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Influence of Pulse Type on Subcellular Selective Photothermolysis of Melanosomes in Adult Zebrafish Skin Following 1,064-nm, Q-switched, Nd:YAG Laser Irradiation: A Pilot Study

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Dear Editor:

In recent years, laser toning using low fluence, multiple-passed, Q-switched, 1,064-nm neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers has gained popularity in Asian countries for the treatment of melasma. However, laser toning often results in hyperpigmentation or mottled depigmentation[1-3]. Therefore, it is critical to determine the optimal conditions for laser toning, including pulse type and irradiation energy density. In our previous study using a zebrafish model[4], we suggested the concept of subcellular selective photothermolysis (SSP) of melanosomes. The recently introduced dual-pulse, 1,064-nm, Q-switched Nd:YAG laser has received considerable attention.

We performed a pilot study to investigate the influence of pulse type on SSP of melanosomes. For this purpose, we used two Q-switched Nd:YAG lasers with different pulse types: (1) conventional pulse (Spectra VRMIII; Lutronic Corp., Goyang, Korea) and (2) dual-pulse laser (Pastelle-PTP mode; Won Tech., Daejeon, Korea). The conventional pulse-type laser had a wavelength of 1,064 nm; fluence of 0.4, 0.5, and 1.0 J/cm^2; and a spot size of 7 mm. The dual-pulse laser had a wavelength of 1,064 nm; fluence of 0.4, 0.5 and 1.0 J/cm^2; and a spot size of 7 mm. We irradiated the caudal peduncle of adult zebrafish with a single light pulse. To identify the fluence level that sufficiently destroys melanosomes, zebrafish were irradiated at fluence levels of 0.5 and 1.0 J/cm^2 with both pulse types, with one zebrafish subjected to each condition. We observed and analyzed temporal changes in melanosomes at the irradiation site, and we examined the tissues 1 week after irradiation when all of the pigment debris had disappeared. The images were developed into black and white photographs to accentuate pigment-free lesions. The relative pigmentation density after laser irradiation was quantified using Quantity One 1-D analysis software (version 4.6.3; Bio-Rad Inc., Hercules, CA, USA). According to the SSP concept, we stained the zebrafish skin tissue with 4′,6-diamidino-2-phenylindole (DAPI) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) 1 day after irradiation at fluence levels of 0.4 and 0.5 J/cm^2 with both pulse types to test whether the laser pulse type has an effect on melanophores and their adjacent cells. The experimental protocols were approved by the Korea University Animal institutional review board (No. KUIACUC-2013-172).

A relative pigmentation density less than 1.0 was observed 1 week after irradiation with light at fluence levels of 0.5 and 1.0 J/cm^2 with both pulse types. Regeneration of melanosomes was observed at 4 and 8 weeks of irradiation at 0.5 J/cm^2 (Fig. 1). A similar change was observed when irradiation was performed at 1.0 J/cm^2 with both pulse

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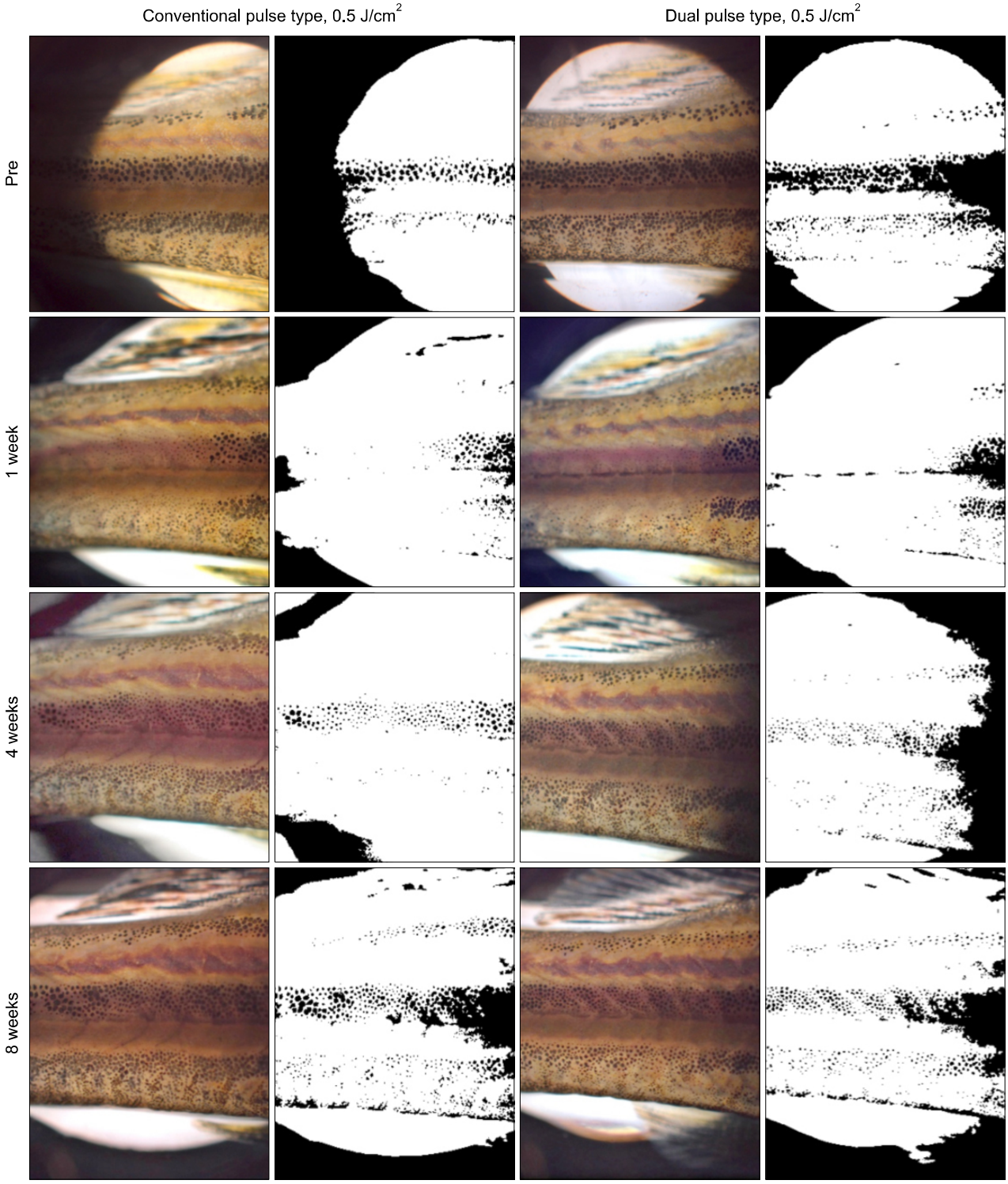

Fig. 1. Changes over time in melanosomes at the site of laser irradiation (×12.5). For the conventional and dual-pulse type lasers, a fluence of 0.5 J/cm^2 was sufficient for the near complete elimination of adult zebrafish melanosomes. Melanosomes regenerated over time.

types. DAPI and TUNEL staining results are shown in Fig. 2. No apoptotic cells were detected for either pulse type at a fluence level of 0.4 J/cm^2. As demonstrated in our previous study, apoptotic cells appeared after irradiation with the conventional pulse type at a fluence of 0.5 J/cm^2; however, no apoptosis was observed at 0.5 J/cm^2 for the dual-pulse type laser.

These results indicate that a fluence of 0.5 J/cm^2 with ei-

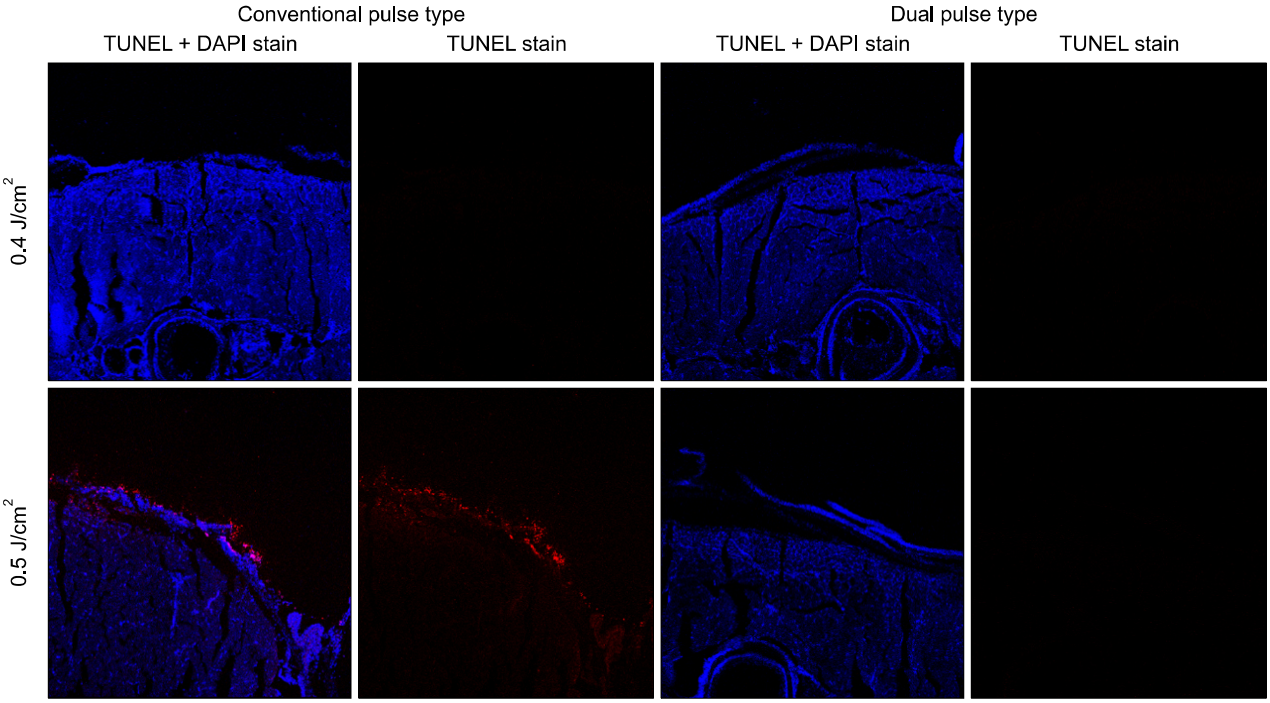

Fig. 2. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and 4′,6-diamidino-2-phenylindole (DAPI) staining of zebrafish skin tissue 1 day after laser irradiation (×20). DAPI staining (blue) shows normal and apoptotic nuclei. TUNEL staining (red) shows apoptotic nuclei. No apoptotic cells were detected with either pulse type at a fluence of 0.4 J/cm^2. Apoptotic cells were present in the conventional pulse type, and no apoptosis was observed with the dual-pulse type at a fluence of 0.5 J/cm^2.

ther pulse type is sufficient for the near complete elimination of adult zebrafish melanosomes. However, a distinct laser pulse type-dependent difference in necrosis and apoptosis of melanophores and other cells was observed at a fluence of 0.5 J/cm^2. The exact reason underlying the results obtained with the dual-pulse laser remains unclear. The dual-pulse method cuts the pulse into two half-fluence pulses, and this produces a 140-μs interval between the dual-pulse beams. Our results suggest that the dual-pulse laser destroys melanosomes with a sub-threshold peak power. Therefore, the dual-pulse method could correspond with the SSP hypothesis; however, further studies are needed to confirm our results. Through SSP, the relatively low irradiation energy could limit thermal diffusion effects to melanosomes without affecting melanophores or other cells. It is presumed that apoptosis of melanocytes after laser irradiation at threshold and supra-threshold fluencies can lead to pigmentary complications[1,2]. We speculate that SSP at a low fluence level could lower the risk of side effects associated with laser toning. In summary, this study suggests that the laser pulse type influences SSP of melanosomes. Future laser toning studies should focus on establishing proper treatment methods that involve pulse type and irradiation energy, to support selective photothermolysis at a subcellular level with the goals of optimizing efficacy and minimizing complications.

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