

CONCISE COMMUNICATION

Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation

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Summary

Background Endothelial cell (EC) proliferation plays a key role in the process of tissue repair. Low-intensity laser irradiation has been demonstrated to accelerate wound healing and to improve microvascularization.

Objectives The present study evaluated a possible stimulatory influence of low-intensity laser irradiation on human umbilical vein endothelial cell (HUVEC) proliferation in a systematic manner.

Methods Subconfluent cultures of HUVEC were irradiated every other day with a 670-nm diode laser (intensity: 10–65 mW cm⁻², dose: 2–8 J cm⁻²) during a period of 6 days. Cell proliferation was evaluated quantitatively by counting in a haemocytometer.

Results Our data demonstrate a dose-dependent and intensity-dependent stimulatory effect of laser irradiation on HUVEC cell proliferation. Doses of between 2 and 8 J cm⁻² induced statistically significant cell proliferation. Testing different intensities at a constant dose of 8 J cm⁻², 20 and 65 mW cm⁻² induced most pronounced cell proliferation.

Conclusions Low-intensity laser irradiation influences EC proliferation and might thereby contribute to the increase in angiogenesis and the acceleration of wound healing *in vivo*.

Key words: angiogenesis, biostimulation, low level laser, microangiopathy

During the process of tissue repair, endothelial cell (EC) proliferation is crucial for the process of angiogenesis.^{1,2} In consequence, numerous disorders of wound healing are associated with reduction of dermal capillary quantity.^{3–5} Low-intensity laser irradiation has been demonstrated to positively influence cellular and humoral components of wound healing (see review⁶). However, to date only a few studies have addressed the direct effects of visible laser irradiation on EC proliferation. Therefore, the present study aimed to determine the effect of low-intensity laser irradiation on HUVEC proliferation.

Materials and methods

Human umbilical vein endothelial cells (HUVEC) were isolated and characterized as described previously.⁷ Cells used for experiments were in passages 3–6. In

order to exclude possible oestrogen-like stimulatory effects of phenol-red,⁸ cultures were switched to phenol-red-free M199 medium 24 h before the first laser irradiation.

Immediately before light exposure, medium was replaced by phenol-red-free Hanks balanced salt solution (HBSS; Sigma, St Louis, MI, U.S.A.) containing antibiotics and 10 mmol L⁻¹ Hepes. A 670-nm diode laser (Helbo, Grieskirchen, Austria) was used for irradiation. This device has a tunable power output (1–250 mW) and a diffuse beam. The required doses and intensities were obtained by adjusting the power output, distance and exposure time. HUVEC were seeded either into 6-well plates at a concentration of 4 × 10⁴ cells per well (for evaluation of different doses) or into 12-well plates at a concentration of 1.6 × 10⁴ cells per well (for evaluation of different intensities) and incubated as described above. Irradiations were performed every 48 h for a period of 6 days in sterile conditions with the dish lid removed. In the first set of

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experiments, doses of 2, 4 and 8 J cm⁻² were tested at a constant light intensity of 20 mW cm⁻². In order to determine the influence of light intensities, further experiments were conducted comparing intensities of 10, 20 and 65 mW cm⁻² at a constant dose of 8 J cm⁻². Paired nonirradiated cultures served as controls.

For evaluation of EC proliferation, cells were detached by trypsin/ethylenediamine tetraacetic acid and 100 µL of cell suspension were pipetted into a haemocytometer and counted by an investigator unaware of the study protocol. Cell viability was determined by trypan blue exclusion test and was > 95% in all experimental groups. All experiments were repeated three times. Data from cell counts are given as means ± SD of three independent experiments. For statistical evaluation ANOVA was used and significance was defined as $P < 0.05$.

Results

In order to evaluate the optimal dose for laser-induced stimulation of HUVEC proliferation, doses of 2, 4 and 8 J cm⁻² were administered at an intensity of 20 mW cm⁻². There was a dose-dependent effect of laser irradiation on proliferation of HUVEC. Significant differences in cell proliferation in comparison to nonirradiated controls were observed at all doses (Fig. 1).

Results of the experiments performed with different light intensities are shown in Table 1. Varying intensities at a constant dose of 8 J cm⁻² revealed that

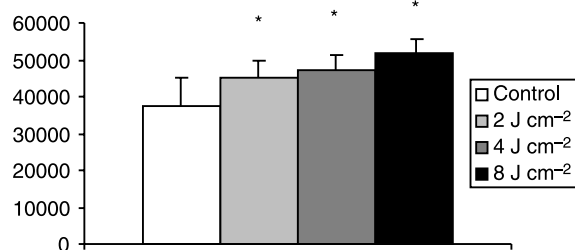


Figure 1. Effect of different doses of laser irradiation (2–8 J cm⁻²) at a constant intensity of 20 mW cm⁻² on human umbilical vein endothelial cell proliferation. Data are given as means ± SD from three experiments.

Table 1. Effect of different light intensities of laser irradiation (10–65 mW cm⁻²) at a constant dose of 8 J cm⁻² on human umbilical vein endothelial cell proliferation. Data are given as means ± SD from three experiments

	Control	10 mW cm ⁻²	20 mW cm ⁻²	65 mW cm ⁻²
Cell count (Mean ± SD)	12143 ± 172	15107 ± 1952	14030 ± 650	14660 ± 802
P-value		0.1	0.05	0.03

intensities of 20 and 65 mW cm⁻² significantly stimulated HUVEC proliferation in comparison to their non-irradiated control cultures.

Discussion

In the present study, we demonstrate that 670 nm laser irradiation at doses of between 2 and 8 J cm⁻² and at intensities between 20 and 65 mW cm⁻² significantly increases HUVEC proliferation. These particular laser parameters were used because of findings from previous *in vitro* and *in vivo* studies. Our results are in line with findings of Ghali and Dyson who used wavelengths of 660 and 820 nm to irradiate bovine aortic endothelial cells and found wavelength- and dose-dependent effects on proliferation.⁹ The highest cell numbers in this study were documented on day 5 at a dose of 8 J cm⁻² and an intensity of 9 mW cm⁻². The 820-nm wavelength suppressed EC proliferation at doses of 1 and 2 J cm⁻².

The underlying mechanisms of action of low-intensity laser irradiation on the cellular and subcellular level are still a matter of ongoing research. However, thermal effects can largely be ruled out.^{10–12} Most likely, a direct or indirect interference of visible and infrared wavelengths with mitochondrial components of the respiratory chain is part of the signal transduction pathway.^{13–15} Moreover, the photodynamic modulation of the quantity of reactive oxygen species may be of importance.^{16–18} Recent studies addressed the possible induction of proangiogenic factors by laser exposure. So far, these factors have been detected after laser irradiation only in culture media of cell lines other than ECs.^{12,19} Bouma *et al.* failed to demonstrate up-regulation of spontaneous and cytokine-induced secretion of interleukin (IL)-6 and IL-8 by HUVEC after pulsed infrared laser radiation,²⁰ which suggests that direct stimulation of EC proliferation following laser irradiation might not be induced by self-expression of proangiogenic factors.

In conclusion, our data demonstrate that low light intensities can interact directly with human EC to stimulate cell proliferation, a critical process in tissue repair.

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