Pulsed Dye Laser in psoriasis

A nerve-wrecking event?

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Publication date
2025

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General Discussion

Preliminary Studies and Background

Before this project, several studies investigated the effectiveness and mechanisms underlying PDL therapy of psoriasis. In 1992, Hacker and Rasmussen found that the highest radiant exposure level they tested— out of 5, 7, and 9 J/cm²—yielded the most effective lesion remission, with significant improvement observed even after a single treatment[1]. Later in 1996, Zelickson *et al.* demonstrated that PDL could induce remission of psoriasis for up to 13 months with minimal side effects[2]. The first studies on the underlying mechanism and histological changes started in 2001 by Hern *et al.*, who showed that a reduction in capillaries was unlikely to be the causal factor in remission of psoriasis after PDL treatment[3-5]. A few years later, in 2009, PDL gained popularity for treating nail psoriasis due to limited treatment options and the smaller treatment area[6-8]. Studies on PDL treatment for nail psoriasis revealed better overall patient group outcomes compared to those for 'regular' psoriasis on the skin, highlighting the effectiveness of PDL in this specific application[9] and making its way to the standard treatment care protocols for nail psoriasis[10,11]. Most studies on psoriatic skin suggested that while PDL did show some improvement, the results were often comparable to other treatments like UVB[12-14]. Additionally, the time investment required for PDL treatment was often deemed not worthwhile compared to simply prescribing a topical treatment or drug[15-17].

With the advent of biologics, supported by large randomized controlled trials and significant industry funding, and the loss of patents around laser technology, interest in PDL treatment diminished and the biologics came to the market from 2006 onward. Besides, hospitals see only a small amount of patients with psoriasis, typically those with extended psoriasis throughout the body for which laser therapy is too labor intensive and takes up too much time. As a result, research on PDL declined and its adoption as a standard treatment option stagnated. However, for some patients, PDL remains the preferred treatment option because it does not involve pharmaceuticals, has minimal side effects, and can lead to long-term treatment-free remission times.

Since the introduction of biologics, interest in optimizing PDL treatment has declined, yet research into understanding the role of nerves in psoriasis has gained momentum during the same period. One of the main observations was that nerve damage could lead to remission of psoriasis, whether through accidental (paralyzing) nerve injury[18,19] by intervening in nerve function using capsaicin[20,21], or through injections with botulinum toxin[22]. Studies highlighted that nerve damage resulted in long-term, and sometimes spontaneous, remission of psoriasis. With the knowledge that both nerve damage and PDL can lead to long-term remission of psoriasis, the central question that I addressed in this project was: '**Does PDL therapy in psoriasis work because it reduces the density of nerve fibers in the skin?**' All chapters in this thesis revolved around this question.

Chapter 1 offers a review of the literature supporting this hypothesis, with possible molecular pathways that could explain the inflammation within psoriasis to be neurogenic in origin, and how PDL might alter the neurogenic inflammation of the skin. **Chapter 2**

demonstrates that cell cultures, when exposed to hyperthermia, show different viability rates with neuronal cells showing significantly lower viability compared to endothelial cells under thermal stress. **Chapter 3** disclosed that similarly to the previous chapter, exposure of 55-60°C results in a significant stagnation in vascular function. But, in contrast to separate cell cultures, we found no cell-type specific significant difference in thermal damage between perivascular nerves and endothelial cells contained in the blood vessel segments. **Chapter 4** describes and improves our understanding of the morphology of the (peri)vascular nerves in psoriatic skin, and the variations in innervation between patients. When comparing the histological findings with the clinical scores, minimal correlation was observed between skin blood volume and plaque redness, as well as between reported pruritus and skin nerve fiber density. **Chapter 5** reports on the morphological changes between baseline and PDL-treated skin. Contrary to our initial hypothesis, the data shows no significant change in the percentage of total nerve fibers, including perivascular nerves, free nerve endings, or reticular nerve fibers, after PDL treatment. However, we noted that many patients exhibited localized clusters of $CD3^+$ immune cells, often associated with blood vessels. We found that PDL treatment significantly reduced $CD3^+$ cells.

This final chapter will not simply recapitulate these results of all individual chapters but instead put our combined results into a broader context and philosophize how they may translate towards an even further understanding of the working mechanism of PDL treatment in skin inflammatory conditions. We first present an updated version of the nerve damage hypothesis, based on the data from this thesis and recent studies from other research groups. We then discuss discrepancies, such as the differences in outcomes between *in vitro* and *ex vivo* experiments. Additionally, we speculate on alternative mechanisms that may contribute to the success of PDL therapy, as suggested by the findings in Chapter 5. Finally, we consider the role of PDL in treating psoriasis and explore how to optimize and expand its therapeutic potential for other inflammatory skin conditions.

Does PDL remove nerves in psoriatic skin?

The aim of this project was to examine whether PDL treatment affects nerve fibers in the skin. Chapter 2 found that cultured nerve cells were more sensitive to thermal stress than endothelial cells, leading us to hypothesize that nerve fibers could tolerate less hyperthermia before being damaged by PDL treatment. However, the *ex vivo* experiments in Chapter 3 showed that nerves did not show a greater loss of function after hyperthermia compared to endothelial cells.

The discrepancy in results may be explained by the fact that the viability and functionality of smooth muscle cells cannot be separated from nerve fibers, as the smooth muscle cells are essential for contraction. Small differences in nerve functionality after hyperthermia may therefore not be detected as they are dependent on the functioning of the smooth muscle cells.

Another factor that may explain the differences between *in vitro* and *ex vivo* is that in cell culture, all cells were grown separately and ATP levels were measured and compared with those at 37°C. In the blood vessels, however, all cell types were contained within the same vessel and each segment of blood vessel acted as its own control, had a different readout (force instead of ATP) and was subjected to more challenges. Aside from the discrepancy between the *in vitro* and *ex vivo* results, the patient study in Chapter 5 showed no significant change in nerve fiber density after two PDL treatments, regardless of the response to therapy. Taken together, these data show that nerve cells do not show a particular sensitivity to thermal damage. Still, these results do not provide conclusive evidence that nerves are unaffected by PDL treatment.

An alternative interpretation that still aligns with the first hypothesis that nerves are damaged upon PDL, is that the visual quantification of nerve fibers might not accurately reflect changes in nerve functionality. It may be that PDL treatment alters nerve activity without visibly changing nerve density. If so, the key changes in nerve activity in successful treatments may involve a reduction in neurogenic inflammation in psoriatic plaques, which would imply a reduced secretion of neuropeptides such as CGRP, VIP and SP.[23,24]. Although immunostainings for these neuropeptides exist, quantifying active neuropeptide secretion and its variation before and after treatment in fixed biopsies remains challenging and labor-intensive. Furthermore, a number of factors, including patient-specific variables, age, and the duration of plaques, impact the density of nerve fibers in the skin. This complexity makes it challenging to identify subtle changes or significant differences within a limited study group, even when each patient serves as their own control.

In order to comprehend the consequences of PDL therapy on nerve fibers, it is essential to initially examine the relationship between the vasculature and the surrounding nerves, as well as to gain insight into the manner in which heat is dispersed through the skin in response to PDL. This process was examined in the *in silico* work of Dr. Leah Wilk, in "Modeling Pulsed Dye Laser Treatment of Psoriatic Plaques by Combining Numerical Methods and Image-Derived Lesion Morphologies."[25]. The work employed the microscopic images from Chapter 4, which were subsequently transformed into a triangular mesh on which thermal models could be implemented (Figure 1). Dr. Wilk demonstrated that varying laser settings produce multiple heat profiles around blood vessels, with thermal stress exceeding denaturation temperatures (>43 °C) extending up to 350 µm from the vessels. The temperature near the blood vessels can reach up to 200 °C (Figure 2), although these high temperatures are maintained for very short durations. Despite this brief exposure, previous studies have documented vascular coagulation of PDL indicating that temperatures reach at least 70 °C[26,27]. Moreover, it is important to note that PDL is unlikely to damage nerves in healthy skin unless there is a sufficient presence of chromophore (oxyhemoglobin) to absorb the laser's energy. Thus, the effectiveness of PDL in altering nerve fiber activity, if any, also depends on the presence of blood vessels and appropriate laser settings. It can be argued that the blood volume at baseline has an impact on the efficacy of the treatment, a topic that will be discussed in greater detail in the final paragraph.

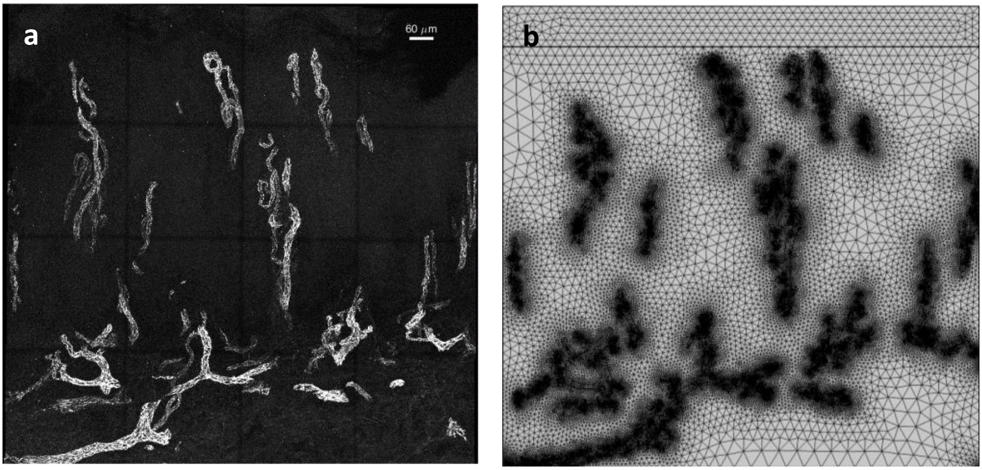

Figure 1. Psoriatic lesion morphology used for the FEM calculations. Confocal fluorescence microscopy image of a psoriatic lesion biopsy, fluorescently labelled using the endothelial marker CD31 conjugated to Alexa Fluor 647 dye (left). Triangular mesh generated from the confocal fluorescence microscopy image consisting of 87518 elements (right). Reproduced with permission from Wilk et al. 2024.

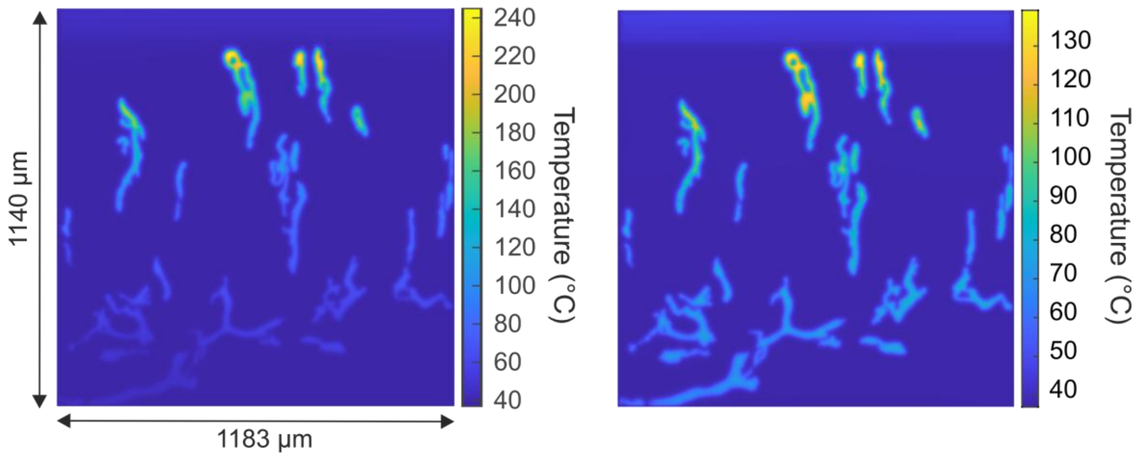

Figure 2. Temperature distribution. Generated temperature field at the end of a 0.45 ms continuous laser pulse at 585 nm (left) and at 595 nm (right) and incident radiant exposure of 8 J·cm^{-2}. Note the difference in temperature scale. Reproduced with permission of Wilk et al. 2024

Is the destruction of local immune cell clusters the key to successful PDL treatment? The results of Chapter 5, which demonstrated no alteration in nerve density following PDL treatment, emphasize the necessity for further investigation into other potential effects of PDL. It is imperative to ascertain whether the efficacy of the treatment may be depending upon the destruction of local immune cell clusters that we observed in our histological sections. One potential mechanism is the PDL-induced selective heating of the vasculature, which could transfer hyperthermia to these clusters, thereby resetting skin inflammation to a non-pathological state. Several findings lend support to this hypothesis, which we will discuss further.

1. Reducing inflammatory cells explains improvement in psoriasis

Psoriasis is a well-documented inflammatory skin condition, and a significant portion of current therapeutic efforts are focused on reducing inflammation. In a study conducted by Hern et al. in 2001, a notable decline in both $CD4^+$ and $CD8^+$ T-cells was observed two weeks following the final PDL treatment (out of three), irrespective of whether the patients exhibited a response or not[3]. Another study demonstrated that PDL led to a downregulation of TNF-α and IL-23, as well as a reduction in $CD3^+$ cells, as early as three hours after treatment[14]. The rapid decline observed suggests that PDL exerts a direct influence on immune cells (and potentially on the inflammatory state) within the skin. Furthermore, by week 7 of PDL treatment, a notable decline in keratinocyte-specific markers, including keratin 17 and β-defensin 2, was observed[14]. This provides additional evidence that the primary biological effect of PDL is damage to inflammatory cells, which subsequently leads to a reduction in keratinocyte hyperproliferation and further normalization of the skin.

These findings lend support to the hypothesis that PDL's primary target are the perivascular immune cells and that thermal destruction is a crucial factor in the clinical improvement of psoriasis (Figure 3). By integrating the findings of previous studies with our own data, we can hypothesize that patients who respond to treatment are those who present with a sustained reduction in immune cells in their skin directly after PDL treatment. Damage to and alterations in nerve function, and a temporary coagulation of blood vessels may aid in preventing the restoration of the immune cell population and leading to (long-term) remission of psoriasis.

2. Positioning of immune cells in the proximity of vasculature makes them prime targets for PDL therapy

Furthermore, our hypothesis that PDL may damage immune cell clusters was supported by the observation that these clusters are surrounded by vasculature, as evidenced by our 3D histology data. The thermal modeling from our studies has also demonstrated that further accumulation of heat occurs in areas where the vasculature exhibits a looping pattern, as heat is produced from all directions. Since the immune cell clusters are frequently situated in proximity to a disordered distribution of vessels, this may suggest that there will be an

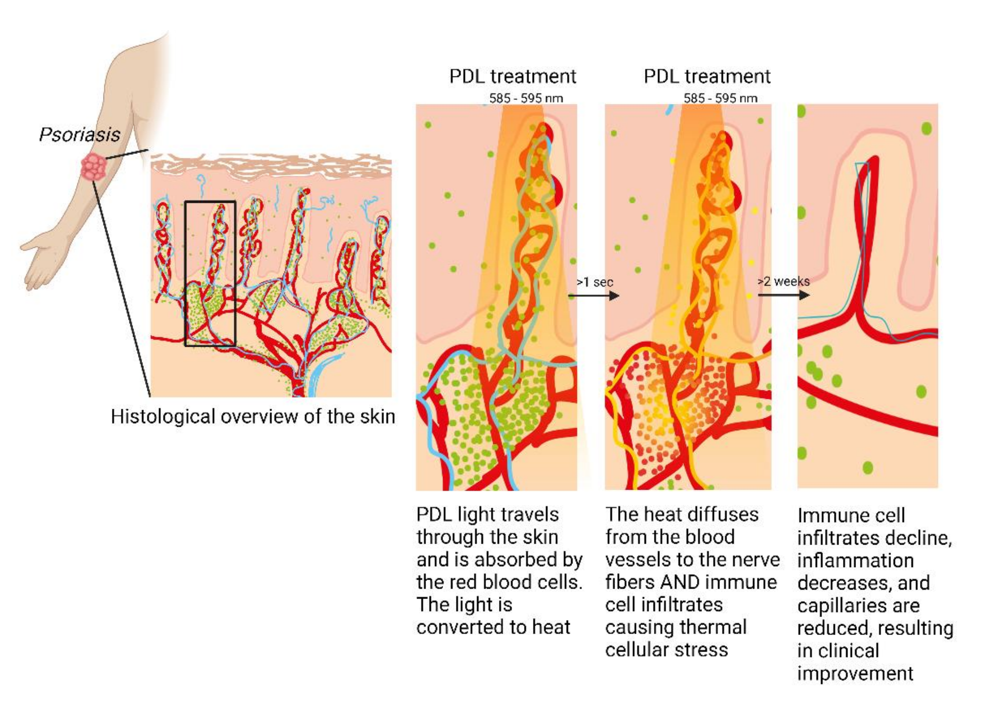

Figure 3. Illustration of possible damage to leukocyte infiltrates during pulsed dye laser treatment of psoriatic skin. The illustration panels on the right show how the vasculature may be a vehicle to bring thermal stress to leukocyte infiltrates that are highly focused around the vasculature. The illustration does not account for light-absorption disturbances such as shadowing effects.

elevated level of thermal stress upon PDL treatment. Additionally, it is plausible that the absorption of PDL light by melanin cells may contribute to the temperature increase in the layer beneath the epidermis, potentially facilitating the heating of immune cell infiltrates[26].

In all histological images, both from our own studies and previous histological studies of psoriatic skin, a typical distribution of immune cells can be observed, with dense clusters located just beneath the epidermis. This distribution is consistent across different studies and a variety of cells can be found within these clusters. One of the cell types that receives a lot of attention in psoriasis are dendritic $CD11^+$ cells which presence in the upper dermis may be essential for cutaneous immune activation[28,29]. Furthermore, T-cells ($CD8^+$ cytotoxic and $CD4^+$ helper) are present in greater numbers at the dermo-epidermal junction than in the epidermis[3].

Further indications of the therapeutic effect by removal of these immune cells can be can be drawn from a series of somewhat unconventional experiments, known as "dermatome shaving." In these experiments, the upper layer of the skin, extending to the middle of the reticular dermis, was surgically shaved, resulting in a local remission of psoriasis in some cases[30,31]. Interestingly, these studies specifically describe that shaving must be performed at least down to the level of the reticular dermis, right below the rete ridges[32]. The area that was shaved off in these experiments is also the most densely populated with inflammatory cells, as previously discussed. Remission times of dermatome shaving are similar to successful PDL treatment and varied between twenty-two[32] to thirty-six months[30], each with a subgroup of patients that showed recurrence of psoriasis that could in some cases be treated with a second shaving.

3. PDL can reach depths of up to 1.2 mm within the skin

The primary uncertainty associated with this novel hypothesis that PDL damages immune cell clusters pertains to the extent of PDL penetration into the dermal layer and the potential for PDL to eradicate these immune cell clusters. Prior research on albino skin pig models has demonstrated that 585 nm PDL can reach depths of up to 1.2 mm into the dermal layer[33]. In contrast, 577 nm, which corresponds to the precise absorption peak of the chromophore oxyhemoglobin, reached a depth of only 0.5 mm. Moreover, 590 nm wavelength demonstrated a reaching depth of 0.8 mm[33]. These measurements were taken at the dermo-epidermal junction (DEJ), which marks the boundary between the epidermis and dermis. A modeling study by van Gemert *et al.* 1997 found a great overlap between the clinical outcomes on the pig skin by Tan *et al.* and the predicted depths of their model, which calculated a predicted maximum depth for vascular injury to 1.16 mm at 585 nm[34]. It should be noted however that in the *in vivo* pig skin studies of Tan *et al.*, pulses were given three times per setting whereas clinical treatment uses a single pulse. Giving a triple pulse may have influenced (and potentially increased) the depth of vascular damage. Other studies showed that with 7.5 J/cm^2, 6 ms, and 10 mm spot size full coagulation of vessels would still occur at 600 µm depth, and partial coagulation at 800 µm depth[35].

In our dataset, no immune cell clusters were observed at a depth of 0.8 mm from the top of the skin, indicating that PDL, at either 585 nm or 595 nm, is capable of inducing thermal damage to the vascular clusters surrounding leucocyte infiltrates. The depth of light penetration is contingent upon a number of factors, including the scaling that can result in the dispersion of light, the thickness of the epidermis through which the light must travel, and the amount of pigment that absorbs the light, which in the case of PDL 585-595 nm includes melanin and oxyhemoglobin. Furthermore, the phenomenon of shadowing must be taken into account, in other words, if multiple layers of blood vessels are present, the uppermost layer of blood vessels will absorb most of the light, resulting in local heating of that layer (in this case, the capillaries) without establishing a thermal increase in the layers below. Shadowing could also provide a rationale for the necessity of multiple or overlapping passes, and/or multiple PDL treatments to eliminate the superficial capillaries, thereby enabling penetration to the lower layers of vasculature in the upper dermis that engulf inflammatory cell clusters. The observation that inflammatory cells are situated in close proximity to the epidermis indicates that PDL may potentially reach a depth of approximately

0.6-0.8 mm, which could enable the indirect targeting of these overactive inflammatory cells through the heat generated by the vasculature. This hypothesis represents a novel vision to the field, whereby the prevailing view that a reduction in inflammatory cells is a secondary effect resulting from a decrease in blood vessels is challenged. Instead, we propose that a decrease in inflammatory cells is a primary effect of PDL. This explanation also appears to be more logical, given that the skin requires adequate vascularization, which provides the possibility for a continuous supply of immune cells if required from the microenvironment. In contrast, the newly formulated hypothesis posits that a PDL exerts its effects by damaging the perivascular immune cell clusters (immediately subjacent to the epidermis), and potentially altering the function of the (peri)vascular nerves in the skin. The destruction of the perivascular nerves and immune cells, we propose, allows for a temporary clearance of the skin and the cessation of positive neurogenic inflammatory pathways, thereby facilitating the regeneration of the blood vessels in a 'normal' non-overstimulated milieu. Further research is required to substantiate this hypothesis, encompassing both the acute phase following PDL treatment and the remission phase, with a particular focus on the underlying biological mechanisms.

Common misconceptions about PDL treatment and maximizing its potential for the treatment of inflammatory skin conditions

One of the most significant misconceptions about PDL is that it is sometimes perceived as an ineffective treatment. However, this conclusion may be premature, as it does not consider the possibility that the observed ineffectiveness may not be due to the treatment itself, but rather to the specific treatment settings that may result in suboptimal outcomes. As with all treatments, the dosage is a significant determining factor in the efficacy of the treatment. In contrast to pharmaceuticals (such as biologics), which undergo rigorous dosage testing to enhance efficacy, the dosage for light and laser treatments like PDL is considerably less standardized. In the case of UVB, a schedule of gradually increasing light energy doses per session is applied to stepwise determine the minimal erythemal dose (MED), which is a measure that prevents undertreatment[11]. A substantial body of evidence from numerous studies has demonstrated that approaching the MED is essential for attaining the optimal treatment outcomes of UVB in the context of psoriasis. However, there is currently no established protocol or standardized settings for PDL treatment in psoriasis. This raises the possibility that suboptimal treatment with PDL may be a key factor contributing to the observed lack of clinical response.

What strategies can be employed to optimize PDL, and what steps are involved in optimizing settings and tailoring the treatment to the individual patient? A variety of approaches can be employed, ranging from non-invasive measurements taken prior to treatment to the manipulation of blood volume in the skin with the objective of adjusting the area of hyperthermia. Other factors to be considered include the method of laser application (i.e., bulk vs. selective photothermolysis), as well as pulses techniques such as pulse-stacking and pulse-overlapping. Additionally, it is important to take into account the recommended session intervals, the optimal treatment duration, and the criteria for discontinuing treatment. The following paragraphs will present a delineation of the stages that could be considered to constitute the patient journey, commencing with the initial visit to the clinic and concluding with the completion of treatment and subsequent care.

Non-invasive measurements prior to treatment

The efficacy of psoriasis PDL treatment is inadequately monitored at this time. The Psoriasis Area and Severity Index (PASI) is unable to provide the necessary information regarding the improvement of individual plaques, as it is more focused on indicating the extent to which a patient has psoriasis on their body. An alternative method is the Physician Global Assessment (PGA) score, which considers the presence and severity of erythema, scaling, and plaque thickness. Our studies have revealed that the PGA is highly variable, with significant discrepancies between different clinicians scoring the plaques. Additionally, the PGA score did not align with the histological features. Therefore, the assessment of the skin and the subsequent improvement thereof appears to be a highly subjective process when conducted by a human being. The mismatch between the clinical image and the histology of the skin makes it hard to track progress of PDL treatment and to adjust settings accordingly. It is essential to collect objective feedback to address the variability in treatment outcome that arises from subjective evaluation. A number of non-invasive devices have been reviewed in the literature for their efficacy in tracking treatment progress of PDL, including laser speckle imaging (LSCI)[36], optical coherence tomography (OCT)[37-39], laser Doppler flowmetry[4], dermoscopy, spatial frequency domain imaging, and more[40].

The utilization of non-invasive modalities for the purpose of optimizing patient settings offers a multitude of potential benefits. OCT and LSCI could be employed to ascertain the extent of plaque penetration, given that psoriasis plaques often recur from outer layers that were inadequately treated[2,32]. It is conceivable that OCT and LSCI could facilitate this process, providing supplementary indications before, during, and after treatment to ascertain the extent of thermal damage. Furthermore, non-invasive measurement of blood volume could provide an indication of when to increase radiant energy in cases where there is a reduction in blood flow.

Modifying the blood volume in the skin to alter the location of hyperthermia

In the event that an insufficient quantity of chromophore is present, it may be beneficial to apply a gentle rub to the skin in order to induce a transient erythema and thereby increase the volume of blood present in the superficial layers of the dermis. In cases of extreme severity, the application of Midalgan® cream may be employed as a means of inducing vasodilation. It may also be of interest to consider the possibility of reducing the blood volume within the capillaries located within the rete ridges. As previously discussed, there is a possibility that PDL is effective when the immune cell clusters below the rete ridges are effectively removed through selective photothermolysis. If this is indeed the case, then the use of a glass plate on the surface of the skin to press out the blood in the capillaries (that have the lowest mm Hg) and prevent shadowing may prove beneficial in preventing the vessels below the rete ridges in the dermis from being obscured by the immune cell infiltrates. Further consideration could be given to the potential of optical clearing with, for example, tartrazine[41], in order to reduce light scattering and facilitate a more comprehensive visualization of the blood vessels. The additional use of dermoscopy in combination with tartrazine may allow for examining coagulation in the skin, thus indicating whether further treatment pulses are required. However, some studies on tartrazine reported neurodevelopmental toxicity and apoptosis in zebrafish[42]. The dosage required to achieve optimal optical clearing of the skin may exceed the recommended levels for safe dietary intake. Further studies are necessary to confirm the safety of chemical optical clearing.

Bulk heating versus selective photothermolysis of the skin

A further crucial aspect of laser treatment is the question of whether the laser should be used for bulk heating or selective photothermolysis. The VBeam Perfecta (Candela Corporation) offers eight pulse duration options (0.45, 1.5, 3, 6, 10, 20, 30, and 40 ms). By adjusting the combination of pulse duration and radiant energy, it is possible to achieve either selective photothermolysis or bulk heating. These two approaches may lead to different biological effects. Selective heating is primarily directed at the vasculature, resulting in localized disruption of blood vessels with minimal thermal diffusion. In this context, one study indicated that complete disruption of the vasculature, indicated by purpura formation, may increase the risk of skin inflammation[43]. It further recommends that intravascular coagulation, which manifests as skin darkening, should be the clinical endpoint rather than purpura.

In contrast, bulk heating produces a more diffuse heating, impacting not only the vasculature but also the surrounding tissue. As previously discussed, the vasculature is encased by perivascular nerves and leukocytic infiltrates, both of which play a role in the inflammatory feedback loops associated with psoriasis. In light of the findings of our study, we put forth the proposition that controlled bulk heating, in comparison to selective heating, is more efficacious in inducing thermal damage to the key elements that contribute to the pathogenesis of psoriasis, thereby reducing its severity. However, bulk heating also carries an elevated risk of complications such as blistering or burns. Clinicians must recognize that thermal effects are influenced not only by pulse duration but also by factors like spot size and radiant energy.

The quantity of pulses and pulse-overlapping

The subsequent aspect to be contemplated is the manner in which light is delivered. In this regard, two principal considerations emerge: firstly, the stacking of pulses (for example, maintaining a fixed position and delivering multiple pulses), and secondly, the overlapping of pulses, which is designed to reduce the extent of untreated areas. Furthermore, the treatment protocol should consider the potential for increasing the radiant energy of the laser in accordance with the chromophore. It should also be considered that if the treatment is effective, this may indicate a reduction in both the vasculature and the blood volume within the skin, which would result in a decrease in the amount of chromophore present. To achieve a comparable degree of therapeutic heating of the skin, it can be theorized that the energy density should be augmented with each subsequent treatment in order to offset the loss of chromophore in the skin (Figure 4).

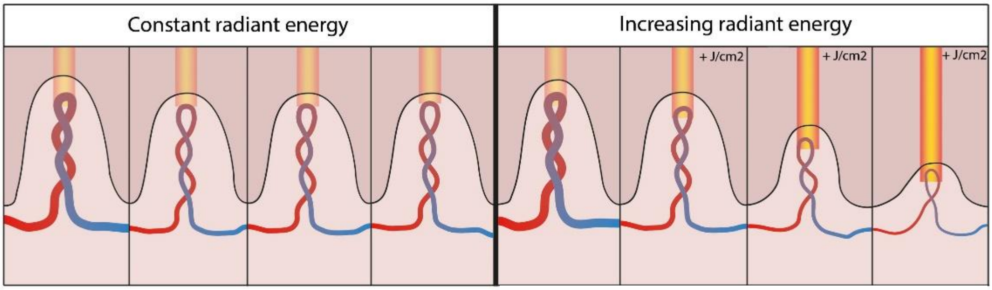

Figure 4. illustration of the impact of radiant exposure (fluence, J/cm^2) on the heat generation within blood vessels when vasculature decreases and/or radiant energy remains constant.

The intervals between sessions, the duration of treatment, and the criteria that should be met before discontinuing treatment

Lastly, the interval between treatments is one of the variables that exhibits considerable variation across studies. In the literature, studies report on a range of interval lengths between PDL treatments, including 2[3,16,44], 3[2,13,14], and 4[45] weeks. The difference of treatment intervals impacts the time and extent of regeneration allowed between treatments, which may be a significant factor for success rate. For example, prolonged regeneration time[46] and lack of stable blood supply[47,48] is known to limit regrowth of nerve fibers. Furthermore, the necessity of repetition of treatment is indicated by the fact that the majority of patients in the clinic require approximately six to eight treatments. The need for multiple PDL treatments before remission occurs could be attributed to the fact that the treatment settings are not optimized for the patient, but there may also be a biological rationale for inducing repeated thermal stress and subsequent damage to the skin in order to induce a more permanent change (i.e., reduction of neurogenic inflammation). It is also noteworthy that a considerable number of plaques exhibit recurrence through growth from the initially seemingly clean edges[49]. The re-growth of plaques from the periphery towards previously cleared areas is indicative of the necessity for clinicians to extend treatment to adjacent edges that lie beyond the delineation of the plaque. Furthermore, it appears that many treatments are discontinued well before remission has occurred. Patients have indicated multiple reasons for not returning, including the experience of pain, the presence of clear skin for two weeks after treatment, which may lead to the perception that there is no need for further treatment, and the absence of results after a course of treatment, which may lead to a lack of trust in the potential efficacy of subsequent treatments. In regard to the first reason, it may be advisable to recommend the use of paracetamol prior to treatment, as this could provide relief. For patients who are satisfied with the outcome, continued monitoring is recommended to ensure the longevity of the treatment's efficacy. Additionally, a notable distinction can be made between patients who exhibit a mild response and those who do not. For the former group, increasing the laser power may be beneficial, and it is important to communicate that optimal results may require time.

In conclusion, our findings indicate that nerves in cell culture are more susceptible to heat than endothelial cells. However, this effect was not observed in our *ex vivo* data. Similarly, no differences in nerve fiber density were observed following PDL treatment. Nevertheless, the lack of unaltered morphological nerve fiber densities does not provide confirmation that their functionality remains unaltered. Furthermore, our microscopic data indicated that PDL may exert its effects through the selective destruction of $CD3^+$ immune cells in the vicinity of blood vessels. The destruction of perivascular immune cells and altered nerve functionality may provide an explanation for the therapeutic effect of PDL treatment. Some studies have provided preliminary support for this hypothesis, although rigorous evidence is currently lacking. Nevertheless, clinicians may still attempt novel approaches to PDL treatment by modifying protocols to enhance targeting of the vasculature in the dermo-epidermal junction, where immune cell clusters are located. In summary, this study paves the way for new insights into and improvements to PDL therapy in the treatment of psoriasis. Future research should continue to elucidate the precise mechanisms through which PDL affects immune cells and evaluate strategies to refine treatment protocols for enhanced patient outcomes.

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