (PBM) and antimicrobial photodynamic therapy (aPDT), enables the combination of wound healing stimulation and reduction of bioburden and will be introduced in the next two chapters.

### 1.1.3. Photobiomodulation (PBM)

The application of light for therapeutic purposes dates way back to the history of the ancient Egyptians, Indians and Chinese [36]. Niels Finsen demonstrated the benefits of blue and red light in the treatment of Lupus vulgaris. This work earned him the Nobel Prize in 1903 [37]. With the development of laser (light amplification by stimulated emission of radiation) technology starting in the 1960s and the further advancement in the following decades, light therapy was revived for medical applications. Multiple research groups around the world started to study the therapeutic effects of PBM [38].

Since the 1990s, when light emitting diodes (LEDs) were introduced, they became the second most used light source for PBM besides lasers. Several properties of lasers were thought to be necessary for successful PBM therapy and to be superior to LED sources (Table 1). Especially, the coherence of lasers was meant to be the important parameter for the photobiological effect [39, 40]. Even though this topic is still controversially discussed, Heiskanen and Hamblin gathered impressive evidence that LED is equally able to stimulate the “photobiological phenomenon” as laser does [41].

**Table 1: Differences between LED and LASER relevant for PBM. Information gathered from ref. [41].**

| ***Parameters*** | **LED** | **LASER** |
|---|---|---|
| *Working principle* | Electroluminescence | Stimulated emission |
| *Bandwidth* | 1-2 nm | down to fractions of nm |
| *Nature of emitted light* | Noncoherent, nonpolarized | Coherent, polarized |
| *Costs and applications* | Low cost | High cost |
| *Irradiation area* | large arrays possible | small spot sizes |

The processes of tissue-light interaction are divided into productive (absorption and scattering) and nonproductive (reflection and transmission). These interactions are

dependent on physical variables like wavelength, irradiance, time and distance of the application, which in turn determine the therapeutic efficacy [42].

PBM exploits the combination of photochemical reactions in cells that follows such tissue-light interactions. For this to happen a chromophore needs to absorb photons, which provide the energy for electrons to become excited to a higher energy level. This energy can then be used for cellular functions. While on one hand, the desired cellular chromophore needs to receive enough energy to trigger a phototherapeutic effect, a loss in energy by other chromophores within the tissue absorbing in the same wavelength range needs to be considered. In human skin the most abundant chromophores are melanin and hemoglobin (oxygenated and deoxygenated; Figure 3). Considering the absorption spectra of these chromophores, a window of wavelengths between 600-1400 nm can be defined, where light can penetrate into the skin to reach the desired chromophores and induce a therapeutic effect. While blue light is mainly absorbed on the surface by melanin in the epidermis and hemoglobin in the dermis, red and especially near-infrared (NIR) light is able to penetrate deeper layers [38, 43].

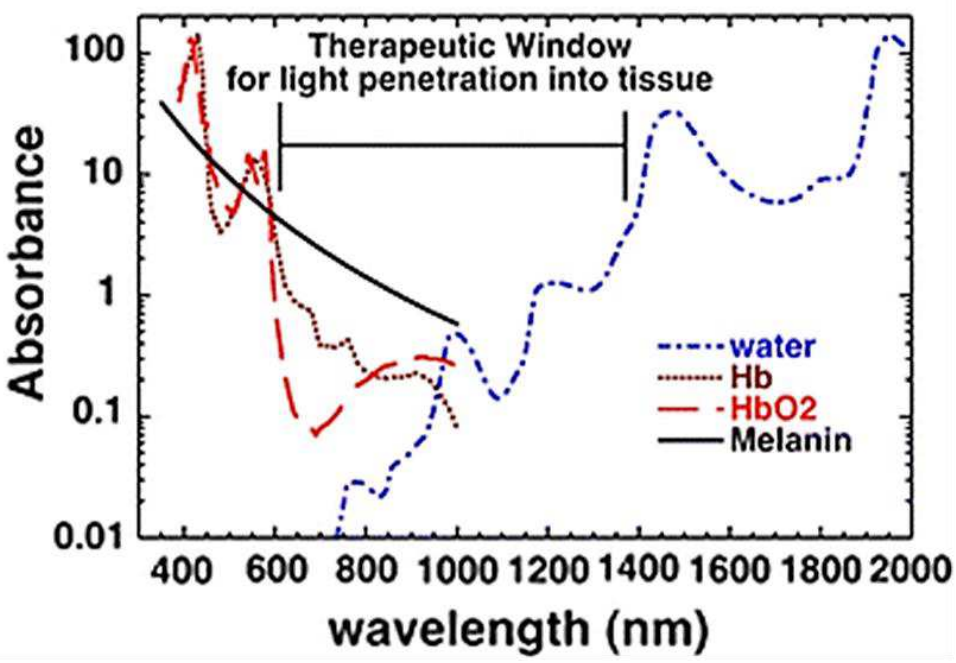


**Figure 3: Position of the optical therapeutic window due to a lower absorption and scattering by abundant chromophores in the human body.** Adapted from ref. [38] with permission from Springer Nature.

In the past decades several molecular mechanisms induced by PBM were explored. The most well-studied is located within the mitochondria and influences an enzyme of the electron transport chain (ETC). The ETC is a series of five complexes within the inner

membrane of mitochondria, that transport electrons in a defined series of redox reactions. This electron transport is coupled to a proton transfer and results in an electrochemical proton gradient that is used to produce adenosine triphosphate (ATP). As the final electron acceptor, oxygen is converted to water. Matching the action spectrum red and NIR light within the therapeutic window, the transmembrane complex IV, also termed cytochrome c oxidase (CCO), is the affected chromophore [44]. Many results of *in vitro* and *in vivo* experiments, like increased adenosine triphosphate (ATP) [45-47] and NO [48-50] levels, gave evidences for this conclusion. An elevated electron transport also means a higher turnover of oxygen and production of ROS, as a natural by-product of the oxygen metabolism. For decades the deleterious effects of ROS and reactive nitrogen species (RNS, various oxidants derived from NO) were in the focus of research. However, nowadays it is known that lower levels of these reactive species induce transcriptional changes by activating several transcription factors and are therefore involved in various signaling pathways [51]. Taken together, these alterations based on photochemical activation lead to changes on a cellular level. the three following mediators are exemplary for the activation by light and were shown to have a positive impact on wound healing.

Arany et al. demonstrated the dose-dependent induction of ROS by laser irradiation, which was able to activate latent transforming growth factor-β1 (TGF-β1) *in vitro* and *in vivo* [52, 53]. As TGF-β is involved in all phases of the wound healing process by affecting endothelial cells, monocytes, fibroblasts and keratinocytes, its influence is of great interest in the research community. Its broad functional impact in wound healing mechanisms like proliferation, migration and the formation of ECM is summarized by Faler et al. [54].

Furthermore, the modulation of the immune response by the downregulation of nuclear factor-κB (NF-κB) after irradiation with NIR laser light has also been shown. Gupta et al. demonstrated faster wound healing as well as reduction of the inflammatory response in the early wound healing process in rats [55], and Rizzi et al. showed an inhibited ROS release followed by a blocked NF- κB activation in traumatized muscle [56]. Hamblin even speculated that PBM might be able to benefit the highly fragile balance of the pro- and anti-inflammatory response during wound healing based on its effects on resolvins and protectins [57].

It is shown that NO is not only released from CCO by irradiation with red and NIR light [58], but CCO was also found to have nitrite reductase function [59]. The release of NO

by irradiation with NIR was found to protect cardiomyocytes from hypoxia and reoxygenation [48]. As *in vivo* studies with inducible nitric oxide synthase (iNOS) knockout mice [60] and the use of NOS inhibitors in rats showed a delay in wound healing [61], PBM in the NIR range releasing NO might be another pathway worth further investigation.

Recent studies suggested additional chromophores like light-gated ion channels [62], opsins [63], water [64, 65] and cryptochromes [66], which have been investigated for their role in PBM effects. Until now, evidence is rare, and the shown effects are mainly based on irradiation with blue and green light, or in the case of water in the infrared region from 1000-10.000 nm. Further research might elucidate the complex context of their role in wound and tissue regeneration or immunomodulation.

### 1.1.4. Antimicrobial Photodynamic Therapy (aPDT)

After an incidental discovery of the “photodynamic phenomenon” by Albert von Tappeiner and his student Oscar Raab [67], von Tappeiner and co-researchers already successfully applied this treatment to skin carcinomas [68] and bacterial inactivation in the early 1900s [69]. Nowadays, photodynamic therapy is well implemented in the clinical treatment of actinic keratosis and basal cell carcinoma, while the antimicrobial use is still intensively investigated as a possible alternative for antibiotic resistant infections.

aPDT requires the interaction of three components, a non-toxic molecule called photosensitizer (PS), visible or infrared light that fits the excitation spectrum of the specific PS and molecular oxygen [70]. The PS absorbs a photon and is elevated to the excited singlet state. There, the PS can lose energy by fluorescence and/or heat and return to the ground state, or it can be shifted to the longer-living triplet state by a transition called inter-system crossing (ISC). From the triplet state, the PS can again return to the ground state emitting phosphorescence or transfer its energy or charge to oxygen molecules (triplet ground state) to generate ROS. Type I mechanism, the transfer of charge, leads to the generation of oxygen radicals, like superoxide ($O_2^{-\bullet}$, hydrogen peroxide ($H_2O_2$) and free hydroxyl radicals (HO•). By the type II mechanism, the transfer of energy, singlet oxygen ($^1O_2$) is generated [70, 71] (Figure 4). After energy or charge was transferred, the PS molecule is shifted back to the ground state and can pass through the whole cycle over and over again, generating thousands of ROS [72]. Depending on the PS, the ratio

of type I and II mechanism is different and is defined by the singlet oxygen quantum yield $\Phi_{\Delta}$ [73].

Regarding resistance development, a major advantage of aPDT over antibiotics is the variety of biomolecules that are attacked by the triggered oxygen bursts, like proteins, lipids and nucleic acids [74, 75]. As ROS have a very short lifetime (e.g. $^1O_2$ in water 4 µs [76]), the photoactivation needs to take place in close proximity of the cells. Depending on the chemical structure and concentration of the PS and the structure of the cell wall, the PS can be localized around, or inside the cells (by diffusion or active transport), or bound to the cellular membrane [77]. The main targets of aPDT are cytoplasmic membranes and cell walls leading to leakage of cellular content, or destruction of membrane transport and enzymes, as well as DNA breakage [78, 79].

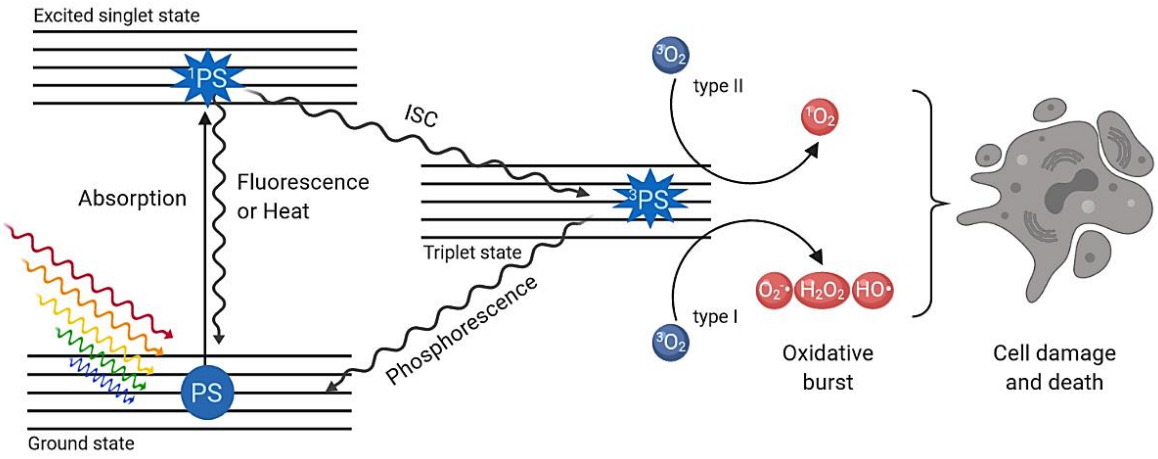


**Figure 4: Mechanism of the photoreaction taking place during photodynamic therapy.** $^1$PS: photosensitizer in the excited singlet state; $^3$PS: photosensitizer in the triplet excited state; ISC: intersystem crossing; $^3O_2$: ground state oxygen; $^1O_2$: singlet oxygen; $O_2^{-\bullet}$: superoxide anion; HO• hydroxyl radical; $H_2O_2$: hydrogen peroxide; created with BioRender.com

Therefore, the difference of the cell walls of Gram-positive and Gram-negative bacteria is reflected in their susceptibility towards aPDT. The cell wall of Gram-positive bacteria consists of a thick peptidoglycan layer, which is permeable to molecules between 30-57 kDa. The Gram-negative cell wall is equipped with a second phospholipid membrane layer on the outside consisting of strongly negatively charged lipoproteins and lipopolysaccharides (LPS) [80]. Negatively charged and neutral PS that are effective against Gram-positive bacteria, show no antimicrobial activity in Gram-negative bacteria. The use of additives that alter the outer membrane of Gram-negative bacteria [81, 82],

as well as the use of positively charged PS like phenothiazines, porphyrins and phthalocyanines [83, 84], are strategies found to overcome the differences and prevent selective treatment.

There is a lot of research dedicated to the development and advancement of PS, as they are key factors deciding upon the success of aPDT. Cieplik et al. [85] and Maisch et al. [86] defined criteria for PS to reach the highest possible antimicrobial activity:

- Hydrophilic, positively charged molecules to assure good binding to negatively charged cell walls [87]
- Small molecules to reach a higher penetration in biofilms [88]
- Low binding affinity and a low toxicity for mammalian cells [89]
- No toxicity before irradiation (dark toxicity) and mutagenicity [89]
- High photostability during irradiation [90]
- High singlet oxygen quantum yield [91]

PS used in aPDT are grouped into the following classes: phenothiazinium derivatives, porphyrin, chlorin and phthalocyanine derivatives, xanthene derivatives, fullerene derivatives, phenalenone derivatives, riboflavin derivatives and curcumin derivatives. Methylene blue, used for the studies of this thesis, belongs to the phenothiazinium derivatives and carries one positive charge, making it suitable for the treatment against Gram-positive and Gram-negative bacteria. Another advantage is that it strongly absorbs in the region of 600 – 680 nm with a peak around 665 nm in the red spectrum which lies within the beforementioned therapeutic window and therefore allows deeper tissue penetration. The singlet oxygen quantum yield is around 0.5 in phosphate buffered saline and therefore type I and II mechanisms are quite balanced [92, 93].

As already mentioned for photobiomodulation, also in aPDT lasers and LEDs represent the most frequently used light sources. Depending on the area of application, also gas-discharge lamps are used [94]. However, the key parameters for choosing a suitable light source are (1) matching of the excitation spectrum of the used PS, (2) light intensity and (3) the required mode of delivery [72].

While topical antibiotics treatment became outdated due the rapid development of resistances [95], aPDT is predestined for the topical use in localized infections [96]. Preventing the commensal gastrointestinal flora damage and systemic toxicity, it provides

great advantage over antibiotic treatment [97]. This, together with its low toxicity towards host tissue, while providing a pronounced antimicrobial effect [98, 99], makes it competitive with topical antiseptics like iodine. However, one important advantage of aPDT over topical antiseptics is its capability to reduce the expression of virulence factors produced by the microorganisms [100, 101]. Despite aPDT was clinically approved only for the use in the field of dentistry, several clinical studies show its great potential also in skin wound healing:

In a randomized, placebo-controlled clinical study, aPDT using the phenothiazinium derivative PPA904 as a PS was applied for the treatment of leg ulcers and diabetic foot lesions [102]. Weekly repeated aPDT treatments for a total period of 12 weeks was shown to significantly reduce the bacterial load in the wounds over a wide spectrum of species compared to the control group (placebo + light). Additionally, the authors reported a pronounced trend towards fastened wound healing, no habituation effect during 12 weeks of treatment, and that aPDT was safe and well-tolerated.

Tardivo et al. performed a randomized clinical study using aPDT to treat diabetic foot ulcers [103]. They used a 1:1 mixture of methylene blue and toluidine blue O as a PS and a halogen as well as an LED lamp for irradiation. Compared to a control group, which was treated with antibiotics and the conventional treatment procedure, the amputation rate in the aPDT treated group was only 2.9 % (1 out of 18 compared to 16 out of 16 in the control group).

Also, the use of δ-aminolevulinic acid (ALA) as PS, which is in general mainly used in anti-tumor PDT, was tested as a treatment against chronic skin ulcers infected with *Pseudomonas aeruginosa* in a clinical study. The patients were treated with aPDT or red light only as a control once a week for two weeks. Lei et al. found that the bacterial count was significantly reduced in the aPDT group 24 hours post treatment and 7 days post treatment also the ulcer area was significantly decreased [104].

### 1.1.5. Aims

This doctoral thesis aimed to investigate phototherapy as an alternative treatment option against wound infections and to improve wound healing. With regard to the publications compiled in this dissertation the following three aims were defined:

i. Evaluation of the impact of photobiomodulation on wound healing-relevant cell types that are subjected to hypoxia/reoxygenation challenge, mimicking certain aspects of ischemic situation in wounds.

ii. Development of a close-to-reality infection wound model in immunocompromised BALB/c mice for the testing of alternative therapy approaches, like aPDT.

iii. Evaluation of aPDT *in vitro* for its bactericidal effects in suspension culture, as well as in wound-relevant assays and subsequent testing of aPDT *in vivo* as a potential treatment option for wounds infected with a polymicrobial fecal suspension under immunocompromised conditions.

## 1.2. Methodology

### 1.2.1. Electron Paramagnetic Resonance (EPR) for the Measurement of Short-lived Free Radicals

Electron paramagnetic resonance is a spectroscopic method based on magnetic resonance and is the only method to directly detect species with unpaired electrons (e.g. free radicals and some transition metal ions). In an external magnetic field, the spin magnetic moment of electrons can have two different energy states: an antiparallel orientation relative to the magnetic field correlates with a lower, and a parallel orientation with a higher energy state. The energy difference between those states is dependent on the strength of the external magnetic field and the electrons' g-factor. In EPR spectroscopy, a sample in an external magnetic field is irradiated with an electromagnetic wave (microwave). If the frequency of this wave matches the energy difference between the states of parallel and antiparallel oriented spins, the energy is absorbed and the spin flips to the higher energy state (resonance). Usually, the frequency of the electromagnetic wave is kept constant, while the strength of the magnetic field is varied until resonance is reached. The absorption signal is detected by a change in resistance and the g-factor of the electron can be calculated from the values of the frequency and the strength of the magnetic field. The g-factor contains the chemical information about the interaction of the electron and the electronic structure of the molecule, constitutes a "fingerprint" and gives information about the species analyzed. It is related to the electronic environment and anisotropy. The amount of absorbed energy (peak height) correlates with the respective concentration [105].

To be able to measure short-lived free radicals, so-called spin traps are used to form stable, covalent adducts that can be analyzed by EPR. The most frequently used spin traps are alpha-phenyl-*N*-tertiary-butyl nitrone (PBN) and 5,5-dimethyl-pyrroline *N*-oxide (DMPO) and 3,5-dibromo-4-nitrosobenzenesulfonic acid (DBNBS) [106]. However, the reaction times of spin probes with many free radicals are slow compared with cellular antioxidants. Therefore, hydroxylamine spin probes, like 1-hydroxy-4-phosphono-oxy-2,2,6,6-tetramethylpiperidine (PPH), that undergo one-electron oxidations with radicals, are preferred. The transformation of the spin probe by reaction with different ROS yields essentially the same nitroxide which can be followed by EPR. However, no differentiation among different ROS is possible, unless specific inhibitors show the inhibition of the EPR signal [107].

### 1.2.2. Respirometry (OROBOROS) for the Detection of Basal Mitochondrial Respiration

A high-resolution oxygraph (e.g. OROBOROS instruments, Innsbruck) can be used to study cellular respiration and mitochondrial function with high accuracy, resolution and sensitivity. The OROBOROS-2k oxygraph consists of two closed chambers that are equipped with polarographic oxygen sensors. These sensors measure dissolved oxygen, which diffuses across a membrane at a rate that is proportional to the pressure of oxygen in the medium. The oxygen is reduced at the cathode, which produces electric current proportional to the oxygen concentration. In this thesis, ROUTINE-respiration of non-permeabilized cells was measured giving information about basal mitochondrial respiration.

### 1.2.3. BrdU Proliferation Assay

ELISA is a method to quantify specific antigens or antibodies in a sample. In this thesis, the antigen 5-bromo-2'-deoxy-uridine (BrdU) was measured in an indirect ELISA procedure using two antibodies. BrdU labelling solution is added to the cultivation medium of the cells for a specific time period and is incorporated into the DNA instead of thymidine during cell proliferation. To detect the incorporated BrdU, the cells are fixed, permeabilized and the DNA is hydrolyzed to make the BrdU accessible to the primary antibody (Ab). After extensive washing to remove unbound primary Ab, a secondary Ab is added, which is specific for the primary Ab and bound to a peroxidase (POD). POD is an enzyme that oxidizes the classical substrate 3,3',5,5'-tetramethylbenzidine (TMB) causing a color reaction from colorless to blue that can be detected with a plate reader. The intensity of the blue color is proportional to the amount of incorporated BrdU and consequently a measure for the cell proliferation rate in the sample.

### 1.2.4. 3rd Generation Sequencing for Microbiological Profiling

The analysis of the order of nucleotides in the DNA is called DNA sequencing. After next-generation sequencing (NGS, 2nd generation), now nanopores are used for sequencing, providing the advantage of analyzing single molecules of DNA and creating ultra-long reads. By this, the amplification and chemical labelling steps are no longer needed, which

makes it a low-cost, rapid method. Before the analysis, DNA is extracted and mixed with processive enzymes, that control the speed of the single DNA strands passing through the nanopore and being sequenced, to form a complex. Nanopore proteins are placed in an electrically resistant membrane and an electric field makes electrolyte ions move through the pore creating an ionic current signal. If a biomolecule is moving through the nanopore, it blocks the flow of the electrolyte ions and the current signal is changed. Depending on the nucleotide, the duration of the change and the amplitude are different and thereby make it possible to identify single bases of the DNA. The translation of the current signals to nucleotide bases is called base calling [108].

### 1.2.5. Autofluorescence and Bioluminescence Imaging of Bacteria

When photons are absorbed by a substance, the electrons are lifted to a higher energy level, the so-called excited singlet state. If this energy is emitted in the form of light, this light is called fluorescence. In most cases, the absorbed light has a longer wavelength than the emitted, which is known as the Stokes shift. Bacteria contain endogenous chromophores, e.g. porphyrins, which absorb light in the violet range. This characteristic is utilized to detect bacteria in wounds. As these chromophores are inherent to bacteria and not externally added, this form of fluorescence is called autofluorescence. After the excitation of their porphyrins, bacteria emit wavelengths in the red range (600-670 nm), which can be filtered from the autofluorescence of the wound tissue and the reflected light from the light used for excitation. These signals have been successfully used for the detection of bacteria and the prediction of infections *in vitro* and *in vivo* [109].

In contrast, bioluminescence is based on chemical processes within the cells that produce energy released in the form of light. The bioluminescence of the bacteria used in the present thesis was not inherent, but induced by a plasmid under the selective pressure of kanamycin. By culturing these bacteria on selective medium containing kanamycin, they are forced to preserve the plasmid for the expression of the kanamycin resistance gene. As a side effect, the genes for the luciferin/luciferase reaction (lux operon), which are encoded downstream of a constitutively active promoter, are also expressed. Thereby the bacterium produces the substrate luciferin and the enzyme luciferase. In the presence of oxygen and ATP, luciferase is able to oxidize luciferin and set free energy in the form of light at a wavelength of 557 nm [110]. This emission gives spatiotemporal information about bacteria *in vitro* and *in vivo*.

### 1.2.6. Flow Cytometry for the Quantification of Bacteria

The principle of flow cytometry is based on the emission of optical signals by cells when they pass a laser beam. By the combination of light scattering signals and signals from fluorophore-tagged antibodies, or dyes, cells can be counted or identified by their size (forward scattered light, FSC), granularity (side scattered light, SSC), surface antigens or differentiation markers. Focused by a laminar flow through a sheath fluid, the cells enter a measurement chamber one after the other and pass the laser beam. The resulting scattered light or fluorescence signal passes filtersets and is captured by photomultipliers, evaluated by a detector. This results in quantitative information about each cell analyzed. In this thesis, a fecal suspension was stained with a nucleic acid stain, that is able to penetrate Gram-negative and Gram-positive bacteria and to produce a green-fluorescence signal at around 500 nm. The fluorescence signal enabled the counting of bacteria within the suspension of microorganisms.

## 1.3. Summary of Articles and Description of Contributions

### 1.3.1. *In vitro* effects of 635 nm photobiomodulation under hypoxia/reoxygenation culture conditions

Especially older people often suffer from chronic wounds, the majority of which occur due to existing underlying diseases such as diabetes mellitus, or as a result of reduced mobility (e.g. pressure ulcers). Photobiomodulation in the red and near infrared range was repeatedly shown to positively influence wound healing in such cases. In this study, the underlying cellular changes after pulsed red LED light illumination were investigated in wound relevant cell lines challenged by hypoxia/reoxygenation (H/R) and nutrient deprivation.

Mouse fibroblasts and myoblasts were cultured in an oxygen deprived starvation medium for 3h, before they were treated with pulsed red LED light at 635 nm for 10 min yielding an overall delivered energy of 24 J/cm². Thereafter, the cells were analyzed regarding their viability, proliferation, mitochondrial respiration, ROS and ATP production.

Myoblasts were found to be less sensitive towards photobiomodulation than fibroblasts, both under normoxic and hypoxic conditions. An enhanced oxygen flux, increased ROS concentrations and rescued ATP levels were found in both cell types. These molecular changes were accompanied by increased cellular proliferation, which was again more pronounced in fibroblasts.

These results suggest that PBM using red LED light significantly effects the proliferation of cells challenged with H/R and nutrient deprived conditions found in ischemic wounds. These findings provide evidence for further expansion of the clinical use of PBM and adds a piece of basic knowledge to the various positive reports from the clinical practice.

#### 1.3.1.1. Detailed Description of Contribution

Sidrah Chaudary and Peter Dungel formulated the hypothesis and designed the experiments. Sidrah Chaudary, Lisa Karner, Barbara Meixner and Stefan Rieger performed the experiments. Sidrah Chaudary and Lisa Karner analyzed the data, performed the statistical analysis, designed figures and wrote the manuscript. Adelheid Weidinger supported with the EPR and OROBOROS measurements and provided critical input to the manuscript. Peter Dungel supervised the work and provided input throughout the process.

### 1.3.2. Contamination of wounds with fecal bacteria in immune-suppressed mice

Especially, due to the increasing occurrence of multi-resistant germs, bacterially infected wounds challenge not only responsible physicians, but also the medical research community. The infection of wounds with mixed cultures is known to increase the persistency and virulence of the pathogens and makes their control explicitly complex. Therefore, this study aimed at the establishment of a mouse model infected with a polymicrobial, fecal bacterial suspension, which reflects a wound situation in immunocompromised patients.

In immunosuppressed, female BALB-c mice, a dorsal median, circular incision wound was created and infected with a polymicrobial suspension. Wounds were covered with hydrogel and dressed with a 3-layered bandage consisting of Suprasorb® F, Hypafix® and Leukoplast®. At given time points, the wounds were analyzed by 3D imaging, microbiological swab analysis, autofluorescence imaging and a wound scoring system.

A careful adjustment of the dose of immunosuppressant and polymicrobial load for infection was found to be critical for reproducible establishment of infection, enabling long-term observations. Infection significantly delayed wound healing leading to larger and slower closing wounds. A newly established wound score, assessing the severity of the wound and the establishment of the infection based on clinical monitoring, confirmed the infection-induced delay in wound healing.

The model presented in this study and the evaluation of appropriate analyses provide a suitable tool to investigate complex microbiological interactions and evaluate new therapy approaches.

#### 1.3.2.1. Detailed Description of Contribution

Lisa Karner and Peter Dungel established the hypothesis and study design. Lisa Karner and Magdalena Metzger supported with the animal interventions and performed the majority of the data analyses. Susanne Drechsler performed the surgeries, supervised animal related concerns and provided critical input to the project and the manuscript. Johannes Zipperle guided the flow cytometry measurements. Guadelupe Pinar and Katja Sterflinger-Gleixner performed the 3rd generation sequencing and supported the associated data analysis. Friedrich Leisch gave input and helped with the statistical analysis of the data. Paul Slezak provided input concerning the surgeries and wound analysis. Marcin Osuchowski co-advised the project. Lisa Karner performed the statistical

analysis, designed figures and wrote the manuscript. Peter Dungel was the lead advisor of the project and provided critical input to the manuscript.

### 1.3.3. Antimicrobial photodynamic therapy fighting polymicrobial infections – a journey from *in vitro* to *in vivo*

ESKAPE pathogens frequently occur in nosocomial infections and show increasing numbers of resistances against antibiotics. The speed of the establishment of such resistances demands new therapies for the treatment of wound infections. Antimicrobial photodynamic therapy is an innovative, biophysical approach that is already widely used in cancer therapy and is now investigated to remedy the problem of infection control. In this publication, aPDT was tested for its efficacy as a treatment option for bacterial infections in both *in vitro* and *in vivo* models.

Gram-positive and Gram-negative single strains, as well as a fecal bacterial suspension were treated using the photosensitizer methylene blue and 10 min of pulsed red LED light in PBS, DMEM and LB medium suspension. In order to more closely mimic wound bed conditions, aPDT was further challenged in solid matrix assays on LB agar surface and in fibrin matrix. Finally, the therapeutic approach was used for the treatment of infected excision wounds in immunocompromised BALB/c mice.

The *in vitro* assays clearly showed a strong dependency of the aPDT efficacy on the surrounding environment during the phototoxic reaction. Especially, on and in solid matrices the effect of aPDT was markedly diminished. *In vivo*, the delayed wound healing caused by infection with the polymicrobial feces suspension, was significantly improved by a two-times application of aPDT.

The results demonstrate a discrepancy regarding the efficacy of aPDT on solid surfaces and clearly demand the further development of close-to-reality *in vitro* assays. Nevertheless, the *in vivo* application showed an impressive impact on wound healing in complicated, infected wounds and the results give an impulse towards its further testing in preclinical and clinical studies.

#### 1.3.3.1. Detailed Description of Contribution

Lisa Karner and Peter Dungel formulated the hypothesis and performed the study design. Lisa Karner and Magdalena Metzger conducted the majority of the experiments and data

analyses and supported with the animal interventions. Barbara Schädl and Carina Wagner helped with the experimental work. Susanne Drechsler performed the surgeries, supervised animal related concerns and provided critical input to the project and the manuscript. Paul Slezak provided input concerning the surgeries and wound analysis. Ara Hacobian guided bacteria related experiments and gave critical input. Johannes Grillari co-advised the project. Lisa Karner performed the statistical analysis, designed figures and wrote the manuscript. Peter Dungel supervised the work and provided critical input to the manuscript.

## 1.4. Significance of Findings

The aging society, as well as the increasing number of chronic diseases and resistances to antibiotics, call for treatment options that withstand these challenges. This doctoral thesis comprises valuable data regarding the use of phototherapy for the treatment of wound infections and wound healing. The data confirms that pulsed red LED light improves wound healing not only by photobiomodulatory impact on wound relevant cell types and mechanisms, but in combination with PS also by its efficacy in fighting bacterial infections.

PBM beneficially effects fibroblasts and myoblasts that are stressed by hypoxia/reoxygenation and nutrient-deprived conditions. It was shown that the impact of PBM on the cellular mechanisms under hypoxia is similar, but more pronounced as in a normoxic environment. The increase in oxygen flux, ATP and ROS concentration further confirm the involvement of the electron transport chain, in particular cytochrome c oxidase, in the proposed mechanism of PBM.

The establishment of a simple and close to reality *in vivo* wound model infected with a naturally occurring polymicrobial suspension, provides the basis for further development of alternative antimicrobial therapeutic approaches. The presented combination of quantitative and qualitative wound analysis allows the use of all kinds of pathogens for wound infection and offers an alternative that is independent of bioluminescence analysis, which became standard in infection wound models.

This is demonstrated by the application of aPDT in this established model. The great potential of the therapy was shown by a significantly improved and faster wound healing of the infected wounds. The discrepancy between the results *in vitro* and *in vivo* repeatedly points out the importance of the multi-phase process in clinical trials and the lack of close to reality *in vitro* wound healing models, which might give an impulse for their faster development.

The gathered information adds important knowledge concerning ischemic and infected wounds and further empowers the development of PBM and aPDT using red LED light. The optimization of treatment modalities and the identification of additional factors affecting the effects and outcomes, increases the reliability of these alternative therapy approaches and help to reach the best possible therapeutic value for the patients in the future.

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# ABBREVIATIONS

| Abbreviation | Term |
|---|---|
| **ALA** | δ-aminolevulinic acid |
| **aPDT** | Antimicrobial photodynamic therapy |
| **API** | Analytical profile index |
| **ATP** | Adenosine triphosphate |
| **BrdU** | 5-bromo-2'-deoxy-uridine |
| **BSA** | Bovine serum albumin |
| **cAMP** | Cyclic adenosine monophosphate |
| **CCO** | Cytochrome C oxidase |
| **CFU** | Colony forming unit |
| **CPA** | Cyclophosphamide |
| **CS** | Cecal slurry |
| **DBNBS** | 3,5-dibromo-4-nitrosobenzenesulfonic acid |
| **dd$H_2O$** | Double distilled water |
| **DECTs** | Dendritic epithelial T cells |
| **DMEM** | Dulbecco's Modified Eagle's Medium |
| **DMPO** | 5,5-dimethyl-pyronine *N*-oxide |
| **DNA** | Deoxyribonucleic acid |
| **DVM** | Doctor of veterinary medicine |
| **EBSS** | Earle's balanced salt solution |
| **ECM** | Extracellular matrix |
| **EEA** | European Economic Area |
| **ELISA** | Enzyme-linked immunosorbent assay |
| **EPR** | Electron paramagentic resonance |
| **EPR** | Electron paramagnetic resonance |
| **ETC** | Electron transport chain |
| **FCS** | Fetal calf serum |
| **HEPES** | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| **H/R** | Hypoxia/reoxygenation |
| **ISC** | Inter-system crossing |
| **LASER** | Light amplification by stimulated emission of radiation |
| **LB** | Lysogeny broth |
| **LED** | Light-emitting diode |
| **LPS** | Lipopolysaccharides |
| **MALDI-TOF** | Matrix-assisted laser desoption/ionization - time of flight |
| **MB** | Methylene blue |
| **MEM** | Minimum essential media |
| **MTT** | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium |
| **NETs** | Neutrophil extracellular traps |
| **NF-κB** | nuclear factor kappa-light-chain-enhancer of activated B cells |
| **NGS** | Next-generation sequencing |
| **NIR** | Near-infrared |
| **NO** | Nitric oxide |
| **NOS** | Nitric oxide synthase |
| **p.o.** | "per os", oral |
| **PBM** | Photobiomodulation |

| | |
|---|---|
| **PBN** | Alpha-phenly-*N*-tertiary-butyl nitrone |
| **PBS** | Phosphate buffered saline |
| **POD** | Peroxidase |
| **PPH** | 1-hydroxy-4-phosphono-oxy-2,2,6,6-tetramethylpiperidine |
| **PS** | Photosensitizer |
| **RNA** | Ribonucleic acid |
| **ROI** | Region of interest |
| **ROS** | Reactive oxygen species |
| **rpm** | revolutions per minute |
| **s.c.** | Subcutaneous |
| **sid** | "semel in die", once a day |
| **SSTIs** | Skin and soft tissue infections |
| **TGF** | Transforming growth factor |
| **tid** | "ter in die", three times a day |
| **TMB** | 3,3',5,5'-tetramethylbenzidine |
| **TNF-α** | Tumor necrosis factor alpha |

# LISA KARNER

## PERSONAL


Wiener Neustadt (11/02/1992)

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## LANGUAGES

| | |
|---|---|
| German | ●●●●● *Native* |
| English | ●●●●○ *Fluent* |
| Swedish | ●○○○○ *Beginner* |

## SKILLS

### IT / Computer

Very good handling of Microsoft Office and GraphPad, basic knowledge in bioinformatics and Python

### Transferable skills

Project management, supervision, written communication, teamwork, presentation, administration and organization

## INTERESTS

Running, football, cooking, reading, playing the guitar, skiing, hiking

## WORK EXPERIENCE

### Product Management Trainee
**Roche Diagnostics Austria** — *02/2020 – present*

### PhD Scientist
**Ludwig Boltzmann Institute of Experimental and Clinical Traumatology** (Prof. Heinz Redl) — *02/2017 – present*

Phototherapy for the treatment of wound infections and the improvement of wound healing

### Master Thesis
**Department of Surgery at the Medical University of Vienna** (Prof. Michael Bergmann) — *03/2016 - 01/2017*

Optimization of the knock-down and the detection of the retrotransposon LINE-1

**Department of Biochemistry at the University of Uppsala** (Prof. Helena Danielson) — *09/2015 - 12/2015*

Evaluation of affinity parameters and determination of interaction kinetics for HCV polymerases with allosteric inhibitors

**Institute of Water Quality, Resource and Waste Management at the Vienna University of Technology** (Dr. Ernis Saracevic) — *09/2014*

Optimization of the HPLC-MS method for the detection of non-detectable traces of hormones and pharmacologically active substances in water

**Ludwig Boltzmann Institute of Experimental and Clinical Traumatology** (Dr. Peter Dungel) — *07/2013 - 02/2014*

Analysis of cellular and sub-cellular effects of Low Level Light Therapy

### Bachelor Thesis
**Institute of Applied Synthetic Chemistry at the Vienna University of Technology** (Prof. Marko Mihovilovic) — *04/2013 - 06/2013*

Enantioselective synthesis of cathinone derivatives for the investigation of monoamine transporters

**Institute of Molecular Diagnostics at the Austrian Institute of Technology** (Prof. Andreas Weinhäusel) — *06/2012 - 07/2012*

Evaluation of serum-autoantibody-biomarkers for early diagnostic testing of colon cancer

## EDUCATION

### Doctoral program in Engineering Sciences in Technical Chemistry
**Vienna University of Technology** — *02/2017 - present*

### Master program Technical Chemistry
Specialization in Biotechnology and Bioanalytics
**Vienna University of Technology** — *10/2014 - 01/2017*

### Bachelor program Technical Chemistry
**Vienna University of Technology** — *10/2010 - 07/2014*

## SCIENTIFIC EXPERIENCE

### Teaching

- **Supervision of Bachelor and Master students**
- **Presentation in the master student lecture “Tissue Engineering and Regenerative Medicine” about Photodynamic Therapy, 2018**
- **Tutoring of the master student laboratory exercise “Methods in Cell Biology”, 2017 and 2018, 2019**
- **Teaching in bachelor student lecture „General, Organic and Polymer Chemistry” at FH Technikum, 2018**

### Conferences and Seminars

- **YSA PhD Symposium, Medical University of Vienna, 2017 and 2019**
- **16th International Photodynamic Association World Congress in Coimbra, Portugal, 2017**
- **Inaugural Meeting of ViCEM – Vienna Center for Engineering in Medicine, Vienna, 2017 (poster presentation)**
- **41st Conference of the Austrian Society of Surgical Research in Schladming, Austria, 2017 (oral presentation)**
- **LBG Meeting for Health Sciences, Vienna, 2018 (oral and poster presentation)**
- **ESP-IUPB World Congress, Barcelona, 2019 (oral presentation)**
- **43rd Conference of the Austrian Society of Surgical Research in Schladming, Austria, 2019 (oral presentation)**

### Trainings

- **5th TERMIS/Expertissues Winter School “In Vitro/In Vivo Preclinical Models and Imaging in Musculoskeletal Tissue Regeneration” in Radstadt, Austria, 2017**
- **PACT Summer School “Primary cells for tissue engineering” at the University of Natural Resources and Life Sciences, Vienna, 2017**
- **5th Summer School of the European Society for Photobiology in Brixen, Italy, 2018**
- **Course on Laboratory Animal Science organized by the Center for Biomedical Research, Medical University of Vienna (Contents and duration equivalent to FELASA Cat. B)**
- **Training in Agile Project Management, Vienna, 2018**
- **Presentation and Media Training, Vienna, 2019**
- **Training in Scientific & PR Writing, Vienna, 2019**
- **Training in Basics of a Management and Business Administration, Vienna, 2019/2020**

### Awards

- **Best oral presentation award at Conference of the Austrian Society of Surgical Research in Schladming, Austria, 2017**
- **ESP Travel Award, 18th Congress of the European Society for Photobiology in Barcelona, 2019**