Received Date : 27-Nov-2015

Revised Date : 28-Feb-2016

Accepted Date : 15-Apr-2016

Article type : Viewpoint

TITLE *Viewpoint*

Photobiomodulation devices for hair regrowth and wound healing: a therapy full of promise but a literature full of confusion

AUTHORS Charles Mignon[+], Natalia V. Botchkareva[+], Natallia E. Uzunbajakava[*], Desmond J. Tobin[+]

AFFILIATIONS [+]Centre for Skin Sciences, Faculty of Life Sciences, University of Bradford, Bradford, West Yorkshire, BD7 1DP, United Kingdom

[*]Philips Research, High Tech Campus 34, 5656 AE Eindhoven, The Netherlands

DATE Feb, 2016

TO Experimental Dermatology

CORRESPONDING AUTHOR Natallia E. Uzunbajakava

High Tech Campus 34

5656 AE Eindhoven

The Netherlands

E natallia.uzunbajakava@philips.com

T 0031 (0) 639768141

FUNDING AND DISCLOSURES

The costs made to conduct this study were paid by European Marie-Curie Actions Programme, Grant agreement no.: 607886, where Charles Mignon is an Early Stage Researcher and Natallia E. Uzunbajakava, Natalia V. Botchkareva and Desmond J. Tobin are the members of a scientific supervisory team.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/exd.13035

Natallia E. Uzunbajakava is an employee of Philips Electronics Nederland B.V. and received salary for this study.

KEY WORDS photobiology, cryptochromes, opsins, optical parameters, skin and hair regeneration

ABBREVIATIONS and DEFINITIONS

ATP	Adenosine triphosphate
Coherence	Degree of similarity between the phase and frequency of the optical wave emitted by a light source
DMEM	Dulbecco's Modified Eagle Medium
ERK	Extracellular signal regulated kinases
IR	Infrared radiation, [700 1e6] nm
Irradiance	Optical power impinging over a defined area ($W.m^{-2}$)
NADPH	Nicotinamide adenine dinucleotide phosphate
NIR	Near infrared radiation, restricted part of the IR [700 2500] nm
NO	Nitric oxide
Optical energy	Energy emitted by a light source in the form of photons (J)
Optical power	Optical energy emitted per unit time by a light source (W)
Optical Transport	Propagation of light photon in a defined medium
Phase	Fraction of a complete cycle corresponding to an offset in the displacement from a specified reference point at time t = 0 (degree or radian)
Polarisation	Direction of variation of the electro-magnetic field
Pulsing	Emission of light characterised by successive emission and stop period
Radiant Exposure	Optical energy received by a defined area ($J.m^{-2}$)
ROS	Reactive Oxygen Species
TRPA1	Transient receptor potential cation channel, subfamily A, member 1

UV Ultra-Violet radiation, [10 400] nm

Vis Visible radiation, [400 800] nm

Abstract

Photobiomodulation is reported to positively influence hair regrowth, wound healing, skin rejuvenation, and psoriasis. Despite rapid translation of this science to commercial therapeutic solutions, significant gaps in our understanding of the underlying processes remain. The aim of this review was to seek greater clarity and rationality specifically for the selection of optical parameters for studies on hair regrowth and wound healing.

Our investigation of 90 reports published between 1985-2015 revealed major inconsistencies in optical parameters selected for clinical applications. Moreover, poorly understood photoreceptors expressed in skin such as cytochrome c oxidase, cryptochromes, opsins, may trigger different molecular mechanisms. All this could explain the plethora of reported physiological effects of light.

To derive parameters for optimal clinical efficacy of photobiomodulation, we recommend a more rational approach, underpinning clinical studies with research of molecular targets and pathways using well-defined biological model systems enabling easy translation of optical parameters from *in vitro* to *in vivo*. Furthermore, special attention needs to be paid when conducting studies for hair regrowth, aiming for double-blind, placebo-controlled randomized clinical trials as the gold standard for quantifying hair growth.

Introduction

The uptake of energy-based home-use devices for medical treatment and personal care is increasing rapidly, due to the appeal of their practicality, simplicity of use, and efficacy (1). Within this trend, skin health attracts particular interest, underlined by a large burden of skin and hair diseases (2).

The non-invasive nature of light, free of potential systemic side-effects, is a very attractive treatment modality, where skin interaction with light in the ultraviolet (UV) to infrared (IR) range with subsequent photochemical, photothermal, and photomechanical effects, drives the therapeutic effects.

Professionals have already successfully exploited the benefits of photothermal, photomechanical and photochemical light-based treatment (3, 4). Some examples include: photothermal effects for skin rejuvenation (5) and for removal of hair and vascular lesions (6); photomechanical skin rejuvenation using laser-induced optical breakdown (7, 8); PUVA-, UVB-, and blue-light-based photochemical treatment of psoriasis (9, 10).

Photobiomodulation describes the therapeutic use of visible (Vis) to NIR light absorbed by endogenous chromophores, triggering non-thermal, non-cytotoxic, biological reactions through photochemical events (11). In November 2015 the term "Photobiomodulation Therapy" is planned to be formally adopted as an official NIH U.S. National Library of Medicine (MeSH) term (12).

The field of photobiomodulation of skin and its appendages, kick-started by a landmark study on hair regrowth in the late 1960s (13), has now expanded to include applications for hair cycle modulation (14), hair regrowth (15), wound healing (16-18), psoriasis (19), skin barrier recovery (20), stem cell regenerative therapy (21), where several books were published on the topic summarizing experimental studies and basic mechanisms (22-24).

As of today, more than twenty light-based devices based on photobiomodulation have been cleared to market by the FDA for the management of hair regrowth (15, 25-27). What is truly fascinating about light-based therapy is that not only is its efficacy reported to be similar to that of existing drugs for hair growth (e.g., minoxidil and finasteride), but that it may even be superior being inherently free of potential side-effects (28).

However, we are very much in the dark regarding how light is 'received' in so-called non-photosensitive tissues (i.e. extra-ocular). We have reason to believe that this light reception may involve the much-vaunted but still inconclusive cytochrome c oxidase (29), nitrosated proteins regulating nitric oxide (NO) bioactivity (30, 31), circadian rhythm regulator flavo-protein cryptochrome (32) and the more recently-considered opsin family photoreceptors (20, 33-36). The field is further complicated as pathways could also be triggered indirectly, e.g. by ROS with the activation of *ERK or production of growth factors and cytokines (37-40).*

Indeed, the difficulty to interpret the diversity of reported photobiomodulation effects may reflect the multiplicity of receptors and downstream mechanisms that can be triggered by light reception.

The purpose of this literature review is to guide clinical and basic science investigators through the last 30 years of photobiomodulation research to aid in the design of more robust clinical studies for more efficacious translation to the clinic (see Supplement, Figure S2). Here we discuss four key elements of current knowledge including; rationality of selected optical parameters; potential photoreceptors; downstream reactions; and the strategies used by industry to translate photobiomodulation science into effective commercial devices.

Inconsistencies in optical parameters applied during *in vitro* studies

Typically, optical parameters for light therapy such as wavelength, irradiance, and radiant exposure are obtained from *in vitro* and *ex vivo* studies. Translation to *in vivo* conditions is needed before the implementation of associated devices in clinical settings. *In vitro* studies focusing on skin and hair health are most often performed on isolated cells including fibroblasts (41), keratinocytes (30), melanocytes (42), mesenchymal stem cells (43), hair follicle dermal papilla cells (14) and others (44, 45).

In the context of the heterogeneity of wavelengths of light reported in 60 studies here, one can see that data span the entire Vis to near-IR range (see Supplement, S1). There is some clustering around 420, 630, and 800 nm, with wavelengths close to 600 nm predominating. Moreover, variation of up to 2 orders of magnitude in irradiance (1 mW/cm^{-2} to 100 mW/cm^{-2}) and radiant exposure (1 J/cm^{-2} to 100 J/cm-2) is reported in 30 *in vitro* studies that use primary or immortalized keratinocyte and dermal fibroblasts (see Figure 1).

Other perplexing features of the current photobiomodulation literature pertain to myriad biological effects associated with the applied optical parameters. A single light parameter can be reported as effecting alternately stimulatory, inhibitory and neutral change for any given phenotypic readout (see figure 1). For example, studies looking at the effects of blue light on human epidermal keratinocytes (30, 34) have used similar but not identical wavelengths (i.e., 453 nm versus 410 nm) and still reported opposite effects on the expression of the keratinocyte differentiation markers such as keratin-1 and -10. This may suggest the existence of narrow wavelength 'windows'. It also reflects the importance of defining the target and culture conditions as they can impact the action of light, illustrated by the treatment of human melanocytes and mouse skin melanoma cells in the presence of retinal and riboflavin, respectively (36, 46).

The effect of other light parameters such as pulsing (47, 48), coherence (49) and polarization (50) have less commonly appeared in studies on photobiomodulation.

Diverse chromophores and photoreceptors mediating responses to light

As biologists we are eager to elucidate the first point of a photon perception in the cell. However there is a wide choice of chromophores and photoreceptors that could potentially mediate the physiological and ultimately, therapeutic effects of photobiomodulation. For example, relevant receptor molecules include photoactive pigments or chromophores like the flavins (51, 52), pterins (53, 54), retinal (55, 56), carotenoids (57) and several metal-containing centres such as hemes and cupredoxins (58, 59). These photoactive pigments represent the photoreactive site of larger molecules called photoreceptors. An extensive, though inexhaustive, list of photoreceptors includes cytochrome c oxidase (29), cryptochromes 1 and 2 (32), and opsin family proteins (I, II, III, IV, V) (20, 33, 34, 60).

Current opinion in the photobiomodulation research community contends that light is absorbed by mitochondrial cytochrome c oxidase around 420 nm, 600 nm and 850 nm (61-63). Photons interacting with this enzyme's metal centres are thought to trigger the increase of ATP via a proton gradient. The process involves the release of NO either via the photodissociation from the complex cytochrome c oxydase or via the catalysis of the reduction of nitrite to NO. The balance results in direct production of ATP and NO as well as ROS as by-product of the respiration metabolism. All are assumed to be responsible for the observed biological and therapeutic effects of light (64). This view is principally based on the work of Karu (29) and is widely

though not universally accepted (15, 26, 29, 65, 66). This is perhaps because we still do not fully understand the structure, optical properties, and function of this enzyme nor of its intermediate forms (67, 68).

The human cryptochromes (32), reported to be involved in circadian rhythm (69, 70), contain two key photoactive pigments, pterin and flavin, which define their absorption spectrum in the UV-blue spectral range (71) with two prominent bands around 350 nm and 420 nm. Their analogues in plants have a clear light-sensitive function (72). As gene transcription regulators, cryptochromes could be very interesting targets in photobiomodulation. Indeed, they have been found to be one of the main circadian clock effectors in mammals (72, 73) and to participate in the regulation of metabolism and immune responses (74, 75). It is currently hypothesized that light interaction with flavin in cryptochrome leads to its conformational change, permitting transcription factors to bind to the C-terminus. Also, redox reaction of flavins and therefore cryptochromes can be accompanied by ROS generation and signalling (76).

More recently, the opsin-family of G-protein coupled receptors responsible for light sensing by retinal cones and rods (77), have come to the attention of photobiomodulation researchers. They include OPN 1 (Short, Middle and Long wavelengths) in cones, OPN 2 (or Rhodopsin) (78) in rods, as well as non-visual opsins, such as OPN 3 or encephalopsin (79), OPN 4 or melanopsin (80-82) and OPN 5 or neuropsin (83). Peaks of OPN1 to OPN 5 absorption spectra span 380 nm to 570 nm (77, 79, 81, 83). Recently, expression of these selected opsin receptors was demonstrated in non-visual tissues, such as mouse aorta (35) and even in the human skin (60), melanocytes and keratinocytes (33, 34, 42, 60), making them an intriguing target for photobiomodulation for skin and hair.

Multiple downstream biomolecular reactions explaining physiological effects

In addition to understanding the role of existing versatile potential photoreceptors present in human skin, the next challenge of the growing field of photobiomodulation is to unravel the exact molecular reaction cascades that mediate the physiological effects of light.

Given the considerable existing complexity, one has to start somewhere. For example, the absorption of red light by cytochrome c oxidase is traditionally accepted to trigger numerous reaction cascades that alter cellular homeostasis (e.g., fluxes in pH, [Ca], cAMP, ATP, NO) (29). Blue light is often reported to photolytically generate NO and ROS from nitrosated proteins and NADPH oxidase, respectively. These can then modulate cell metabolic activity, vasodilatation and improve wound healing (30, 84). IR light has been shown to activate epidermal keratinocytes *in vitro* (85, 86) and to induce ROS production in mouse skin (65).

In addition to these more 'traditional' downstream cascades, new intriguing insights are emerging in the literature. Interestingly, red light has been reported to induce rhodopsin-mediated, phosphodiesterase-dependent recovery of skin barrier function (20, 87). In contrast, violet light of 410 nm suppresses human epidermal keratinocyte differentiation by the activation of rhodopsin, probably involving specific signalling

pathways via (G_{α_i}) (34). Expression of rhodopsin was also detected in human epidermal melanocytes that can be activated by violet-blue, 315 to 400 nm light. This leads to the activation of a transduction pathway involving $G_{\alpha q}$-protein and TRPA1 eventually leading to intracellular calcium influxes and melanogenesis (33, 42).

The diversity of molecular reactions potentially stimulated by light, and the paucity of information about the specific pathways underpinning the observed phenotypic change(s) makes it difficult to rationally choose appropriate readouts for assessment of light effects. Currently, multiple cellular readouts have been evaluated where the impact is assessed via cell viability (88-91), proliferation (41, 86, 88, 89, 91-98), differentiation (30, 34, 99), morphology (90, 91, 95), and apoptosis (30, 94). However, more specific methods are now needed to elucidate the stimulated pathways, including the assessment of specific gene expression in particular skin cell subpopulations for selected application (93, 100, 101).

Pitfalls of translational research

Despite significant difficulties in interpreting the available published data derived from laboratory-based studies, there appears to be no doubt that photobiomodulation is indeed a real phenomenon, and that has already begun to be translated for the management of a variety of dermatological conditions such as hair loss (15, 25, 102), acceleration of healing in various skin wounds (17, 18, 103) and improvement of psoriasis (19). However, efforts for greater clinical efficacy of photobiomodulation-based therapies appear to be disengaged from the pursuit of greater understanding of the complex interactions between biological and optical parameters. This significant gap in our knowledge may confer risks for potential user of so-called photobiomodulation devices.

One of our aspirations was to see how to achieve greater consistency in optical parameters for *in vivo* studies. Indeed, the 'optical window' as reported for hair regrowth and wound healing appears slightly narrower than for other applications (Figure 1, a and b).

A key question here is how (and if) high levels of inter- and intra-study variability in the devices' optical performance affect their reported therapeutic efficacy. To tackle this we examined photobiomodulation in hair growth, as hair follicle is an excellent model to interrogate how light may influence complex biological tissues in health and disease. Also there are now at least twenty FDA-cleared light-based devices for the treatment of androgenetic alopecia in males and females, where reported efficacy was similar to that of existing drugs for hair regrowth (e.g., minoxidil and finasteride) (28). Data on long-term effect of light treatment are needed for solid conclusions of its efficacy in comparison to that of drugs. Here we focused on three of them, a comb (device A), and two helmets, (devices B and C), which all use red light, albeit with somewhat different wavelengths: 660 nm (device A), 670 nm (device B) and 650 nm (device C) and emit beams of 5mW power in a continuous wave mode (with exception of device C which has extra pulsing LEDs).

However, the devices differ significantly in the number of beams per device (comb A emits 9 beams, helmet B emits 80, and helmet C emits 51 beams) and application duration to cover the area of treatment (helmets B and C directly treat a significant area of the scalp, while the comb (A) must be moved each 4 seconds to cover the same area). As such, treatment time per spot is longer for the devices B and C (see Supplement table S1). Furthermore, the devices differ in their beam size and profile at the skin surface (Figure 2 a-to c). The corresponding maxima of irradiances are 130, 35 and 2 mW/cm^{-2} and of radiant exposures *at the skin surface*, 0.5, 42, and 2.7 J/cm^{-2} for devices A, B, and C, respectively.

Calculated photon densities inside the skin at the assumed level of the terminal scalp anagen hair bulbs (e.g., around 3-4 mm) for devices A and C differ by 10-fold (see Figure 2d-f, where Monte Carlo methods of light propagation in tissue were used). Given the exposure times per treatment session, these devices will have as much as 40-fold variability in radiant exposure at the target.

These are significant differences in irradiances and radiant exposures (per treatment session and per total treatment period). From our perspective it is rather perplexing that these devices offer comparable clinical hair growth-stimulating efficacy. In particular, for devices A and C the results of published clinical trials report a maximal increase of around 20 hairs per unit area (cm^{-2}) compared to a sham-control group in male and female cohorts (15, 25, 26) (see Supplement, table S1). This uncanny inter-device similarity suggest that they may be stimulating entry of kenogen hair follicles into anagen rather than having any effect on miniaturised hair follicles *per se* (104). We perhaps could be excused for concluding, at least for applications of photobiomodulation in hair growth studies, that there is a pitiful lack of rationality for the choice of treatment optical parameters.

Concluding remarks and recommendations

We have surveyed the last 30 years of published literature pertaining to skin photobiomodulation, and have focused on some of the unresolved complexity that retards rational development of this field. First, optical parameters remain poorly characterised and ill-defined. The range in wavelengths, irradiance and radiant exposures used for stimulating skin cells remain very wide and indeed we struggled to find evidence of any reported rational path for choosing light parameters for skin and hair follicle photobiomodulation, i.e. studies that could convincingly show the effect of particular optical parameters in any systematic way.

Second, skin cells express several potential photoreceptors covering the entire visible and infrared parts of the spectrum. The fact that most are reported to be involved in a particular photobiomodulation process, suggests that light, even at a particular wavelength, may trigger several photoreceptors simultaneously. This highlights the need to examine the presence and action spectrum of different photoreceptors as well as their physiological significance for specific applications.

Third, the mechanisms of action of light 'treatments' are currently very poorly understood. Few if any of the assumptions made on their behalf have been proven. Two potential mechanisms, however, stand out. Red and NIR light, absorbed by cytochrome c oxidase, may trigger reaction cascades that alter cellular homeostasis (e.g., via changes to pH, [Ca], cAMP, ATP). Blue light photolytically generates NO and ROS from nitrosated proteins and NADPH oxidase, respectively. These effectors can initiate physiological effects in cells (e.g., effects like proliferation, vasodilatation or wound healing). However, neither of the mechanisms provide a convincing explanation for all the physiological effects observed. Thus, there remains a pressing need to identify the potential molecular mediators of these processes, and for their mode of action.

While our understanding of photobiomodulation still has numerous gaps, its translation to commercial and therapeutic solutions continues apace against this backdrop of suboptimal characterization. Further translation should ideally follow a more rational route, both by identifying the key targets of light, *in vitro* or *in vivo*, and involving the application of light stimuli in a controlled way. The latter is likely to require the use of optical modeling tools for light propagation in tissue as was successfully done for light-based hair removal (105), where melanin, haemoglobin, and purulent discharge would be strongly affecting light transport.

We conclude a concerning lack of consistency in experimental and translational approaches in photobiomodulation studies both *in vitro* and *in vivo* including experimental conditions, treatment methods, clear translation between *in vitro* study and *in vivo* study. Not only should the optical parameters be rationally selected, but so also should the biological models under study. Most *in vitro* studies do not even closely approximate *in vivo* conditions. Thus, coupling both approaches (i.e., unravelling the photochemical reaction cascades involved as well as controlling the amount of light delivered to a selected target *in vivo*) will be required to improve the efficacy of existing devices and identify new light-based treatment opportunities for skin and hair health. Similar unresolved challenges are applicable to the entire field of photobiomodulation-based therapies, where a large leap forward in basic understanding is required prior it finds itself in the mainstream of therapies.

We remain very confident that future is very bright for light's contribution to skin health.

Author contributions

Mr Charles Mignon performed literature research, measurements of devices and wrote the manuscript together with Dr Natallia E. Uzunbajakava. Prof Desmond J. Tobin and Dr Natalia V. Botchkareva contributed to writing the manuscript and are senior authors.

Supporting Information

Supplement: Methodology of the literature review and characterisation of light-based devices for hair regrowth.

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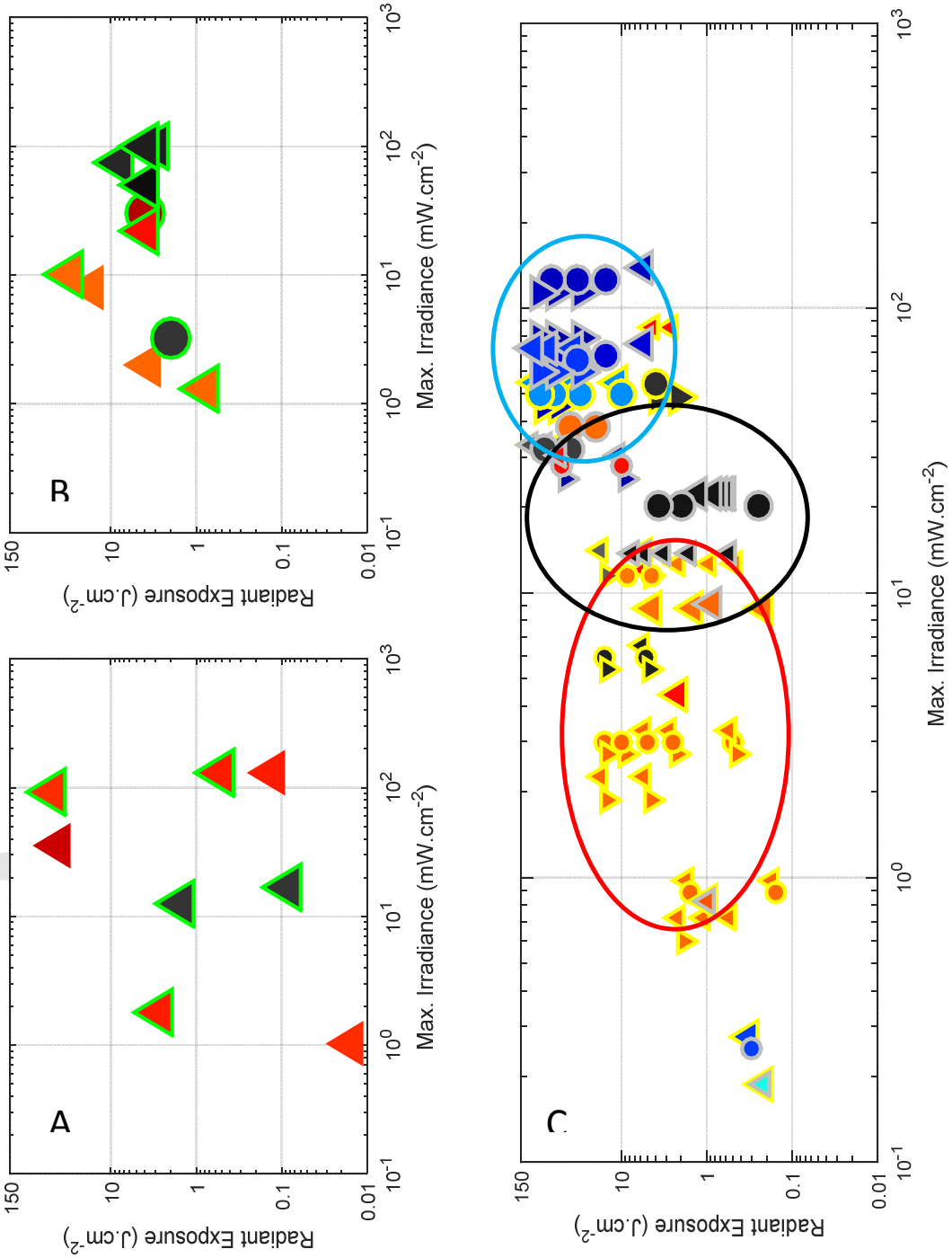

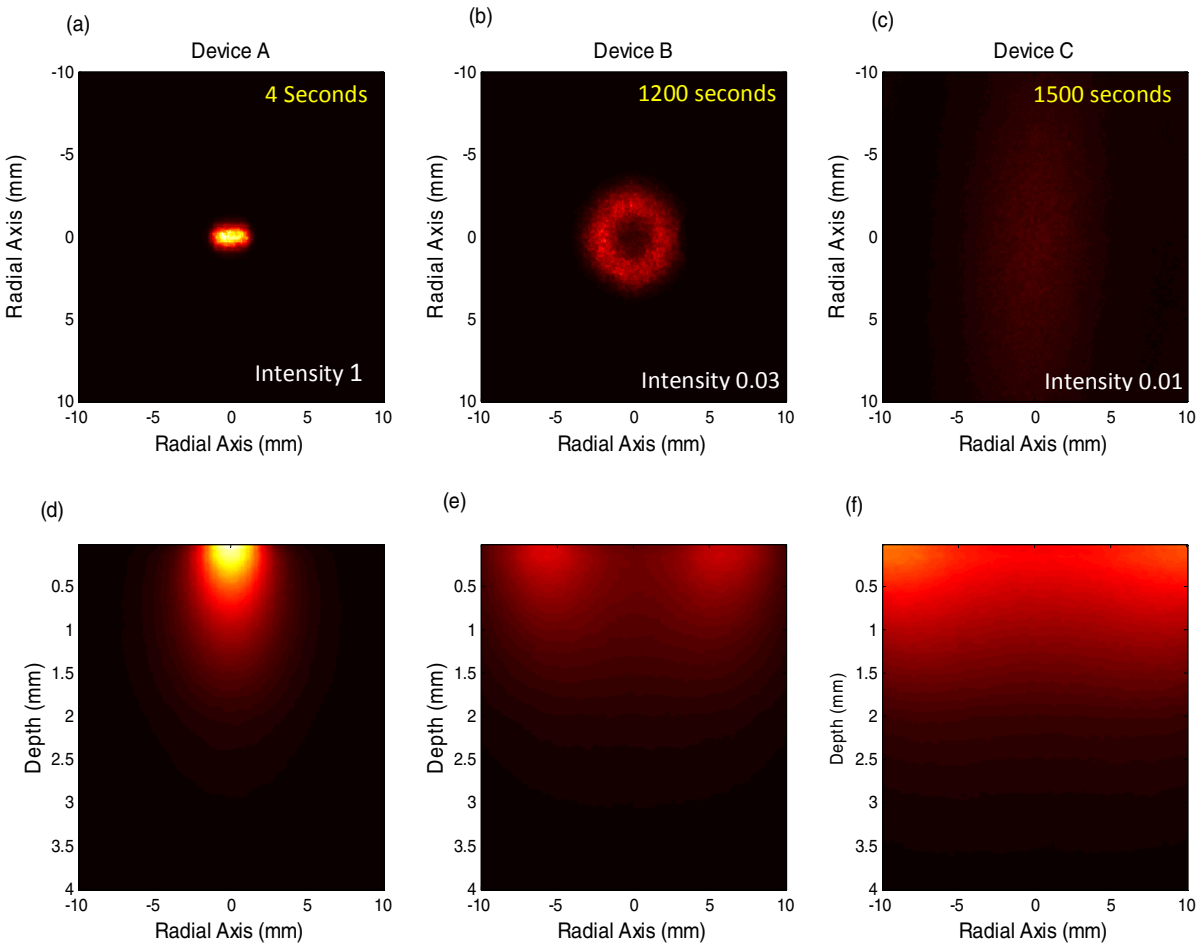

Figure 2: Measure beam profile and irradiance at the skin surface as determined based on the measured optical output and beam profile (from a to c) and irradiance inside the skin estimated using Monte Carlo method of light propagation in turbid medium (from d to f) for three commercial FDA-approved light-based devices for hair regrowth, noted as Device A, Device B and Device C, respectively. The recommended treatment durations and the relative maximum intensity are also shown in the upper right and the lower left corner, respectively. The relative maximum intensity was obtained by normalizing the photon density of each of the device on that of the Device A.