Epidermal and dermal changes in response to various skin rejuvenation methods

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Abstract

During the last years, a number of new devices have been developed to improve the dermal and epidermal signs of photo- and chronological skin ageing. There are well-established ablative and non-ablative skin resurfacing options using different lasers and light sources, but side effects have been observed frequently. A recently developed photorejuvenation method using non-thermal stimulation of skin cells with low energy and narrow band light has been termed photomodulation. Light emitting diodes are the ideal source of this kind of light that stimulate mitochondrial cell organelles leading to up-regulation of cytochrome electron transport pathway leading to mitochondrial DNA gene modulation. This paper reviews the most current knowledge of intrinsic and extrinsic changes of ageing and summarizes different systems for skin rejuvenation with focus on non-thermal non-ablative skin rejuvenation modalities.

Résumé

Durant les dernières années, un certain nombre de nouveaux dispositifs ont été développés pour améliorer les signes dermiques et épidermiques du photo vieillissement et du vieillissement cutané chronologique. Parmi celles-ci, il y a les techniques bien connues de traitement de surface plus ou moins invasives utilisant différents lasers et sources lumineuses, mais des effets secondaires ont été fréquemment observés. L’utilisation d’une méthode de photo rajeunissement a récemment été développée. Sans stimulation thermique des cellules cutanées, utilisant une faible énergie et une bande étroite de lumière, elle est désignée sous le nom de photo modulation. Les diodes électroluminescentes (LED) sont la source idéale pour cette lumière qui stimule la voie électronique du cytochrome pour conduire à la modulation génétique de l’ADN mitochondrial. Cet article passe en revue les éléments actuels connus sur les modifications du vieillissement intrinsèques et extrinsèques et recense les différents systèmes utilisés pour le rajeunissement cutané en se focalisant sur les techniques non-invasives et sans stimulation thermique.

Introduction

The human integument forms the most visible indicator of age. Ageing skin presents various morphologic changes such as wrinkles, skin atrophy or thinning next to thickening (both epidermal and dermal compartments), dyspigmentation, teleangiectasia and loss of elasticity [1–3]. With increasing age of the population, the demand for minimal invasive treatments to preserve or improve skin smoothness and tonicity is increasing. Various rejuvenation modalities have attempted to reverse the dermal and epidermal signs of photo- and chronological ageing. There are different well-established ablative skin resurfacing options for the repair of rhytides and
photoaged skin including conventional and fractional ablative laser interventions using CO₂- or Er:YAG-lasers. Using this technique, controlled collateral dermal heating is achieved next to microscopic ablation zones (MAZ) which is followed by a wound healing response ultimately leading to re-epithelialization and dermal remodelling [4] (Fig. 1). Common side effects are pain, long-lasting erythema, infections, hypo- or hyperpigmentations and sometimes scarring [5–7].

Categories of thermal but non-ablative devices include intense pulsed lights (IPLs), infrared lasers (1064, 1320, 1450 and 1540 nm), visible light lasers (532 and 585 nm) and radiofrequency [8–11]. They affect the wound healing cascade by a thermal or photothermolysis type of injury. Selective photothermolysis destroys skin structures with little or no damage of the surrounding tissue. The target structures hemoglobin, melanin or water are reached depending on the wavelength of the light source used (Fig. 2). Thermal but non-ablative photorejuvenation typically involves a confined selective thermal injury to the papillary and upper reticular dermis which contains the majority of solar elastosis in photodamaged skin without any epidermal damage leading to fibroblast activation and synthesis of new collagen [8, 12–17]. The underlying molecular changes of both ablative and non-ablative epidermal and/or dermal remodelling are not fully understood but have been postulated to be induced by a time dependent release of heat-shock protein 70 (HSP70) among others [18–24].

![Figure 1](image1.png)  
**Figure 1** Immunoreactivity (magnification x20) before and after AFP: 64 mJ: 8 ms and 8 W: (a) native (untreated skin), (b) day 0 (directly after AFP), (c) day 3 (3 days after AFP), (d) day 7 (7 days after AFP).

![Figure 2](image2.png)  
**Figure 2** Absorption spectra of the different targets.

Light emitting diodes (LED) photobiomodulation is the newest category of non-thermal light therapies with no side effects reported in the published literature. It has been reported to accelerate cutaneous wound healing after various injuries including thermal-based rejuvenation treatments leading to a more rapid wound healing [25–31] by activation of fibroblasts [32, 33] and demonstrated anti-inflammatory potential [34]. A significant increase in collagen production after LED treatment has been shown in various experiments, including fibroblast cultures, third-degree burn animal models, and human blister fluids and skin biopsies [35–37]. Furthermore, LED brings several advantages to enhance the clinical efficacy of photodynamic therapy (PDT): progressive photoactivation of photosensitizers, large uniform beam profile, reduced procedural pain and multiple wavelengths available. Thereupon, LED light

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sources had been used in many different indications alone or in combination with a photosensitizer including photorejuvenation [32, 33]. But, the decision for a specific photomodulation parameter protocol for a particular cell target seems to be crucial for the results because it is possible with an identical LED light source and identical energy fluence to see either an increase or decrease of collagen synthesis in cell culture.

In this review, we will summarize the current knowledge about skin ageing and the different treatment modalities with different light sources and focus on non-thermal non-ablative skin rejuvenation modalities.

**Intrinsic and extrinsic skin ageing**

Both, intrinsic and extrinsic influences are involved in the ageing process of the skin. Intrinsic structural changes occur as a natural consequence of ageing and are genetically determined whereas extrinsic factors are, to varying degrees, controllable and include exposure to sun light, pollution or nicotine, repetitive muscle movements like squinting or frowning and miscellaneous lifestyle components such as nutrition, sleeping position and overall health [38].

Intrinsic ageing expresses by smooth, thinned skin with exaggerated expression lines and the occurrence of benign neoplasms such as seborrheic keratoses and cherry angiomas. Histological changes include a flattening of the rete ridges with reduced surface contact of the epidermis and dermis which results in a reduced exchange of nutrients and metabolites between these two parts. Besides a decreased number of melanocytes and Langerhans cells can be detected in the epidermis. In the dermis, several fibroblasts may be seen as well as a loss of dermal volume along with a reduced number of blood vessels. Furthermore, terminal hair converts to vellus hair with a loss of melanocytes in the bulb [39]. In addition, ageing is significantly different among different anatomical sites within one individual [40, 41] and influenced by hormonal changes, primarily of oestrogen and testosterone [42–44]. The natural process of ageing contributes to the generation of reactive oxygen species (ROS) that stimulate the inflammatory process in the skin with activation of transcription factors that regulate the proteolytic degradation of the extracellular matrix. An interlinked network of enzymes that convert ROS to harmless water and molecular oxygen regulates the antioxidant defence system which is reduced in the skin of aged individuals [45].

Extrinsically aged skin is characterized by signs as wrinkles, dyspigmented lesions, telangiectasia and loss of elasticity, especially in facial skin [1–3]. Characteristic histological features, observed in photodamaged skin, are a reduced amount and fragmentation of collagen fibres, elastotic degeneration of elastic fibres, an increased amount of glycosaminoglycans, dilated and tortuous dermal vessels as well as atrophy and eventual loss of epidermal polarity [2, 46, 47]. Corneocytes in sun-exposed areas become pleomorphic with increasing anomalies: retention of nuclear remnants, loss of lines of overlap and roughening of border edges [38].

It has been shown a clear dose–response relationship between a loss of dermal collagen and development of elastosis and telangiectasia and smoking with smoking being a greater contributor to facial wrinkling than even sun exposure [48–50]. It causes skin damage primarily by decreasing capillary blood flow which in turn creates oxygen and nutrient deprivation in cutaneous tissues.

UV light exposure initiates a flurry of molecular and cellular responses in which UVA, penetrating into the dermis, is responsible for most of chronic skin damage. Matrixmetalloproteinases (MMP1, 2, 3, 8, 9 and 13) [51–53], serine proteases (neutrophil elastase), fibroblast elastase [54], free radicals, ROS and the xeroderma pigmentosus factor are induced and subsequently degrade or damage dermal collagen leading to epidermal–dermal invagination representing the beginning of wrinkles [55–57]. Additionally, UV light causes depletion of cellular and enzymatic antioxidants (SOD, catalase), activates the neuroendocrine system leading to immunosuppression and release of neuroendocrine mediators. At cellular level, UV irradiation triggers cytokine production [58], induces surface expression of adhesion molecules [59] and affects cellular mitosis, apoptosis and necrosis [60]. MMP 1 initiates cleavage of fibrillar collagen type I and III in the dermis which is then further degraded by MMP 2 and 9 [61]. Simultaneous expression of MMP 2, MMP 3 and MMP 9 results in degradation of non-collagenous components of dermal ECM including basement membrane glycoproteins and proteoglycans. MMPs in aged skin may already be in a more active state because of the decreased levels of tissue inhibitor of MMPs (TIMPs) [62].
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Additional acute effects of UV irradiation are direct effects on keratinocytes such as the induction of several interleukins and tumour necrosis factor (TNF) α leading to the infiltration of the skin with phagocytic cells which themselves secrete these cytokines [63]. IL 1 and TNF α increase the rate of degradation of proteoglycans and inhibit proteoglycan biosynthesis. In contrast, TGF β and IGF 1 have the opposite effect and thus induce proteoglycan synthesis [64]. TGF β and TNF α are known to suppress keratinocyte proliferation. On top of this, prostaglandines and other inflammatory mediators such as histamine and leucotrienes [65] as well as granulocyte–macrophage colony-stimulating factor are also increased in response to UV [66]. Other growth factor agonist/antagonists in response to UV include: IL 1 receptor antagonist [67], α-melanocyte-stimulating hormone [68], vascular endothelial growth factor, nitric oxide [69], basic fibroblast growth factor, nerve growth factor, endothelin 1 and proopiomelanocortin derived peptides and β-endorphin [70].

The pro-inflammatory mediators increase the permeability of capillaries leading to infiltration and activation of neutrophils and other phagocytic cells into the skin. ROS are primarily produced by phagocytic cells and polynuclear lymphocytes in response to inflammation [71] leading to oxidative damage to cellular proteins, lipids and carbohydrates and DNA [72]. One of the primary events in ROS-mediated inflammation is the activation of transcription factors. Nuclear factor kappa B (NFκB) and activator protein 1 are transcription factors that are involved in regulation of gene expression of a variety of genes involved in immunological and inflammatory responses including genes that encode cytokines, matrix degrading metalloproteases, adhesion molecules and regulators of cell growth, differentiation and cell death. [73]. The free radical-induced peroxidation of membrane lipids contributes to increased phospholipase A activity leading to more production of prostaglandins [74]. Phagocytic cells further stimulate keratinocytes to synthesize and secrete elastin, an inhibitor of human neutrophil elastase which eventually limits the damage caused by the inflammatory neutrophils [75].

Non-thermal non-ablative skin remodelling

The treatment of facial rhytides has traditionally centered around methods that remove the epidermis and cause a dermal wound with resultant dermal collagen remodelling. These methods have included dermabrasion, chemical peels and the use of the charfree pulsed CO2 and Er:YAG lasers [76, 77]. Fractional laser skin treatment has been shown high clinical efficacy for the ameliorization of photodamaged and scarred skin and low post-operative side-effect rates compared with conventional non-fractionated laser therapies [78]. Despite the limited recovery period after fractional laser resurfacing, patients are often inconvenience by skin erythema and oedema that prevent them from immediately pursuing their activities of daily living anyhow, although severe or long-standing complications are rare.

In the late 1960s, during a series of mouse experiments on the carcinogenic potential of lasers by using a low-powered ruby laser (694 nm) improved hair growth could be observed. This was the first demonstration of ‘photobiostimulation’ with low-level laser therapy. Because of this casual observation, medical treatment with coherent light sources (lasers) and non-coherent light (LED) had been expanded and had been introduced in the treatment of skin rejuvenation, too [25–31].

LED sources produce narrow band light that typically range 10–20 nm on either side of the dominant emissive wavelength listed. This produces a profile of photons of different wavelengths that is broader than typical laser sources but much narrower than typical IPL or other medical light sources. Different wavelengths have different chromophores and can have various effects on tissue. For best effects, the wavelength used should allow for optimal penetration of light in the targeted cells or tissue and should also be within the absorption spectrum of the chromophore or photoreceptor molecule. Red light can be used successfully for deeper localized targets (e.g. sebaceous glands), and blue light may be useful for the treatment of skin conditions located within the epidermis in PDT (e.g. actinic keratoses). To reach as many fibroblasts as possible, a deeply penetrating wavelength is desirable [79, 80]. The therapy is painless, safe and the entire face can be treated in a few minutes because of large LED panels available. But, it is important to recognize the specificity of the photomodulation parameter protocol for a particular cell target because it is possible with an identical LED light source and identical energy fluence to see either an increase or decrease of collagen synthesis in cell culture
[79, 80]. Laser power below 2.91 mW enhanced cell proliferation, whereas higher laser power (5.98 mW) had no effect. Collagen type I production was affected in the opposite direction to cell proliferation: when the cell proliferation was increased, collagen type I production was decreased [79, 80].

Dose reciprocity effects were examined in a wound healing model and showed that varying irradiance and exposure time to achieve a constant specified energy density affects LED therapy outcomes [81]. In practice, if light intensity (irradiance) is lower than the physiological threshold value for a given target, it does not produce photostimulatory effects even when irradiation time is extended. Moreover, photoinhibitory effects may occur at higher fluences. Furthermore, accurate positioning or working distance is important for the proper amount of photons delivered to the treated skin to avoid hot or cold spots in the treatment field to trigger the expected cell response.

Photomodulation occurs via stimulation of mitochondrial cell organelles which results in an up-regulation of the mitochondrial cytochrome electron transport pathway and associated mitochondrial DNA gene modulation. Energy for conversion of ADP to ATP provides energy for non-mitotic cell processes. Cytochrome molecules, in particular cytochrome oxidase within the mitochondrial membrane, synthesized from protoporphyrin IX, are believed to be responsible for the light absorption in the mitochondria.

Different clinical trials using the 590 nm wavelength LED and a very specific pulsing time code (total output: 0.1–0.9 J cm\(^{-2}\), 2 pulses per cycle with 100 cycles delivered over 35 s, on time for each pulse: 250 ms, off time: 100 ms. 8 treatments delivered over 4 weeks) were highly effective for the stimulation of collagen synthesis and clinical improvement of photoaged skin with a reduction of elastosis, erythema and pigmentation in 60–90% of patients [28, 31]. Results peaked between 4 and 6 months and declined slowly over the following 6–12 months after completion of treatment. In contrast, application of continuous LED light had minimal effect [36]. A randomized, placebo-controlled clinical trial comparing an 830 nm (55 mW cm\(^{-2}\), 66 J cm\(^{-2}\)), 633 nm (105 mW cm\(^{-2}\), 126 J cm\(^{-2}\)) or both LED treatments (2×/week over 4 weeks) showed significant reductions of wrinkles (maximum: 36%) and increases of skin elasticity (maximum 19%) compared with baseline and sham treatment measured by profilometric evaluation and cutometer. Histologically, a marked increase in the amount of collagen and normal elastic fibres was observed 2 weeks post-treatment with the most significant changes perifollicular and in the papillary and upper reticular dermis. But changes of collagen network appeared even deeper than 500 μm extending to almost the entire dermis, not restricting dermal matrix remodelling to the areas affected by thermal damage as shown in other studies [82, 83]. Combination of the 633 nm (105 mW cm\(^{-2}\), 126 J cm\(^{-2}\) on days 8, 10 and 12) and 830 nm LED treatment (55 mW cm\(^{-2}\), 66 J cm\(^{-2}\) on days 1, 3, 5, 15, 22 and 29) represented an effective and acceptable method for photorejuvenation [33] and led to the greatest percentage reduction in wrinkle severity [82, 83].

Ultrastructurally, highly activated fibroblasts, surrounded by abundant elastic and collagen fibres could be observed. Immunohistochemistry demonstrated an increase of TIMP1 and 2, inhibiting MMP activity protecting the newly synthesized collagen. RT-PCR showed an increase of the proinflammatory cytokines IL 1β and TNF α [37]. Furthermore, Cx43 mRNA increased after LED therapy, probably enhancing cell–cell communication between fibroblasts synchronizing their cellular responses to the photobiostimulation effects. Cx43 is the main protein of gap junctions [84, 85], primarily located in the interfollicular epidermis, throughout the spinocellular and granular cell layers and focally in the basal cell layer, but is also expressed in fibroblasts, hair follicles, smooth muscle cells and endothelial cells [84]. During the early wound healing process, up-regulation of Cx43 also is observed in smooth muscle cells and endothelial cells in the dermis suggesting mediating transendothelial migration of leucocytes through gap junctional intercellular communication [86–88]. This could be the reason for the neocollagenesis even in areas not irradiated directly [37].

**Heat-shock proteins**

HSP coordinate the molecular signal transduction in response to different stress factors and during wound healing [89]. These stress proteins tend to be up-regulated in all cell types exposed to increasing temperatures (i.e. 4–6°C above their physiologic temperature) or other forms of physical and chemical stress, thereby facilitating various aspects
of protein maturation [90–95]. Increasing levels of HSP enhance the ability of cells to deal with
the resultant accumulation of abnormally folded proteins, either facilitating the refolding of dam-
aged proteins or participating in the synthesis of new proteins to replace those irreparably dam-
aged [96].

HSP control cell growth and apoptosis regulate steroid receptors, kinases and increases cell resis-
tance against several other stress factors, e.g. reactive oxygen species [97, 98]. The most important
HSP are the family members of HSP70 (72 kDa = HSP72 and 73 kDa = HSP73) with extremely
high sequence homology (95%) and similar bio-
chemical properties [94] and HSP47. Both, HSP72
and HSP73 are present in the cytoplasm and the
nucleus of keratinocytes, fibroblasts and adipocytes
[99, 100]. HSP73 is synthesized constitutively in
all mammalian cells and therefore is often referred
to as the ‘constitutive HSP70’. The synthesis of
HSP72 is usually restricted to the cell experiencing
stress and therefore often is referred to as the
‘inducible HSP70’. Several studies showed an
up-regulation of HSP72 in human keratinocytes
by heat, UV-irradiation, wounding, inflammation
and ablative fractional resurfacing of skin [23,
101–103]. Different laser therapies have been
shown to increase the expression of HSP70 within
the epidermis and around the ‘microscopic thermal
injury zones’ 2–48 h post-treatment leadingulti-
ately to collagen remodelling via an up-regulated
expression of HSP47 [19, 20, 22–24, 104–110].

In previous studies, we could show a clear time-
dependent HSP70 expression profile post-AFP per-
formed by a scanned 250 μm CO₂-laser beam and
a 1550 nm Er:Yag laser in a human skin explant
model and in a clinical trial using three different
fractionated CO₂-laser treatment regimens. Using
the skin explant model, one aliquot of the explants
was fixed in 4% buffered formalin immediately
after laser procedure whereas the others were sub-
jected to cell culture medium (DMEM, enriched
with streptavidine and 10% foetal calf serum) for
1, 3 or 7 days at constant temperatures of 31–
32°C corresponding to an average skin surface
temperature before immunohistochemical staining.
Another non-laser-treated aliquot of the explant
served as baseline control. Further studies could
show that pre-treatment with heat as well as cool-
ing lead to cellular thermotolerance by the induct-
on of HSP70 protecting the tissue of a more
severe secondary heat stress [111, 112].

Mitochondria have a crucial function in initiati-
ing the cascade of caspase activation in response
to different apoptotic signals [113]. Disruption of
the outer mitochondrial membrane by apoptotic
stimuli results in the release of cytochrome c into
the cytoplasm [114]. Cytochrome c binds to the
cytosolic apoptotic-protase activating factor 1
(Apaf-1), thereby promoting Apaf-1-mediated acti-
vation of caspase 9 in an ATP/dADP-dependent
manner [115, 116]. Active caspase 9 can subse-
quently amplify the caspase cascade by its ability
to process its own proenzyme as well as the effec-
tor caspase 3 [117]. Association of procaspase 9
with Apaf-1 is an essential step in its initial activa-
tion and is mediated by the caspase recruitment
domain (CARD) sequence located at the amino ter-
mini of both proteins [118]. HSP70 has been dem-
onstrated to directly bind to the CARD of Apaf-1,
thereby preventing the recruitment of oligomeriza-
tion of Apaf-1 and association of Apaf-1 with pro-
caspase 9 [119–121] obviating apoptosis (Fig. 3).

In the case of PDT-induced apoptosis translocated
cyttoplasmic HSP70 to the cell surface has been
shown to have a crucial role in treatment response
[122, 123] because of its inhibitory effect of both
apoptotic and necrotic pathways [124]. Non-thermal
non-ablative treatments using two LED (LEDA
SCR red light: 635 nm, 40–120 W cm⁻², 40–1-
20 J cm⁻²; LEDA SCR yellow light: 585 nm,
16–35 W cm⁻², 20–100 J cm⁻²; spot size: 16 ×
10 cm) did not lead to a HSP70 up-regulation in
our skin explant model described above.

Figure 3 Apoptotic pathways.
Future indications of LED in dermatology

At the beginning, LED studies mainly focused on its wound healing properties. Wound size had been shown decreased and acceleration of wound closure had been demonstrated in various in vivo animal [125] and human models [126]. The magnitude of the biostimulation effect depends on the targeted molecules and cells with their own thermal relaxation times [127] and the physiological condition at the moment of irradiation [128]. Compromised cells and tissues respond generally more than healthy cells or tissues.

Besides, a series of studies demonstrated the anti-inflammatory potential of LED in the treatment of different inflammatory diseases, e.g. diffuse type rosacea, acne, atopic eczema or keratosis pilaris rubra [127, 129–131]. It has been further shown to accelerate the resolution of erythema and reduce post-treatment discomfort in pulsed dye laser-treated patients with photodamage [127]. In our own investigations, we could observe anti-inflammatory and anti-proliferative effects in several psoriasis patients using a 308 nm LED. Because LED is known to reduce MMPs, it might be useful in conditions in which MMPs are implicated like lupus erythematosus (LE) or photodamaged human skin [132].

The induction of cellular resistance to UV insults by LED may possibly be explained by the induction of a state of natural resistance (possibly via the p53 cell signalling pathways) without the drawbacks and limitations of traditional sunscreens [133]. These results are encouraging to expand the potential applications of LED therapy in the treatment of patients with anomalous reactions to sunlight such as polymorphous light eruption or LE. Furthermore, photoprophylaxis could be reached administering a LED therapy several times prior to an UV irradiation, a mechanical trauma such as a CO₂ laser treatment or surgery [127, 134] and there is some evidence of a preventive and complementary approach to thermal laser induced post-inflammatory hyperpigmentations [135].

An imbalance between collagen biosynthesis and degradation superimposed on the individual’s genetic pre-disposition has been implicated in the pathogenesis of hypertrophic scars and keloids, probably because of interleukin (IL)-6 signalling pathways. For that reason, IL-6 pathway inhibition could be a promising therapeutic target for scar prevention [136] by LED therapy that has been shown to decrease IL-6 mRNA levels [37].

LED used for PDT can complement other skin rejuvenation therapies or topical agents used to enhance collagen production. Red wavelength (630 nm) can reach the sebaceous glands and blue (405 nm) light photobleaches any residual protoporphyrin IX in the epidermis, thereby reducing post-treatment photosensitivity. LED-based PDT showed remission rates and cosmetic results similar to incoherent light systems [137–139]. There are multiple innovative methods such as photophyllaxis, photopreparation and photoregulation for LED use with no or little side effects. Future research should focus on investigating specific cell-signalling pathways involved to better understand the mechanisms of action, search for cellular activation threshold of targeted chromophores, as well as study its effectiveness in treating a variety of cutaneous problems as a stand alone application and/or complementary treatment modality. LED seems to be a multifunctional treatment approach which can be used as a substitute for or in combination with many laser therapies reducing side effects by easy practicability.

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