

Study of the effect of laser pulse duration in the ultraviolet spectral range on fibroblasts

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Abstract—We report the different pulse duration of UVB laser radiation cytotoxic effect on human skin fibroblasts. The shorter the pulse, the less viable the cells. According to MTT test examined 24 hours after irradiation cells have the potential to recover. Regarding flow cytometry, we concluded that the toxicity is concerned with the lipid membrane destruction.

Vitiligo is the depigmentation disease characterized by loss of epidermal melanocytes [1]. The two most effective methods of vitiligo treatment are psoralen plus ultraviolet A (PUVA) and narrowband ultraviolet B (NB-UVB), but nowadays PUVA practically not used because of its aggressive phototoxicity and carcinogenic effects on cells [2]. NB-UVB first used in vitiligo in 1997 [3]. The wavelength which used was 310-315 nm with peak emission at 311 nm. This unique wavelength is effective possibly because it can stimulate non-active epidermal melanocytes and modulate cutaneous immune system [4]. However, simultaneously with “treatment” of skin melanocytes most of other cells are negatively affected by UVB-irradiation. Reactive oxygen species (ROS) are formed in fibroblasts, that causes various damages [5]. In this work we studied new perspective methods of vitiligo treatment which will cause less or no side effects.

II. METHODS AND RESULTS

We investigated a relation between cells viability and time of irradiation, impulse's duration and timing of the MTT assay. We have used the laser radiation of LiLiYF₄:Ce+Yb active medium, which provides generation of sub-nanosecond pulses and wavelength tuning in UVB range [6,7]. It has been shown that the survival of fibroblast cells is less when irradiated with UV light with a shorter pulse duration (1 ns) Fig. 1(a). When we examined cell viability 24 h after irradiation Fig. 1(b) we found increasing in HSF cells viability. As shown in Fig. 1(c) After 24 h viability of HSF cells that were irradiated at wavelength 300 and 310 nm was less than control. Whereas, in the last sample (325 nm) viability was increased quite a lot. It was established by flow cytometry that as a result of UV laser irradiation of fibroblasts, the cytoplasmic membrane is damaged in 80% of cases Fig. 1(d).

Fig. 1. Cell viability right after irradiation (a) Cell viability 24 hours after irradiation (b) Effect of different wavelength to HSF cells viability 24 hours after irradiation (c) Table of the results of flow cytometry, DiOC₆+PI- (alive) I, DiOC₆-PI- (with damaged mitochondria) II, DiOC₆-PI+ (dead, late apoptosis) III, DiOC₆+PI+ (perforated cytoplasmic membrane) IV (d)

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