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Intense Heat-Shock Biomodulation (i-HBM) of Skin and Mucous Cells with the Fotona SMOOTH® Er:YAG Laser Modality

Matjaz Lukac,[1,2] Irena Hreljac,[3] Sasa Terlep,[3] Marcelo Tettamanti[4,5]

[1] Institute Jozef Stefan, Jamova 19, 1000 Ljubljana, Slovenia
[2] University of Ljubljana, Jadranska 19, 1000 Ljubljana, Slovenia
[3] Fotona d.o.o., Stegne 7, 1000 Ljubljana Slovenia
[4] Biotechnolaser, 15 Grosbeak Way, Landrum, SC 29356, USA
[5] Laser and Health Academy, 1000 Ljubljana, Slovenia

ABSTRACT

The safety and excellent clinical results of the non-ablative Fotona SMOOTH® Er:YAG laser therapy for soft tissues have been attributed to the unique dual regenerative characteristic of this Er:YAG laser modality, whereby the standard deep-thermal coagulation is accompanied and enhanced by an additional superficial heat-shock triggering mechanism.

In this paper, the mechanism of superficial heat-shock triggering is analyzed from the viewpoint of indirect biomodulation in response to alarm signals activated by Er:YAG laser-generated intense heat-shocks at the tissue surface. The uniqueness of this intense heat-shock biomodulation (i-HBM) mechanism is in its ability to effectively yet safely regenerate superficially located tissues, and as well in its self-regulatory characteristics, which ensures that the intense i-HBM hyperthermic therapy always remains within the safe hormetic zone.

Key words: Er:YAG, biomodulation, photo-biomodulation, hormesis, hyperthermia, Fotona SMOOTH®, SMOOTH, smooth-resurfacing, heat shock triggering.

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I. INTRODUCTION

a) Biomodulation in tissue response to stress

In medicine, biomodulation describes a biological change in cells and tissues in response to pathologic or therapeutic stimuli [1], such as elevated temperatures [2], mechanical damage [3], laser ablation [4,5], reactive oxygen species (ROS) [6,7], UV light, pathogens, etc. Biomodulation includes, but is not limited to photo-biomodulation (PBM), which has been adopted as a term for non-thermal (cold) low-level laser therapy (LLLT) [8-10]. In general, biomodulation is involved whenever environmental stresses induce danger/alarm signals in distressed or injured cells [11–13]. These cells can react either by induction of repair and regeneration processes or by induction of cell death, depending on the extent of the damage. The injured cells also transduce danger signals to their neighbors, resulting in a protective response gradient in adjacent tissue, including in cells that have not been directly damaged by the environmental stress. The mechanisms of cellular responses to stress are complex and highly conserved throughout evolution, demonstrating their importance for survival.

b) Fotona SMOOTH® Er:YAG laser therapy

The non-ablative Fotona SMOOTH® Er:YAG laser therapy of the skin and mucosa has in recent years attracted significant attention due to the technique's safety and excellent clinical results [14–30]. The Fotona SMOOTH® therapy consists of delivering a non-ablative Er:YAG laser pulse sequence to the treated soft tissue (see Fig. 1), consisting of a controlled number (N) of individual laser pulses of very short duration (0.3 - 0.6 ms), with cumulative fluences (F_s) below the ablation threshold.

In spite of the technique's "smoothness" the regenerative response of the tissue to this therapy is significant [32–34]. This characteristic of the non-ablative Fotona SMOOTH® therapy has been attributed to the unique dual-regenerative characteristic of this Er:YAG laser modality, whereby the standard deep-thermal coagulation resulting from the long-duration temperature exposure is accompanied and enhanced by an additional superficial heat-shock triggering mechanism resulting from the exposure of the superficial tissue to very short duration-high temperature peaks.

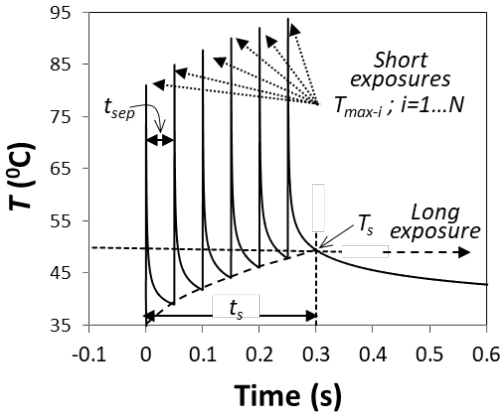

Fig. 1: Fotona SMOOTH® Er:YAG laser's pulse sequence (example for N = 6 is shown) and resulting temperatures [31]. Due to fast thermal diffusion from the heated ≈ 3 μm thin superficial tissue layer, the duration of the thermal exposure (t_{exp}) to high temperature peaks T_{max-i} separated by t_{sep} is extremely short (t_{exp} < 1 ms). The temperature T_s is the final sequence temperature, representing the long-duration temperature exposure.

In this review, we focus on the mechanisms, effects, and novel developments in Fotona SMOOTH-induced hyperthermia used in rejuvenation and functional regeneration of human skin and mucosa.

II. MATERIALS AND METHODS

In this section, the basic characteristics of hyperthermia, hormesis and the biophysics of tissue damage are presented.

a) Cellular response to hyperthermia

It is well known that hyperthermia induces a multitude of complex and integrated signaling pathways. In mammals, exposure to temperatures above 40-41°C in the seconds-to-minutes range results in cellular damage due to partial denaturation of folded proteins [35,36]. At the same time, temperature elevations up to only about 39°C already activate heat-sensitive receptors in the cellular membrane, mostly belonging to the TRP receptor family [37]. Some of these receptors are also responsive to light and mechanical stimuli, and different stressors can induce activation of different subsets of receptors. Both protein denaturation and TRP receptor activation further activate the expression of a class of proteins known as heat shock proteins (HSP) – the main players in orchestrating the so-called heat shock response. The activation of HSPs during and following hyperthermia has been found to perform a dual function [37–39]: i) HSPs activated and synthesized within the cells act as molecular chaperons or guardians of the proteome, working to repair (refold) partially denatured proteins, facilitate the degradation of irreversibly denatured proteins, and inhibit protein aggregation, protecting cells from further damage.; and ii) extracellularly released HSPs act as a danger or an alarm signal to adjacent tissue, leading to biomodulation of neighboring cells and stimulation of the innate immune system (see Fig. 2).

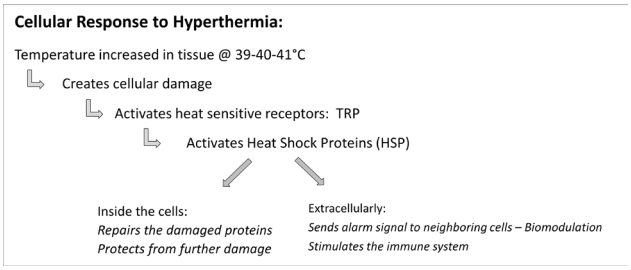

Fig. 2: Cellular Response to Hyperthermia. Heat shock proteins (HSP) get generated inside damaged cells, as well as extracellularly by the alarm signals sent to neighboring cells.

Once triggered, the enhanced synthesis of heat shock messages persists for about 24 hours following the heat shock, with the rate of synthesis reaching its peak after around 6 hours (see Fig. 3) [40].

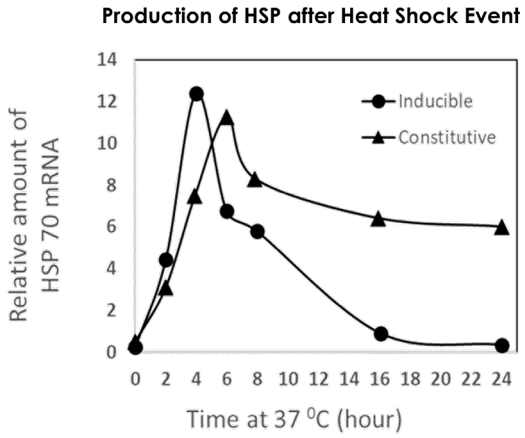

Fig. 3: Kinetics of the heat shock protein induction following a 15 min heat shock at 45°C [17]. Once the heat shock response gets triggered during a 15 min heat shock, most of the HSPs' upregulation occurs during the hours immediately following the heat shock. Both the inducible variants of HSPs, which are normally expressed only in response to stress, and the constitutive variants of HSP, which are constantly expressed in cells, follow a similar delayed timeline of gene upregulation in response to a short-duration heat shock.

Despite their name, HSP proteins can be expressed in response to any kind of stress signal that compromises cell survival. This is likely because different stressors have been shown to cause similar biochemical reactions, such as the creation of reactive oxygen species (ROS) and protein denaturation. Both ROS and the presence of denatured proteins have been shown to directly induce HSP expression. In addition, HSP expression is also induced following activation of different environment-sensing cell membrane receptors, such as the above-mentioned TRP thermoreceptors [37,38].

b) Hormesis

Aging is characterized by a decrease in adaptive capabilities due to progressive failure of cellular maintenance and repair mechanisms. Rejuvenation of human tissues, characterized by functional regeneration of their structure and function, can be stimulated by exposing the tissue to brief periods of mild stress, which stimulates the activation and synthesis of repair and regenerative mechanisms [11][41]. Stimulative response to mild doses of otherwise harmful conditions is known as hormesis. Hormesis follows the rules of the "biphasic dose–response" [42,43], a principle that states that there is a specific "optimal" stress dose, which provides a therapeutic and positive action on the targeted tissues, while in high doses, the stimulus/therapy may lead to inhibitory and even damaging effects.

c) Critical temperature

When stress is delivered by means of hyperthermia, the stress dose is defined by the value of elevated temperature (T) and the thermal exposure time (t_{exp}), i.e., the duration of the tissue's exposure to the elevated temperature.

For typically studied thermal exposure times (seconds to minutes range), the tissue injury grows exponentially with elevated temperature, and linearly with the time of exposure [44,45]. However, the most recent research studies involving extremely short exposure times (micro- to millisecond range) demonstrated that the critical temperature (T_{crit}, representing the temperature at which the concentration of the undamaged tissue is reduced to approximately 35%) follows the VHS (Variable Heat Shock) dependence on the exposure time, as shown in Fig. 4 [46,47].

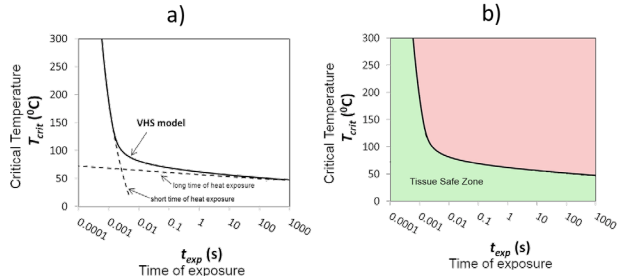

Fig. 4: a) VHS model's critical temperature as a function of the exposure time [48]. Damage depends on the LEVEL of temperature and the TIME it is exposed to the tissue. The Variable Heat Shock (VHS) model is built by 2 curves: short heat exposure time and long heat exposure time. b) The maximum volume of Heat Shock Proteins (HSP) is created when the temperature generated is slightly below the level that produces significant, but reversible cellular damage (the green tissue safe zone closest to the VHS model curve).

The HSP synthesis was shown to be largest when the elevated temperature was just slightly below the temperature characterized by a significant but still reversible protein degradation [36]. As the aim for the hyperthermic biomodulation for rejuvenation is to be minimally invasive and optimally effective, the elevated temperature needs to be high enough to elicit the regenerative response, but far enough from the critical temperature to avoid extensive tissue damage.

The other component of the thermal stress dose, the thermal exposure time t_{exp}, can be broken down to 3 phases: i) a temperature ramp-up heating phase during which the temperature reaches its maximum, ii) a constant elevated temperature phase, and iii) a temperature ramp-down cooling phase during which the temperature returns back to its initial temperature T_0 [46,49].

As can be seen from Fig. 4, the duration of thermal exposure is of critical importance for reaching the optimal hormetic dose. While the first two phases of thermal exposure can be controlled to a certain degree by the duration and method of heat delivery, the cooling phase is determined predominantly by the rate of the heat flow away from the heated tissue volume. This is because once the tissue is heated up, its temperature persists after the heat delivery has ended until the tissue is cooled down by the process of heat conduction into the surrounding colder volume. It is therefore the thermal conduction process rather than the duration of the heat delivery itself that typically sets the lower limit for the achievable duration of a thermal pulse [31,46,48]. The larger the volume of the heated tissue, the longer is the cooling time, and consequently also the exposure time.

Since most published studies on heat shock tissue effects use long-exposure hyperthermic methods, the volume of the heated tissue is large, requiring a long time to cool down. As a result, most critical temperature experiments have been made for longer thermal exposures above 1 s, mostly in the minutes range [46]. As is shown further below, using lasers for inducing hyperthermia provides a unique possibility to perform hormetic therapies with shorter thermal exposures and therefore also with higher treatment temperatures.

III. RESULTS

In this section, the basic principles of hormetic hyperthermia are applied to situations where hyperthermia is induced by a laser.

a) Biophysics of laser-induced hyperthermia

A major advantage of performing hyperthermia using lasers is the ability to control the volume of the heated tissue, and therefore the thermal exposure time, by the wavelength of the laser light. Namely, when using laser light to heat up the treated tissue, the volume

of the tissue exposed to the elevated temperature is limited by the optical penetration depth (δ) of the delivered laser light within the tissue. Approximately, the time required for the laser-heated tissue layer to cool down by heat diffusion into the deeper lying bulk tissue is equal to $t_{cool} = \delta^2/D$, where D = 0.1 mm²/s is the thermal diffusivity of the soft tissue. This time represents the shortest exposure time achievable by a particular laser wavelength. The table below shows approximate minimal exposure times ($t_{exp} = t_L + t_{cool}$) achievable by some of the common medical lasers operating at their typical pulse durations (t_L).

Table 1: Estimated minimal exposure times as achievable by common medical lasers, characterized by different optical penetration depths δ.

Laser	Wavelength (μm)	δ (mm)	t_{exp} (s)
Nd:YAG	1.064	10	15
Nd:YAP	1.34	0.6	4
Diode	1.47	0.4	1.5
CO_2	10.6	0.02	0.01
Er:YAG	2.94	0.003	0.0006

The corresponding critical temperatures based on the VHS model are presented in Fig. 5.

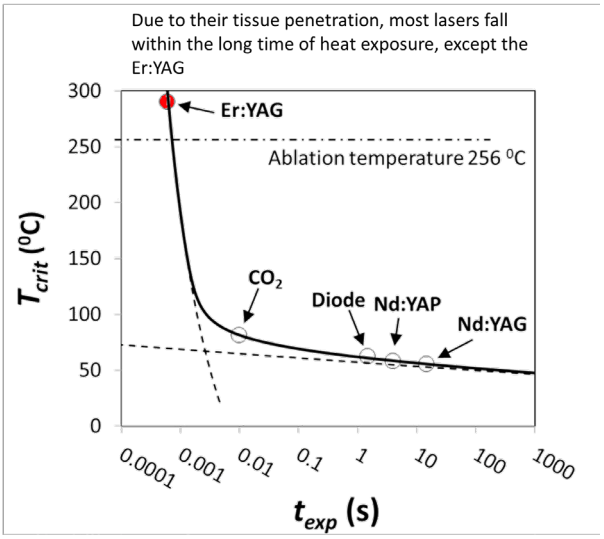

Fig. 5: Critical temperatures T_{crit} (presented by grey circles), belonging to shortest exposure times achievable by different laser wavelength devices as presented in Table 1. Lasers that are not highly absorbed by water will penetrate deeper in the tissue. Once the heat has penetrated deeply it will dissipate slowly, providing a long time of heat exposure. The Er:YAG, with only 3μm of optical penetration in tissue, has a very short heat exposure time (cools very quickly) and is the only laser that can safely elevate skin temperatures above 100°C. Even when the ablation temperature of 256°C (represented by the dashed line) is reached, the skin cools down immediately, and this prevents any irreversible damage to deeper tissue, keeping the treatment within a safe hormetic zone [46].

As can be seen from Table 1 and Fig. 5, the Er:YAG laser wavelength of 2.94 μm is the only wavelength with which it is possible to perform intense heat-shock biomodulation (i-HBM). The Er:YAG laser with its extremely short optical penetration depth within the human soft tissue of about 3 μm allows human cells to be safely exposed to temperatures above 100°C and even up to the ablation temperature of 256°C, owing to its uniquely short thermal exposure time. The importance of this characteristic can be seen in Fig. 6, which shows the clinical effect on human skin following a single pulse with 2.94 μm Er:YAG laser and with 1.34 μm Nd:YAP laser, both delivered with single-pulse fluences reaching the same skin ablation threshold temperature of 256°C [46]. Despite the same maximal temperature, the longer cooling time following the Nd:YAP laser irradiation results in a significantly longer thermal exposure time and consequently a higher dose and more extensive damage to the tissue, while the Er:YAG laser's pulsed-induced hyperthermia remains well within the safe hormetic dose window.

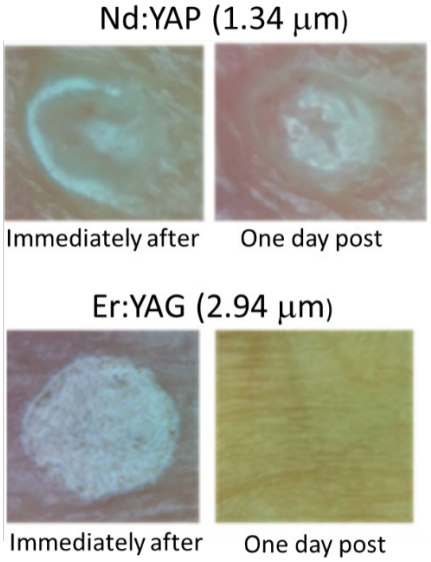

Fig. 6: Observed clinical effect on human skin following a single pulse of the same duration with Er:YAG and Nd:YAP, both reaching the skin ablation threshold of 256°C [46]. The longer cooling time of Nd:YAP results in long thermal exposure with extensive damage while Er:YAG remains safely in the hormetic dose window thanks to its fast cooling time.

b) Intense Heat-Shock Biomodulation (i-HBM)

When skin or mucosa is treated with a laser, a wound-healing response is triggered. The response to injury is largely dependent on the extent of tissue damage. In general, the skin wound healing response consists of three distinctive overlapping phases – inflammation, proliferation, and remodeling. Although dermal fibroblasts are critically important in all steps, epidermal keratinocytes also act to recruit, stimulate, and coordinate the actions of multiple cell types

involved in healing [50–52]. Keratinocytes and fibroblasts communicate with each other via double paracrine signaling loops, known as cross talk or dynamic reciprocity and coordinate their actions to restore normal tissue homeostasis after wounding [52, 21]. In response to paracrine signaling from keratinocytes and activated extracellular signaling molecules, fibroblasts respond with increased proliferation, migration to the injured site, activation of collagen synthesis and cross-linking to form new extracellular matrix [50].

A spatially localized, laser-generated supra-physiological level of heat can induce a transient heat shock response, characterized by the activation of HSPs and other alarm signals, initiating temporary changes in cellular metabolism that result in the release and production of growth factors and an increase in the rate of cell proliferation [44,45,53]. This indirect tissue regeneration mechanism is based on stimulating signal transduction processes for transcription factor activation, gene expression and fibroblast growth, leading to cell proliferation and ECM synthesis, restoring healthy epithelial structure. Low amounts of ROS that have been shown to follow laser-induced hyperthermia, can also stimulate wound healing pathways, resulting in increased proliferation of keratinocytes and fibroblasts, which in turn generate collagen and other ECM proteins [54–57].

The Er:YAG laser possesses a unique advantage when considering generating spatially localized transient heat-shocks on a tissue's surface. As can be seen from Fig. 5, the Er:YAG laser wavelength of 2.94 µm is the only wavelength with which it is possible to achieve heat shocks in the sub-millisecond exposure duration range. Therefore, it is only with this wavelength that it is possible to perform intense heat-shock biomodulation (i-HBM) by delivering intense (T_{max} = 85°C - 250°C) heat shocks to the tissue surface without exceeding the critical temperature (T_{crit}) for tissue damage.

It is important to note that although the heat-shock triggering is caused by the absorption of Er:YAG photons at the tissue surface, the processes in the cells deeper within the tissue are not modulated by direct thermal and non-thermal action of photons, but rather by the alarm signals triggered by the very short intense heat-shocks produced from the superficially absorbed Er:YAG photons (Fig. 7). As has been shown, a low intensity irradiation of skin with Er:YAG can induce tissue remodeling gene expression and collagen production in the dermis, although the initial laser pulses only directly reach the superficial keratinocytes [33,58].

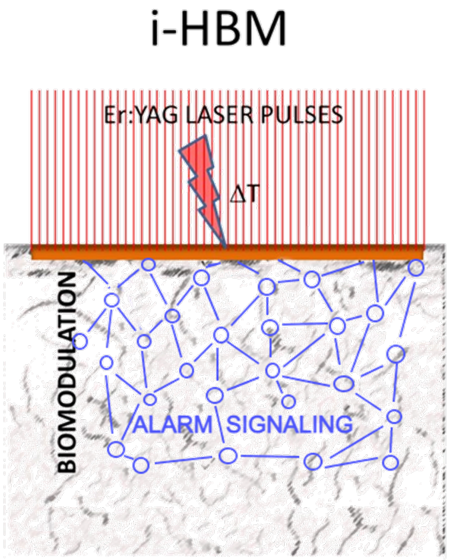

Fig. 7: Fotona SMOOTH® mode i-HBM. The i-HBM is generated by intense, very short thermal pulses of up to 250°C that create a superficial heat-shock event. This stress will generate a cascade of inter-cellular communication that carries alarm signals that triggers the body's biomodulative repair mechanisms, including fibroblast proliferation, increased collagen and vascularization. Lasers with deeper skin penetration are not capable of producing this effect since they are limited by the maximally allowed temperature at the tissue surface and the patient's pain threshold.

It is also to be noted that since the elevated temperatures during the intense heat shocks remain significantly below the critical temperature for tissue damage, the i-HBM stress reaction gets triggered even in the absence of any significant protein denaturation. This phenomenon resembles the reported triggering of the immune response to skin grafts in mice, in spite of the absence of an actual injury [13,59]. We attribute the i-HSB alarm triggering to the fact that protein denaturation (injury) takes place mainly during the actual duration of the heat shock, as can be concluded from the strong dependence of the critical temperature on the exposure time (Fig. 5). On the other hand, most of the HSPs' accumulation due to the upregulated protein synthesis occurs during hours after the heat shock has already ended (see Fig. 3). This indicates that the dependence of the HSP's induction on the exposure time is not as strong compared to the HSP's dependence on the process of protein denaturation (i.e., tissue injury). Intense heat shocks of very short duration can thus trigger alarm signals even at minor levels of protein denaturation that do not significantly damage the tissue.

The i-HBM is also self-regulating in terms of keeping the therapy within the safe hormetic zone since the ablation temperature of T_{abl} = 256°C is below the critical temperature of about T_{crit} = 290°C. Therefore, in case the heat shock temperature would reach T_{abl}, the ablation process would cool down the skin and prevent

further temperature growth, limiting the maximal temperature to $T_{abl} < T_{crit}$.

Figure 8 graphically depicts the general principle of the Er:YAG laser's superficial i-HBM triggering of the tissue regeneration process. The stimulation mechanism of superficially generated heat shocks resembles the observed stimulation effect of exogenous free radicals (ROS) at the tissue surface in plasma medicine [6,7]. A major difference is that with the plasma treatment, it is extremely difficult to deliver just the right ROS dose before causing an irreversible tissue injury. On the other hand, the i-HBM treatment is self-regulating, keeping the stress dose safely within the hormesis zone [31].

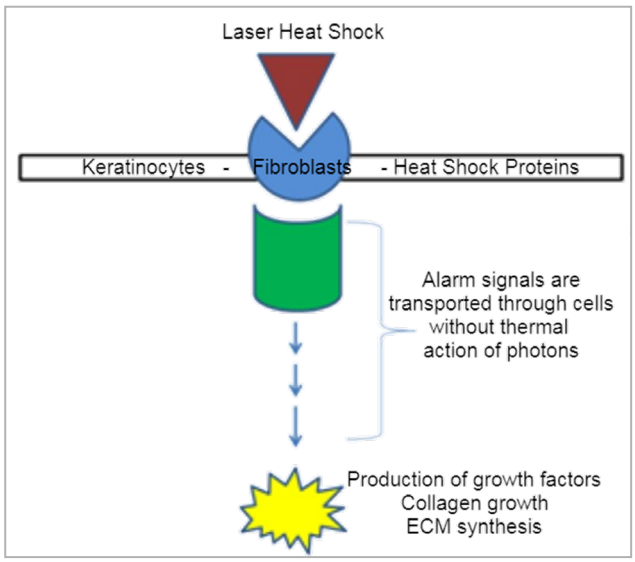

Fig. 8: The principle of intense heat-shock biomodulation (i-HBM). When the tissue receives stress signals, dermal fibroblasts and epidermal keratinocytes cross-talk to initiate a wound healing process. A laser can generate a short-duration Heat Shock trigger, creating alarm signals that will activate HSPs, releasing growth factors and increasing cell proliferation and development of a new extracellular matrix (ECM).

IV. DISCUSSION

a) Intense heat-shock biomodulation (i-HBM) with Fotona SMOOTH® therapy

The non-ablative Fotona SMOOTH® Er:YAG laser therapy (see Fig.1) for skin and mucosa has been shown to result in a significant regenerative response of the tissue [34]. For example, in a recent immunohistology study [32], the tissue regeneration process was observed to persist for more than 3 months following the Fotona SMOOTH® therapy, with the total amount of positive fibroblasts (i.e., newly synthesized pro-collagen type I fibroblasts) that extended up to 1600 µm within the skin, being measured to double at the 21-day follow up and to increase by 4-fold at the 3-month follow up (Fig. 9) [32].

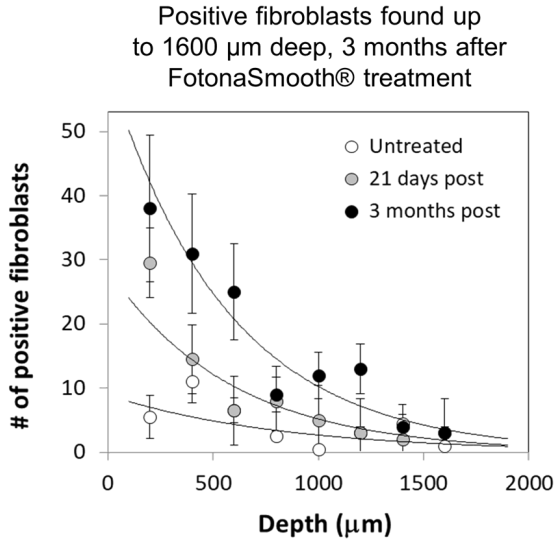

Fig. 9. Measured spatial distribution of pro-collagen type I positive fibroblasts for untreated skin, and at 21 days and 3 months following the Fotona SMOOTH® treatment [32]. The curved lines are a visual aid only.

The observed pronounced regenerative effects are attributed to the unique Er:YAG laser-generated heat dynamics resulting in a dual tissue-regeneration phenomena (see Fig. 10):

Intense heat-shock biomodulation (i-HBM) (Fig.10a): Intense short-duration thermal pulses resulting from individual laser pulses, with peak temperatures (T_{max-i}) up to 250°C at the surface (see Fig. 10a), leading to superficial heat-shock alarm triggering of the regeneration of deeper-lying tissues [31,32,46].

Deep hyperthermia/coagulation (Fig.10b): Slow gradual build-up of the spatial temperature distribution over the total duration of the sequence, extending several hundred microns deep into the tissue, with the long-duration surface temperatures (T_s) typically below 48-52°C [31,46].

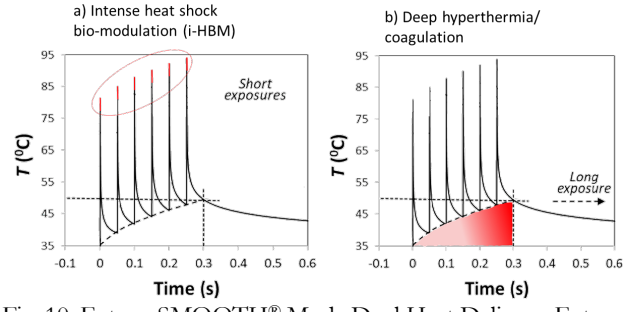

Fig. 10. Fotona SMOOTH® Mode Dual Heat Delivery. Fotona SMOOTH® emissions produce 2 very distinct types of heat depositions at different tissue depths: a) Intense Heat-Shock Biomodulation (i-HBM): High peak temperatures on the tissue surface that last for a very short time (short exposure); b) Deep hyperthermia/coagulation: lower levels of gradually increasing deeper heat deposition that has longer exposure time.

The intense heat shock biomodulation (i-HBM), characterized by intense temperatures pulses of up to about 250°C, represents an important contributing mechanism for the observed positive clinical effects of Fotona SMOOTH® procedures. Although the intense heat shocks are limited to keratinocytes on the skin surface, it is known that not only fibroblasts, but also the superficially located keratinocytes can be initiators of the wound healing process following epithelial injury [51,60]. The critical contribution of i-HBM to the total Fotona SMOOTH®-induced regeneration process has been recently evaluated by comparing treatment efficacies of three Fotona SMOOTH® biomodulation modalities, in terms of the number of generated positive fibroblasts per delivered laser fluence [32]. Figure 11 shows the results of this in-vivo immune-histology study for protocols designed to generate either predominantly i-HBM or predominantly hyperthermic biomodulation, and for a protocol combining the i-HBM and hyperthermic biomodulation effect. In order for these three protocols to be comparable, the cumulatively delivered fluences during each of the three protocols were set to be just below the patient's pain threshold. In order to prevent deeper heat deposition, the i-HBM protocol consisted of only N = 12 intense Er:YAG laser pulses, while the hyperthermic protocol consisted of N = 120 low-fluence pulses resulting in an approximately 8-times deeper heat of penetration and about a 20-times lower level of estimated heat shock triggering. And the combined protocol was performed with N = 66 medium-fluence pulses, each being sufficiently intense to generate significant i-HBM alarm signaling in addition to deep hyperthermia.

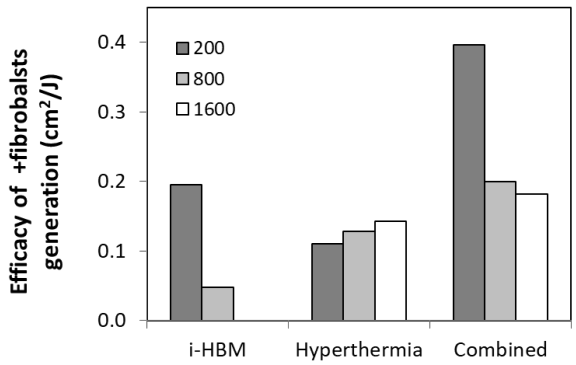

Fig. 11. Comparison of treatment efficacies of three Fotona SMOOTH® biomodulation modalities, in terms of the number of generated positive fibroblasts per delivered laser fluence, for skin depths of 200 μm, 800 μm and 1600 μm. The results of an in-vivo immuno-histology study [32] are shown for protocols designed to generate either predominantly i-HBM or predominantly hyperthermic biomodulation, and for a protocol combining the i-HBM and hyperthermic biomodulation effect. Results show that combining the i-HBM and hyperthermic biomodulation mechanisms results in a significant synergistic enhancement of tissue regeneration.

As can be seen from Fig. 11, a considerably higher new fibroblast-generation efficacy was observed at the skin surface for the prevalently intense heat-shock protocol in comparison to the hyperthermic protocol. Even more importantly, the results show that combining the i-HBM and hyperthermic biomodulation mechanisms results in a significant synergistic enhancement of the tissue regeneration.

b) Comparison of PBM, PLLT and i-HBM

Although the intense heat shock biomodulation (i-HBM) is performed using laser light (i.e., photons), this mechanism of stimulating tissue response is different from photo-biomodulation (PBM) and PLLT. With PBM, the tissue gets stimulated directly by the absorbed low-intensity photons, with the intensity of light being so low that there is no appreciable increase in the tissue temperature (Fig. 12a). Similarly, with PLLT, the laser light gets absorbed throughout the treated tissue's volume, however, due to the higher light density the tissue also gets moderately heated up, with the tissue temperature increase (ΔT) falling exponentially with depth within the tissue (see Fig. 12b). The typical temperature increase during PLLT is several degrees [49], however, higher temperature PLLT treatments in the range of 45-55°C are also possible.

On the other hand, with i-HBM, the photons are absorbed only at the surface of the tissue being treated, with the resulting short-duration heat shock temperatures at the surface in the range of 80-250°C. The deeper-lying tissue does not get stimulated directly by photons, but indirectly by alarm signals resulting from the superficially generated very intense heat shocks (Fig. 12c).

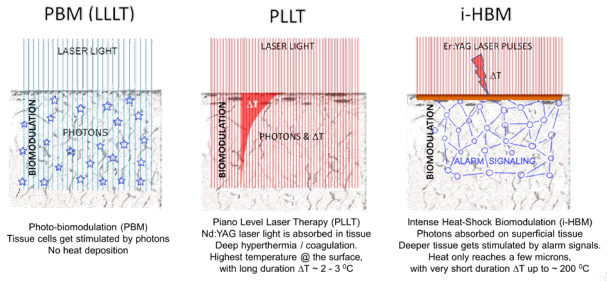

Fig. 12. Comparison of PBM, PLLT and i-HBM. Left: Photo-biomodulation (PBM). The tissue cells get stimulated directly by low intensity photons. Middle: Piano Level Laser Therapy (PLLT). The laser light is absorbed throughout the treated tissue's volume, with the resulting tissue temperature increase (ΔT) falling exponentially with depth within the tissue; Right: Intense Heat-Shock Biomodulation (i-HBM). Photons are completely absorbed within a several-microns-thick layer of the superficial tissue. The deeper-lying tissue does not get stimulated directly by photons but indirectly by alarm signals resulting from the superficially generated very intense heat shocks.

When considering using PLLT instead of i-HBM for stimulating superficially located (1-2 mm deep) tissue layers, it is important to note that the maximal laser intensity allowed during PLLT is limited by the maximally allowed temperature (T_{max}) at the tissue surface, where the temperature increase is highest. This temperature is limited by the pain threshold, and ultimately under local anesthesia by the critical temperature T_{crit} shown in Fig. 4. This maximal temperature limitation cannot be significantly overcome by using a laser wavelength with a penetration depth of $\delta \leq 1$ mm. This can be seen from Fig. 13, which shows the temperature distribution within the tissue for an exemplary diode laser wavelength with a penetration depth of $\delta = 0.4$ mm. For this penetration depth, the critical temperature of 62°C (Fig. 7) is not significantly higher than $T_{crit} \sim 55$°C for the more deeply penetrating ($\delta \sim 10$ mm) Nd:YAG.

Therefore, the i-HBM is currently the only laser method that enables very intense (with maximal temperatures up to ~ 250°C) stimulation of superficially located tissue layers, as required, for example, for tissue tightening. On the other hand, when pain reduction affecting deeper lying muscles, or very deep body sculpting is desired, the deeply penetrating Nd:YAG laser may be the wavelength of choice. This applies especially since in this case the superficial maximal temperature limitation can be overcome by cooling the tissue surface [65], while this approach would be counter-productive when attempting to stimulate superficial layers by heat.

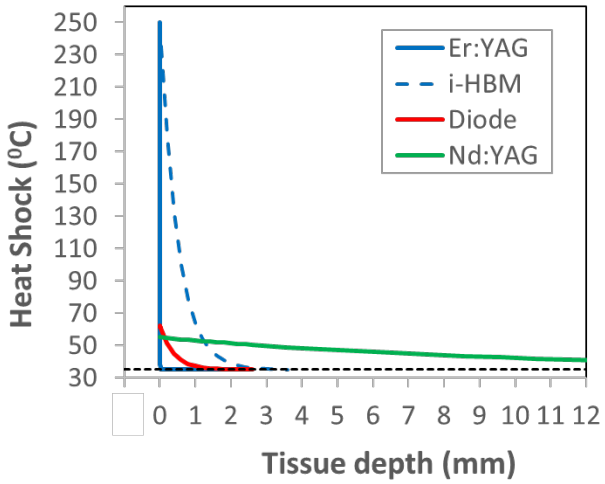

Fig. 13. Approximate temperature distributions within the depth of skin or mucosa, for Nd:YAG laser (1064 nm; δ = 10 mm; green line), diode laser (1440 nm; δ = 0.4 mm, red line) and Er:YAG (2940 nm; δ = 0.003 mm, full blue line). For comparison, the dashed blue line illustrates the intensity and spatial reach of the i-HBM mechanism resulting from intense heat shocks generated by Er:YAG laser pulses.

In conclusion, when attempting to regenerate tissue cells located within a depth of approximately 1-2 mm, the i-HBM method is a preferred method in comparison to the direct PLLT. The reasons are as follows:

- The measured range of i-HBM is optimal for stimulating the initial, about 1 mm thick layer of tissue (see Fig. 7).
- The intensity of heat shocks can be safely increased up to the ablation temperature of 256°C.

The i-HBM method is self-regulating, ensuring that the treatment always remains within the safe and effective hormetic zone. With PLLT, the safe hermetic window can be easily exceeded, especially when attempting to perform a more intense biomodulation.

The extremely short duration superficial heat shocks are painless [48]. The patient's discomfort results only from the slow gradual build-up of the spatial temperature distribution (deep hyperthermia) over the total duration of the heat shock sequence.

c) Clinical application of Fotona SMOOTH® dual-tissue regeneration

There are four clinical areas where the Fotona SMOOTH® protocol with combined effects of i-HBM and deep hyperthermia (see Fig. 11) is most commonly being performed: skin tightening, gynecology, ENT and intra-oral aesthetics. For skin tightening [31,32,64], special scanners have been introduced that automatically distribute high repetition rate Er:YAG laser pulses over a larger skin treatment area, in a manner which ensures that each treatment spot experiences irradiation by a large number of pulses at an appropriately reduced repetition rate [48,64]. This prevents any undesired local superficial temperature build-up, while keeping the overall area treatment speed at the laser's maximal output power capability. Using this technique, it is possible to deliver relatively high cumulative fluences, as required for deep skin coagulation, without the need for local or general anesthesia, since the delivered energy can be distributed over a large number of low-fluence pulses. The remaining treatments consist of non-ablative resurfacing of the mucosal tissue to thermally initiate a process of cell activation, production of extracellular matrix, and tissue remodeling that continues for up to 6 months following a treatment. The treated mucosa undergoes rejuvenation consisting of an increase in epithelial thickness, fibroblast proliferation, an increase in the amount of collagen, and vascularization [34,14], resulting in improved tissue tightness and elasticity. The rejuvenation effects of the minimally invasive Fotona SMOOTH® resurfacing procedure, which represents an alternative to more aggressive and risky surgical procedures, are temporary, lasting for up to several years,

after which an additional touch-up procedure may be needed. In gynecology, the tissue rejuvenation results in an alleviation of symptoms of the genitourinary syndrome of menopause (GSM), pelvic floor dysfunctions (stress urinary incontinence (SUI), pelvic organ prolapse (POP) and other urinary symptoms such as frequency, urgency and dysuria) as well as vaginal relaxation syndrome [14–23] and the treatment of lichen schlerosus. In ENT, non-ablative resurfacing of soft palate, uvula and tonsillary regions has been reported to significantly reduce the symptoms of chronic snoring-related sleep disorders [24–28]. And in aesthetics, intraoral Fotona SMOOTH® resurfacing has been demonstrated to represent a safe, painless, and effective treatment option for accentuated nasolabial fold (NLF) wrinkles [29,30]. Finally, studies have shown that Fotona SMOOTH can be used for non-surgical stimulation of hair growth renewal, reducing the need for surgical transplantation and potential post--operative complications [66,67].

V. CONCLUSIONS

The Fotona SMOOTH® treatment represents a unique combination of the actions of two regenerative mechanisms involving both a short-exposure intense heat-shock biomodulation (i-HBM) and a long-exposure hyperthermic biochemical process, characterized by a medium- to long-term activation of collagen synthesis. Although the i-HBM is activated by the absorption of Er:YAG photons at the tissue surface, the processes in the cells deeper within the tissue are not modulated directly by the thermal and non-thermal action of photons, but rather by alarm signals triggered by very short intense heat-shocks produced from the superficially absorbed Er:YAG photons. The intense heat-shock biomodulation is made possible by the unique absorption characteristics of the Er:YAG laser wavelength, enabling very intense short-duration heat shock exposures, with elevated temperatures remaining below the critical temperature for irreversible tissue damage.

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