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Biostimulatory Windows in Low-Intensity Laser Activation: Lasers, Scanners, and NASA's Light-Emitting Diode Array System

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ABSTRACT

***Objective*: The purpose of this study was to assess and to formulate physically an irreducible set of irradiation parameters that could be relevant in the achieving reproducible light-induced effects in biological systems, both in vitro and in vivo. *Background Data*: Light–tissue interaction studies focusing on the evaluation of irradiation thresholds are basic for the extensively growing applications for medical lasers and related light-emitting systems. These thresholds are of central interest in the rejuvenation of collagens, photorefractive keratectomy, and wound healing. *Methods*: There is ample evidence that the action of light in biological systems depends at least on two threshold parameters: the *energy density* and the *intensity*. Depending on the particular light delivery system coupled to an irradiation source, the mean energy density and the local intensity have to be determined separately using adequate experimental methods. *Results*: From the observations of different research groups and our own observations, we conclude that the threshold parameters energy density and intensity are biologically independent from each other. *Conclusions*: This independence is of practical importance, at least for the medical application of photobiological effects achieved at low-energy density levels, accounting for the success and the failure in most of the cold laser uses since Mester's pioneering work.**

INTRODUCTION

Ameliorated wound closures have been achieved at energy densities between 1 and 4×10^4 Jm^{-2} in the therapy of ulcera cruris with 50-mW He/Ne-lasers.[3] This and further evidence have led to the establishment of one basic Arndt-Schultz curve (Fig. 1) showing different modes of cell reaction at different levels of energy density.[1–8] When energy densities were too small, there were no observable effects. Higher energy densities resulted in the inhibition of cellular functions. So far, the energy densities of reproducible photobiological effects that could be of therapeutic relevance[8–21] were generally in accordance with the effective energy density range described in the basic Arndt-Schultz-curve. The influence of the light intensity on the irradiated cells was subsequently demonstrated in fibroblast cultures (Fig. 2),[22] and possibly as well in animal experiments: The mast cells of irradiated mouse tongues showed progressive degranulation with increasing laser power (4 mW, 50 mW) where the locally administered energy density was kept at the same level.[23] Assuming that the cross sections of both laser beams were of the same magnitude, this experiment would hint at the intensity dependence of photobiological cell membrane effects in vivo. This activation of mast cells could also represent a significant mechanism in the acceleration of wound healing under the correct laser light irradiation. Observations in patients also revealed that thresholds of light intensity (presumably wavelength dependent) have to be surpassed to achieve reproducible biostimulatory effects. However, there is no clinical documentation of the precise threshold values. What had been repeatedly found was that the clinical use of lasers with a power smaller than 4 mW in the field of application induced no reproducible biological effects, independent of the length of the total

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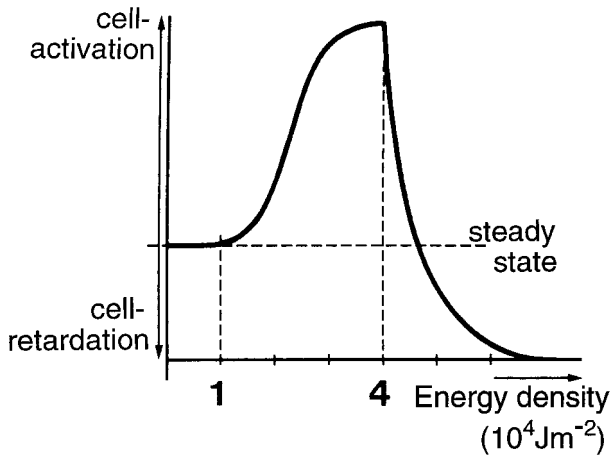

FIG. 1. Basic Arndt-Schultz curve.[1–8]

irradiation time.[24,25] Unfortunately, the results were never correlated with the value of the laser beam cross section; otherwise, the importance of laser light intensity in low-level laser therapy (LLLT) would have been ascertained earlier.

THE LILAB EQUATION

It becomes clear that the stimulative effect of laser light in biological tissues depends on a set of at least four parameters, besides the wavelength of the light: a light intensity threshold I_0, the beam cross section a, the total irradiation time Δt_{tot}, and the energy density $(E/a)_{act}$ required for activation. The stimulation parameters relevant for activation are interrelated according to the low-intensity laser-activated biostimulation (LILAB) equation:

$$(E/a)_{act} = I_{stim}.\Delta t_{tot}, \quad (1)$$

where intensities necessary for stimulation I_{stim} have to surpass the threshold intensity I_0.[26,27] The majority of the published results with a negative outcome stem from ignoring the importance of the relation:

$$I_{stim} \geq I_0 \quad (2)$$

in photobiological experiments. Light intensities lower than threshold values I_0 obviously do not produce biostimulatory effects, even under a prolongation of the irradiation time Δt_{tot}. The effective range of $(E/a)_{act}$ in equation (1) is given by the particular Arndt-Schultz curve. Although equation (1) is physically surprisingly simple, the biological implications are by no means trivial. Biologically, the parameters $(E/a)_{act}$ and I_{stim} are clearly independent from each other, an important consideration at least for the medical applications of photobiological effects with the use of lasers (including noncoherent light sources) at low-energy density levels.

APPLICATIONS OF THE LILAB EQUATION

In practice, it is of great importance to apply the laser light to a much greater area than the laser beam cross section itself. Due to the cooperative behavior of photostimulated cells,[28,29] it seems to be important to irradiate the application field simultaneously to avoid adverse effects with respect to the intended aims.[30] Consequently, the application field would have to be irradiated in the shortest possible period, creating a homogenously distributed mean energy density with the necessary local light intensity, as required for activation.

Scanners have been developed for these practically important cases and have been used in vivo with satisfactory results. The suitability of conventional scanners for medical applications depends, besides the values of the local light intensity, on the uniformity of the mean energy density in the application field, thus implying a high scanning velocity of the laser beam.

The successful application of conventional scanners is, however, hampered by an increasing energy density difference between the periphery and the center of the application field. This energy density difference was shown to increase with increasing scanner speed,[8] and can easily be understood because the mean irradiation time in the vicinity of the periphery of the scan increases as the scanning speed slows down due to the approach of the laser beam to its turning point. Energy density ratios of 5:1 from the periphery to the center of the scan have been reported for linear scanners. Therefore, energy densities within

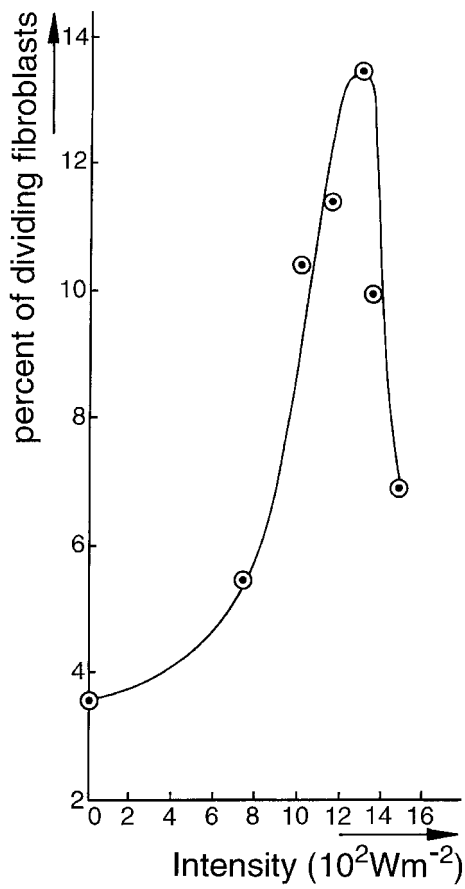

FIG. 2. Percentage of dividing fibroblasts, 24 h after irradiation at 540 nm at a constant energy density of 4×10^4 Jm^{-2}.[22] Principal result of the preliminary study was the experimental demonstration of the existence of a light intensity window in vitro.

the activating fluency range[8] in the central field of laser application would be followed by potentially damaging higher energy densities with opposite effects in the collateral field. Thus, an adequate aperture is used to shield and protect the peripheral tissue, a waste of expensive scanner power. Studies comparing the biological results of laser irradiation applied via scanners to the spot by spot method are missing in the accepted literature available (MedLine).

In agreement with equation (1), the biologically effective light intensities can also be applied on greater areas by use of high-power lasers in combination with optical lenses (beam-diverging systems). This is presently still a very expensive solution due to the cost of high-power lasers. However, beam-diverging systems could be adequate with suitable semiconductor lasers, as reported on the successful photobiostimulation treatment of General Motors workers and other patients with carpal tunnel syndrome.[31] Potential light sources promising for photobiostimulation of extended wound areas with homogeneous mean field intensities and energy densities within the activating range,[8] appear to us to be light-emitting diodes (LED) and NASA's lightweight light-emitting diode array systems in particular.[17,18]

In contrast to the threshold intensity necessary for activation I_{stim}, a quantity directly calculable from the technical data of the laser, the mean field intensity I_{field} in any application field A greater than the cross section of the laser beam can only be determined accurately by measuring the mean energy density (E/A). The determination of this quantity is relatively simple in cases of the spot surfaces generated by beam-diverging systems, and more complicated in case of the light patterns generated by scanners as described in the literature.[8,32,33] The question of whether (E/A) is an activating energy density or not depends not explicitly on the particular magnitude of the associated I_{field} value, but primarily on the value of the local light intensity I_{stim} and the total duration of the local light stimulus per activated field.

The LILAB System,[37] being also an exemplary model to demonstrate the interplay between biologically relevant irradiation parameters, permits, besides the periodical photobiostimulation of large surfaces, avoiding the aforementioned disadvantages commonly associated with medical lasers used in wound healing. The LILAB System is based on a very fast beam distributor designed for homogeneous irradiation of arbitrarily large application fields.[8] This beam distributor (Fig. 3) consists in its simplest version of the following basic components: a small electric motor, two mirrors, and a swinging rod system. The electric motor, attached to the laser housing via a rubber shock absorber, drives a rotating mirror holder with mirror M_2. The motor speed is continuously variable. The mirror holder is attached to the end of the shaft of the motor. The mirror holder has a ring with a sinusoidal outer-edge profile. This sinusoidal ring profile rides on a suspended swinging rod system, also attached to the laser housing via a similar rubber shock absorber. On the other end of the rod, the second mirror (M_1) is mounted. The laser beam reflected by the mirror M_1 paints, depending on the drive rotation speed, a dense light pattern on the mirror M_2, subsequently directed toward the field of application. Using a prototype LILAB System based on a 25-mW He/Ne laser (632.8 nm), we could generate various energy density fields nearly homogenously spread over the total irradiation field. A simple variation in rotation speed of the drive allowed arbitrary high-beam velocities without the energy density gradients prevalent with conventional scanner systems.[8,32] The energy density variation within the periodic light patterns generated by the LILAB system, scanned so quickly that it could mimic locally pulsed lasers, amounted to a maximum of 30%.[32] For mean field intensities of biological relevance generated by the LILAB System (72 Wm^{-2} at 632.8 nm),[33] we realized and described dynamic light intensities similar to the magnitude of those administered via NASA's irradiation system, ranging from 24 Wm^{-2} to 743 Wm^{-2}. These intensities were found to be effective in fibroblasts, osteoblasts, and skeletal muscle cells.[16–18] Recent laboratory results observed in murine osteoblasts irradiated with the NASA LEDs, and the associated experimental protocol, accounting for the irreducible set of the three biologically independent parameters (wavelength, energy density, intensity) necessary for complete characterization of the irradiation, are shown in Fig. 4.[16]

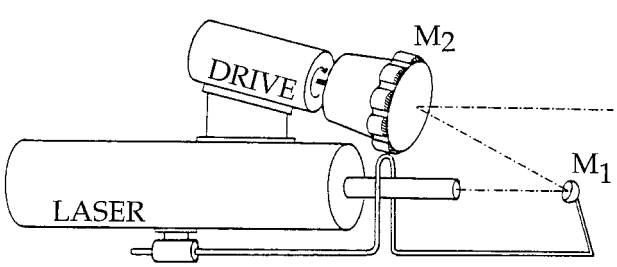

FIG. 3. Beam distributor for lasers with principal components: drive, rotating mirror M_2, and swinging rod system ending in mirror M_1.[26]

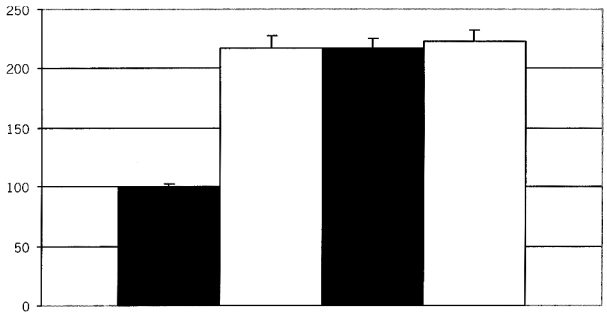

FIG. 4. DNA synthesis for 24 h ([^{3}H]thymidine incorporation).[16] Results as percent change relative to control: histogram from $1 \rightarrow r$. Control = 100%: no LED treatment. LED treatment (670 nm) at 4.0×10^4 J/m^2 and 743 W/m^2. LED treatment (728 nm) at 4.0×10^4 J/m^2 and 400 W/m^2. LED treatment (880 nm) at 4.0×10^4 J/m^2 and 530 W/m^2.

DISCUSSION

To illustrate the practical implications of the equations (1) and (2) and their implementation in irradiation systems applied in LLLT, we described the LILAB System. This irradiation system is well suited for generating homogeneous energy densities

at stimulating local intensities, thus satisfying the conditions and the generally accepted basic principles necessary in experimental and clinical work. The existence of an upper limit for the applicable light intensity, as found in cell culture experiments,[22] could not be observed in clinical practice, presumably because of the change of the intensity with the depth of penetration due to absorption. However, with an upper limit for the stimulating light intensity, as demonstrated by Lubart,[22] we could now be, in principle, in the position to evaluate effectively *activating energy density thresholds/stimulating intensity windows* at the surface and in deeper layers of any biological tissue.

Realizing the importance of intensity and energy density, there is no way to circumvent in future laser experiments the specification of the laser beam diameter and, in using scanners, the measurement of the mean energy density. The method for the measurement of the mean energy density generated by scanners and its validation has been published.[33] Besides the light intensity thresholds and the activating mean energy densities, determined in case of the scanners by the cumulation of the duration of local light stimuli, certain beam repetition frequencies with extended influence on activation seem to exist. There is also evidence from literature for their existence. The biological effect of the pulse frequency received support from the experimental side from the observation of additional Ca^{2+} uptake in macrophages[34] and an enhanced chemiluminescence in murine splenocites[35] after irradiation with pulsed semiconductor lasers of suitable pulse duration and repetition frequency. There has also been support from the clinical side.[36] Thus, the periodical stimulation of extended tissue areas with maximum local photon density, uniform energy density, and minimum thermal effects as realized, e.g., with the LILAB System is a powerful method for the achievement of photobiological results with lasers.[37] We are now studying the molecular mechanisms behind these results, also in case of selectively stimulatory irradiation parameters. At the European Nearfield Scanning Optical Microscopy Application Laboratory (ENSOMA), we are attempting to establish a connection between low-intensity light-activated biostimulation, and near-field optical methods. This study could facilitate our direct access to topographical changes on nanoscale levels and reveal detailed morphological responses, observable in cells reacting to their far-field stimulation with light.

CONCLUSIONS

The present study suggests that the irradiation of areas exceeding the cross section of laser beams with homogenous energy densities must be paralleled in practice by the precise measurement of at least two independent threshold parameters: the local intensity of the laser beam, respective diode field, and the mean energy density in the application field.

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REFERENCES

1. Mester, E., Szende, B., and Gartner, P. (1968). The effect of laser beams on the growth of hair in mice. Radiobiol. Radiother. 9, 621–626.
2. Mester, E., Spiry, T., Szende, B., and Tota, J.G. (1971). Effect of laser rays on wound healing. Am. J. Surg. 122, 532–535.
3. Mester, E. (1981). Über die stimulierende Wirkung der Laserstrahlung auf die Wundheilung, in: *Der Laser: Grundlagen und Klinische Anwendungen.* K. Dienstl and P.L. Fischer (eds.). Berlin, Heidelberg, New York: Springer, pp. 109–118.
4. Mester, E., Mester, A., and Toth, J. (1983). Biostimulative Effect of Laser Beams, in: *New Frontiers in Laser Medicine and Surgery.* K. Atsumi (ed.). Excerpta Medica, pp. 481–489.
5. Mester, A.R., Nagylucskay, S., Mako, E., et al. (1998). Experimental immunological study with radiological application of low power lasers, in: *Laser in Medicine.* W. Waidelich (ed.). Berlin, Heidelberg, New York: Springer, pp. 502–512.
6. Ohshiro, T. (1988). *Low Level Laser Therapy: A Practical Introduction.* New York: John Wiley and Sons, p. 30.
7. Mester, E., Mester, A.F., and Mester, A. (1985). The biomedical effect of laser application. Lasers Surg. Med. 5, 31–39.
8. Sommer, A., and Franke, R.P. (1993). LILAB—a new System for low intensity laser activated biostimulation. Biomed. Tech. 38, 168–171.
9. Yamada, K. (1991). Biological effects of low power laser irradiation on clonal osteoblastic cells (MC3T3-E1). J. Jpn. Orthop. Assoc. 65, 787–799.
10. Trelles, M.A., and Mayayo, E. (1987). Bone fracture consolidates faster with low power laser. Lasers Surg. Med. 7, 36–45.
11. Wu, W., Naim, J.O., and Lanzafame, R.J. (1994). The effect of laser irradiation on the release of bGFG from 3T3 fibroblasts. Photochem. Photobiol. 59, 167–170.
12. Rosner, M., Caplan, M., Cohen, S., Duvdevani, R., Solomon, A., Assia, E., Belkin, M., and Schwartz, M. (1993). Dose and temporal parameters in delaying injured optic nerve degeneration by low energy laser irradiation. Lasers Surg. Med. 13, 611–617.
13. Pinheiro, A.L., Cavalcanti, E.T., Pinheiro, T.I., Alves, M.J., and Manzi, C.T. (1997). Low level laser therapy in the management of disorders of the maxillofacial region. J. Clin. Laser Med. Surg. 15, 181–183.
14. Bihari, I. (1994). CO_2 laser in low power applications in wound healing. Laser Therapy, 6, 43.
15. Sroka, R., Schaffer, M., Fuchs, C., Pongratz, T., Schrader-Reichard, U., Busch, M., Schaffer, P.M., Duhmke, E., and Baumgartner, R. (1999). Effects on the mitosis of normal and tumor cells induced by light treatment of different wavelengths. Lasers Surg. Med. 25, 263–271.
16. Whelan H.T., personal communication. NASA-LEDs presented in refs. 17 and 18 were used to irradiate the osteoblasts.
17. Whelan, H.T., Houle, J.M., Whelan, N.T., Donohoe, D.L., Cwilinski, J., Schmidt, M.H., Gould, L., Larson, D.L., Meyer, G.A., Cevenini, V., and Stinson, H. (2000). The NASA light-emitting diode medical program—progress in space flight and terrestrial applications. Space Tech. & App. Int'l. Forum-2000 504, 37–43.
18. Whelan, H.T., Houle, J.M., Donohoe, D.L., Bajic, D.M., Schmidt, M.H., Reichert, K.W., Weyenberg, G.T., Larson, D.L., Meyer, G.A., and Caviness, J.A. (1999). Medical applications of space light-emitting diodes technology—space station and beyond. Space Tech. & App. Int'l. Forum-1999, 458, 3–15.

19. Pinheiro, A.L.B. personal communication. Irradiation of mouth ulcers in 57 HIV-positive patients, an average of three ulcers per patient (23 patients with lesions strongly related to the HIV infection), using 5-mW lasers (635 and 670 nm, beam cross sections ~1 mm^2) and local light doses between 0.9 and 3.4×10^4 Jm^{-2}, revealed generally an approximately 50% reduction of the total healing period of the ulcers, when compared to nonirradiated cases. In the HIV related cases, the healing times depended upon the actual status of infection of the patients: AIDS-related complex (ARC) patients reacted in general better to the laser therapy than patients having developed full AIDS.
20. Pinheiro, A.L.B. personal communication. Laser irradiation of tissues contacting implants improved osteointegration (animal experiment): laser power 40 mW, wavelength 830 nm, beam cross section ~1 mm^2, dose per spot 1.2×10^4 Jm^{-2}, and total dose per session 4.8×10^4 Jm^{-2}. Abstract in: Martins, P.P.M., Pinheiro, A.L.B., Oliveira, M.A.M., and Gerbi, M.E.M. (2000). P.P.M.M. implant system have osteointegration improved by LLLT. World Association for Laser Therapy, Third World Congress. Athens, 11–13 May.
21. Tunér, J., and Hode, L. (1999). *Low Level Laser Therapy—Clinical Practise and Scientific Background.* Sweden: Prima Books, pp. 161–162.
22. Lubart, R., Friedmann, H., Peled, I., and Grossman, N. (1993). Light Intensity Effect on Cell Proliferation. Fifth Congress of the European Society for Photobiology. Marburg/Lahn, September 19–26.
23. Trelles, M.A., and Mayayo, E. (1992). Mast cells are implicated in low power laser effect on tissue. A preliminary study. Lasers Med. Sci. 7, 73–77.
24. Mester, A.R. (1992). Modalities of low power laser applications. Laser Applications in Medicine and Surgery. Laser Bologna 92. Third World Congress—International Society for Low Power Laser Applications in Medicine. Bologna, September 9–12.
25. Mester, A.R. (1994). Low power laser in the complex wound management. Laser Ther. 6, 39.
26. Sommer, A. (1993). Fast Surface Covering Beam Distributor for Lasers. German Patent Office, Munich, Patent Nr. 4308474/2000.
27. Lubart, R., Friedmann, H., Peled, I., and Grossman, N. (1993). Fibroblasts proliferation and light—a non linear interaction. Lasers Surg. Med. 9, 143.
28. Costato, M. (1992). Laser light and biological response: cooperative phenomena vs. order and disorder. Laser Bologna 92. Third World Congress—International Society of Low Power Laser Application in Medicine. Bologna, September 9–12.
29. Dahle, J., Kaalhus, O., Moan, J., and Steen, H.B. (1997). Cooperative effects of photodynamic treatment of cells in microcolonies. Proc. Natl. Acad. Sci. USA 94, 1773–1778.
30. Longo, L., and Corcos, L. (1992). Defocused CO_2 Laser therapy in pathologic wound healing, in: *Laser in Medicine.* W. Waidelich (ed). Berlin, Heidelberg, New York: Springer, pp. 409–412.
31. Gwynne, P. (1994). Cold laser uses move beyond carpal tunnel. Biophotonics 1, 28–29.
32. Sommer, A., and Franke, R.P. (1993). Evaluation of the energy density distribution in laser light fields generated with the LILAB System. Biomed. Tech. 38, 240–242.
33. Sommer, A., and Franke, R.P. (1995). Determination of the energy density of a periodical homogeneous laser light pattern used in medical applications. Biomed. Tech. 40, 133–136.
34. Young, S.R., Dyson, M., and Bolton, P. (1991). Effect of light on calcium uptake by macrophages. Laser Ther. 3, 1–5.
35. Karu, T., Andreichuk, T., and Ryabykh, T. (1993). Changes in oxidative metabolism of murine spleen following laser and superluminous diode (660–950nm) irradiation: effects of cellular composition and radiation parameters. Lasers Surg. Med. 13, 453–462.
36. Goldman, J.A., Chiapella, J., Cassey, H., et al. (1980). Laser therapy of rheumatoid arthritis. Lasers Surg. Med. 1, 93–101.
37. Sommer, A., and Franke, R.P. (1994). The low intensity laser activation biostimulation (LILAB) system. Laser Ther. 6, 23.

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