Cellular and biomolecular comparison of a novel, dual-pulsed Q-switched 1064 nm Nd:YAG laser with conventional Q-switched 1064 nm Nd:YAG laser

Sir,

A recent clinical and histopathological study in melasma patients using a low-fluence, 1064 nm Q-switched neodymium-doped yttrium aluminum garnet (QS Nd:YAG) laser has shown effectiveness in reducing the number of melanosomes and expression of melanogenesis associated proteins.[1] Compared to the high-fluence 1064 nm QS Nd:YAG laser treatment, using the low-fluence, multiple pass and repeated version (called 'laser toning') has a lower risk of adverse events in the treatment of melasma. However, the treatment outcomes are inconsistent. Adverse events including post-inflammatory hyperpigmentation and mottled hypopigmentation by conventional laser toning may occur, especially when the melasma lesions are accompanied by erythema.[1-3] To overcome these pitfalls, a dual-pulsed (or twin-pulsed) mode of 1064 nm QS Nd:YAG laser was devised. This mode delivers the desired fluence in two evenly divided pulses, separated by a very short time interval. In a previous study, the dual-pulsed mode was clinically effective in treating hyperpigmentary disorders such as post inflammatory hyperpigmentation and Riehl's melanosis.[4,5] However, its effect has not been demonstrated at a histological or molecular level.[1,4,5] Therefore, we examined the efficacy of the dual-pulsed 1064 nm QS Nd:YAG laser at a histological and molecular level.

The laser device (Tri-beam®, Jeisys Medical Inc., Korea) used in this study provides a dual-pulsed mode with a 140 μs pulse interval. For this study *in vivo*, pre-tanned brown guinea pig skin using UVB handisol (300 mJ/cm^2, 9 times over 3 weeks; National Biological Corporation, OH, USA) and naturally-tanned human skin was used. The right forearm of a 45-year-old Korean male volunteer was chosen for this purpose. For *in vitro* tests, cultured melanin-rich mouse melanocytes (Melan-A cell line) were used. All of these were irradiated separately by a 1064 nm QS Nd: YAG laser system using the following two parameters, parameter 1: dual-pulsed mode, 3 J/cm^2 (two evenly divided pulses of 1.5 J/cm^2 delivered with a time gap of 140 μs between the pulses), 1 pass and parameter 2: conventional mode, 3 J/cm^2, 1 pass.

After laser irradiation, the guinea pig skin (at day one, and two weeks after laser irradiation) and cultured Melan-A cells (one hour after laser irradiation) were subjected to electron microscopic examination. In addition to this, skin biopsy samples were taken from the treated and non-treated areas of the human skin and evaluated using Fontana-Masson staining and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for mRNA (seven days after laser irradiation).

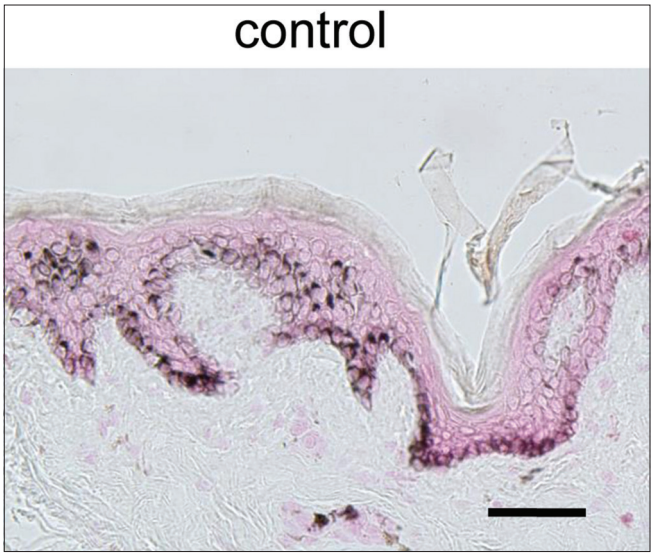

Figure 1a: Histopathology of the non-treated human forearm skin specimen (Fontana-Masson, ×200, scale bar: 50 μm)

Fontana-Masson staining of the human skin revealed a similar effect of pigment destruction across both parameters used [Figure 1]. The quantitative RT-PCR showed an increase in type III collagen levels in both modes of laser irradiation. However, the dual-pulsed mode elevated the type III collagen level three times more than the conventional mode [Figure 2]. The protease-activated receptor (PAR)-2 levels were lower by 50% in the dual-pulsed mode, compared to the conventional mode. Pro-inflammatory transcription factor such as nuclear factor κB p65 subunit (NF-κBp65, RelA) and tumor necrosis factor-α (TNF-α) were also remarkably lesser in the dual-pulsed mode compared to the conventional mode [Figure 2].

On electron microscopy of guinea pig skin, the dual-pulsed mode was shown to have lesser damage to epidermal keratinocytes and melanocytes compared to the conventional mode. At one day

Table 1: Expected clinical differences and observed biomolecular differences between the dual-pulsed *versus* conventional mode of 1064 nm QS Nd:YAG laser

Mode of 1064 nm Q-switched Nd:YAG laser	Dual-pulsed mode *versus* Conventional mode
Expected clinical differences	
Erythema	Less
Dyspigmentation (PIH or hypopigmentation)	Less
Biomolecular differences	
Collagen III	Significantly higher
Protease-activated receptor-2 (PAR-2)	Significantly lower
TNF-α	Significantly lower
NF-κBp65	Lower

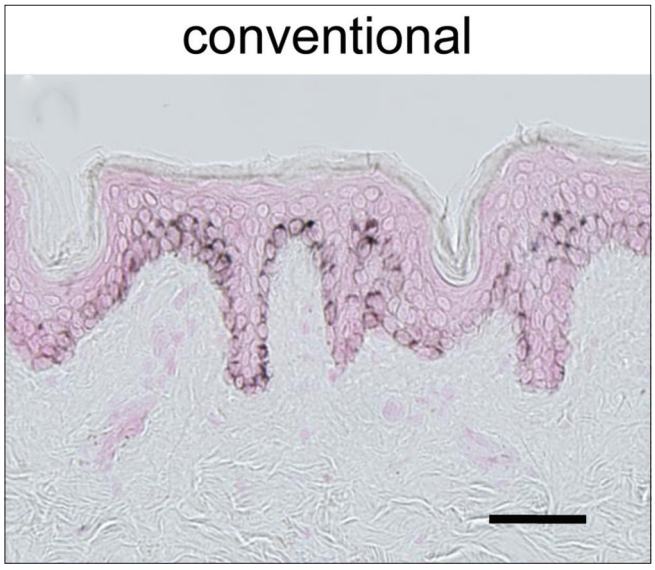

Figure 1b: Melanosomes markedly decreased by conventional mode seven days after laser irradiation (Fontana-Masson, ×200, scale bar: 50 μm)

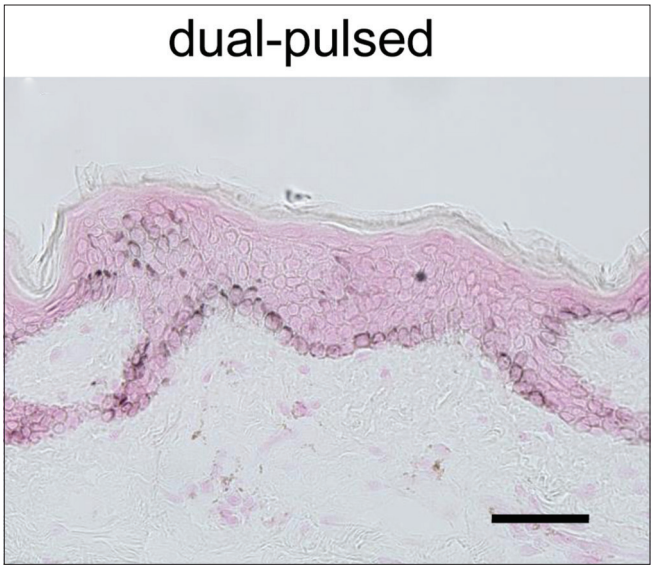

Figure 1c: Melanosomes also markedly reduced with the dual-pulsed mode seven days after laser irradiation (Fontana-Masson, ×200, scale bar: 50 μm)

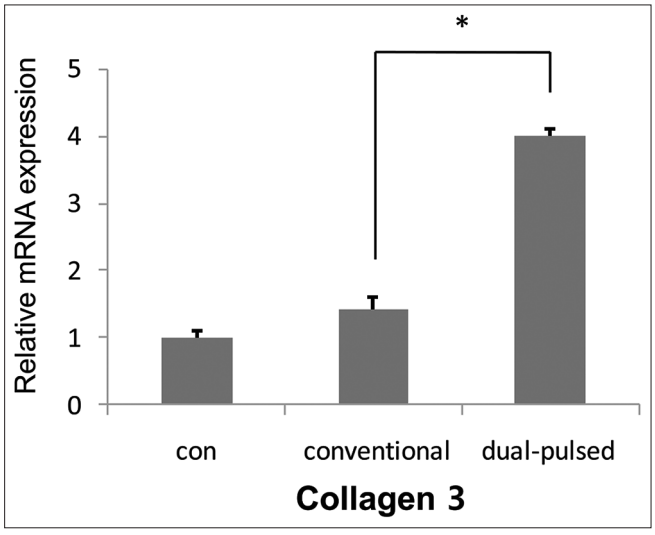

Figure 2a: Quantitative RT-PCR results in human skin biopsy samples seven days after laser irradiation. Compared to the untreated control, increase in type III collagen levels were seen in both modes of laser irradiation. Dual-pulsed mode elevated type III collagen level to a higher degree

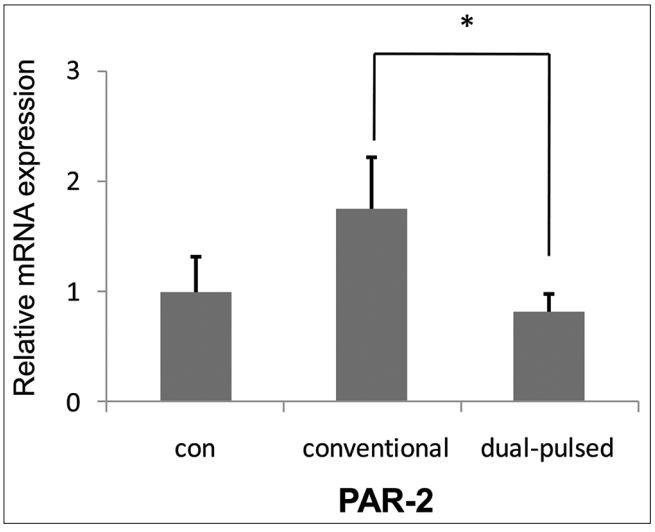

Figure 2b: The PAR-2 levels were much lower in the dual-pulsed mode than the conventional mode seven days after laser irradiation

after laser irradiation, spongiosis and damaged mitochondria in keratinocytes were noted in both modes [Figure 3a and b]. In addition, melanosomes in the upper dermis, with vacuolar changes at the dermo-epidermal junction were observed in specimens irradiated by the conventional mode [Figure 3a]. These are thought to be melanosomes that have fallen into the dermis due to an excessive laser photomechanical effect, clinically appearing as post-inflammatory hyperpigmentation. Interestingly, at two weeks after laser irradiation, the dual-pulsed mode showed less prominent spongiosis and intracellular vacuoles compared to the conventional mode. The basement membranes looked partially damaged only in the conventional mode[Figure 3c and d]. On electron microscopy of laser-irradiated cultured Melan-A cells at 1 hr after irradiation, the vacuoles were located mainly within the intra-cytoplasmic spaces when irradiated with the conventional mode, whereas it was found within the inter-cellular spaces with the dual-pulsed mode. Disrupted melanosomes and peripheral condensation of chromatin was observed in both modes, with no obvious differences [Figure 4].

Our study sheds light on the cellular and biomolecular differences between the dual-pulsed and conventional modes of 1064 nm QS Nd:YAG laser [Table 1]. The higher levels of type III collagen demonstrated after dual-pulsed mode irradiation is believed

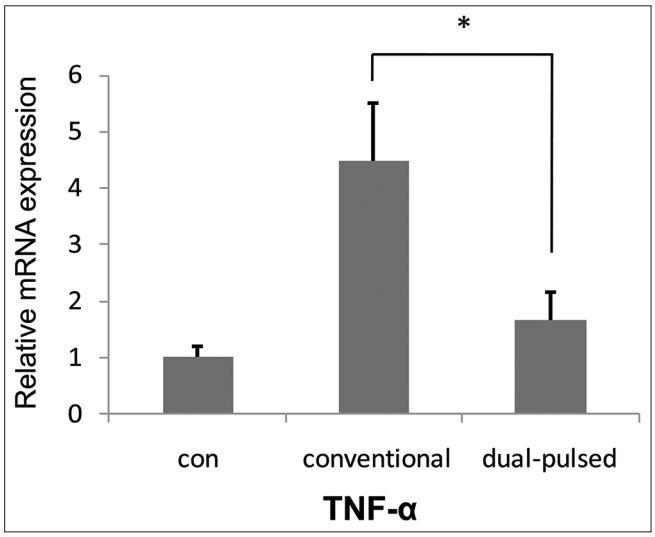

Figure 2c: The level of TNF-**α** was also remarkably lower in the dual-pulsed mode compared to the conventional mode seven days after laser irradiation

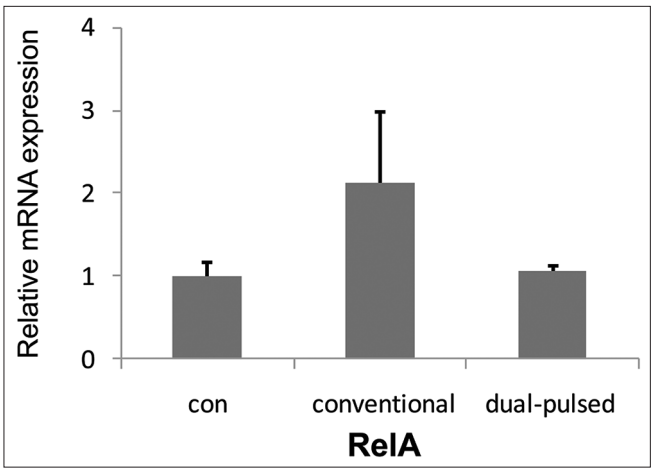

Figure 2d: By the seventh day post-irradiation, NF-κBp65 (RelA) was less expressed in the dual-pulsed mode compared to the conventional mode, though the difference was statistically insignificant

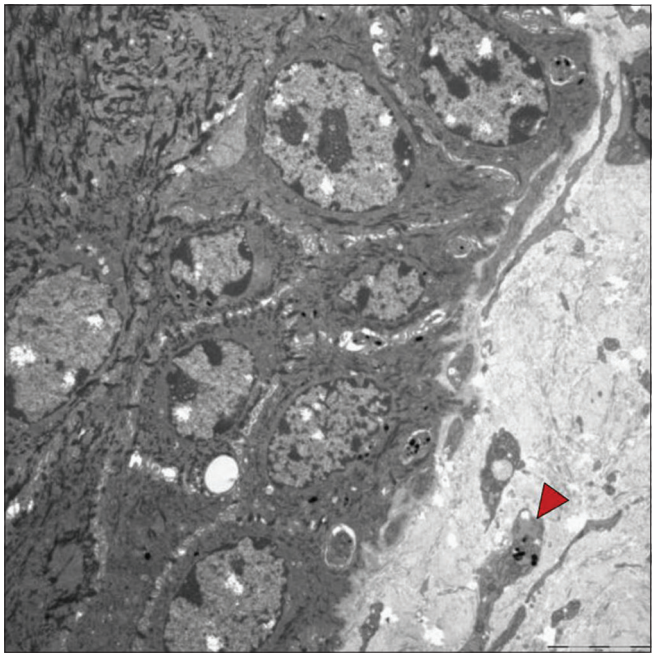

Figure 3a: Electron microscope image (×3000 magnification) of guinea pig epidermis after irradiation with conventional mode of 1064 nm QS Nd:YAG laser at 1 day after laser irradiation. Inter-cellular vacuolar spaces and melanosomes in the upper dermis (arrow), with vacuolar changes at the dermo-epidermal junction are observed

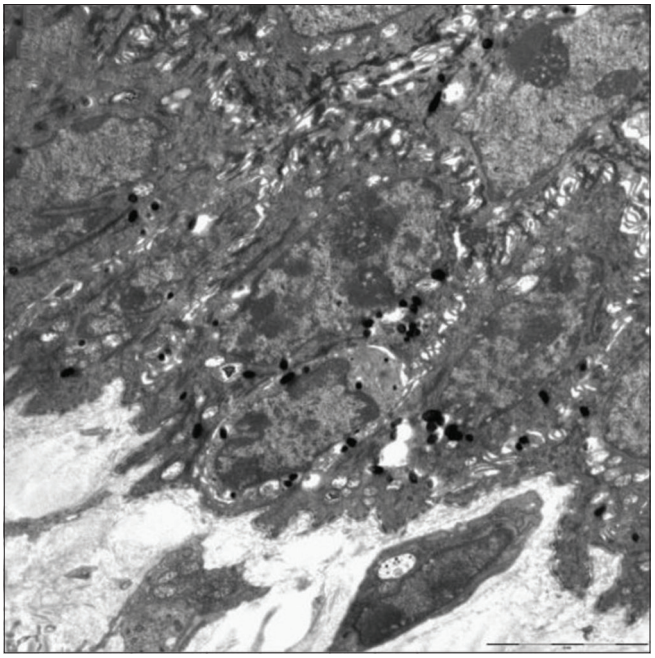

Figure 3b: Electron microscope image (×3000 magnification) of guinea pig epidermis after irradiation with 1064 nm QS Nd:YAG laser at 1 day after laser irradiation in dual-pulsed mode. Inter-cellular vacuolar spaces in keratinocytes were noted

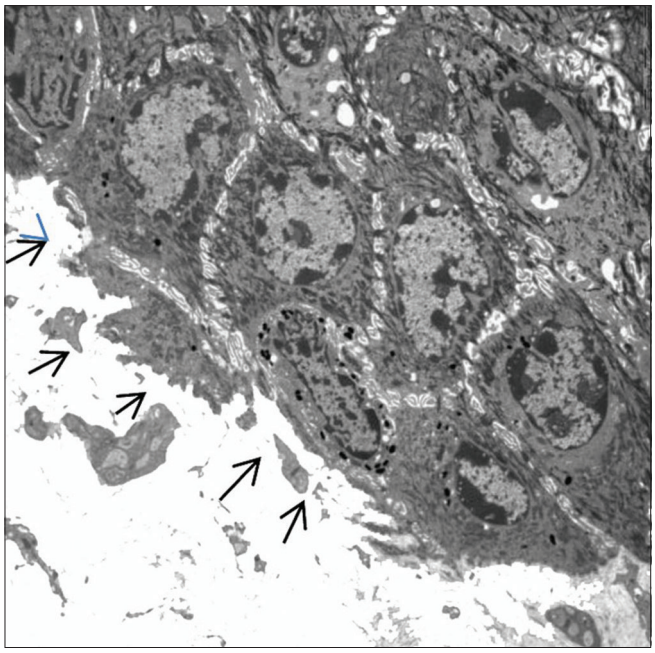

Figure 3c: At 2 weeks after laser irradiation, the conventional mode still showed prominent inter-cellular vacuolar spaces and intra-cytoplasmic vacuoles and the basement membranes are damaged (arrow) (×3000 magnification)

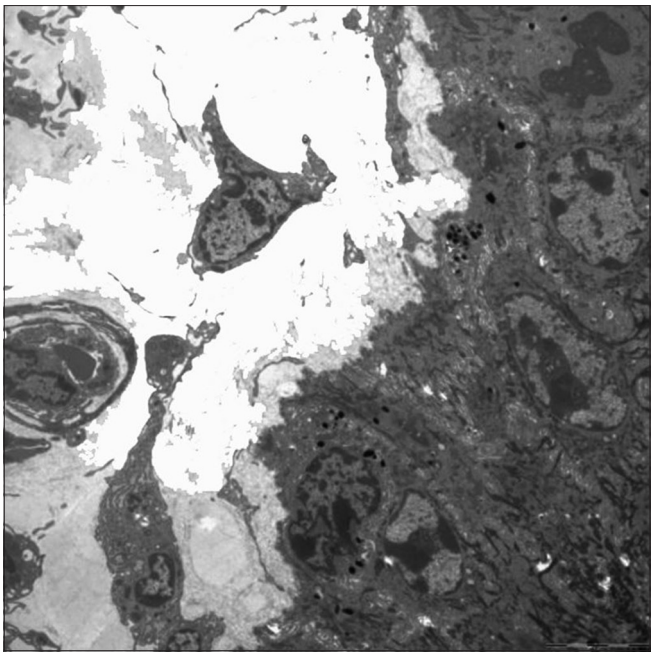

Figure 3d: At 2 weeks after laser irradiation, the dual-pulsed mode showed rare inter-cellular vacuolar spaces and intra-cytoplasmic vacuoles (scale bar: 5 μm) (×3000 magnification)

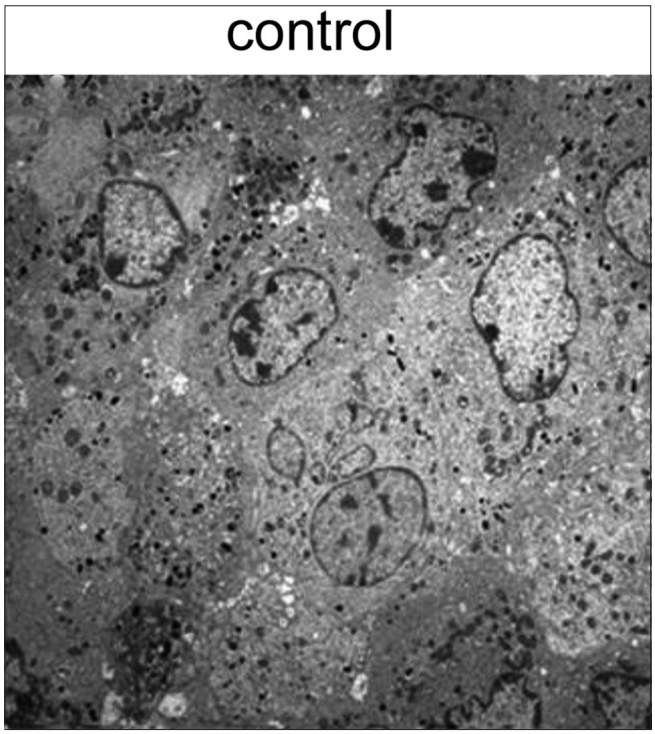

Figure 4a: Electron microscope images (×3000 magnification) of non-treated control cultured Melan-A cells

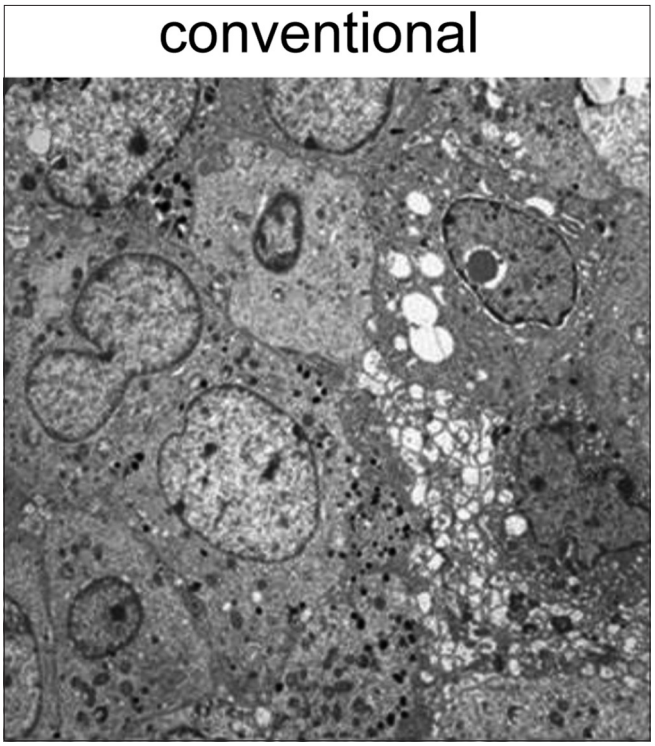

Figure 4b: One hour after irradiation with the conventional mode, many intra-cytoplasmic vacuoles and disrupted melanosomes were observed (×3000 magnification)

to result from the additional photo-acoustic waves generated by dividing the pulse. The relatively lower PAR-2 levels, pro-inflammatory transcription factors and pro-inflammatory cytokines following dual-pulsed mode indicates this mode is less susceptible to skin erythema/inflammation or resulting dyspigmentation. Electron microscopy of the irradiated guinea pig skin shows that the dual-pulsed mode exhibits gentle treatment delivery in terms of reducing damage to epidermal keratinocytes and melanocytes compared to the conventional mode. This fits well

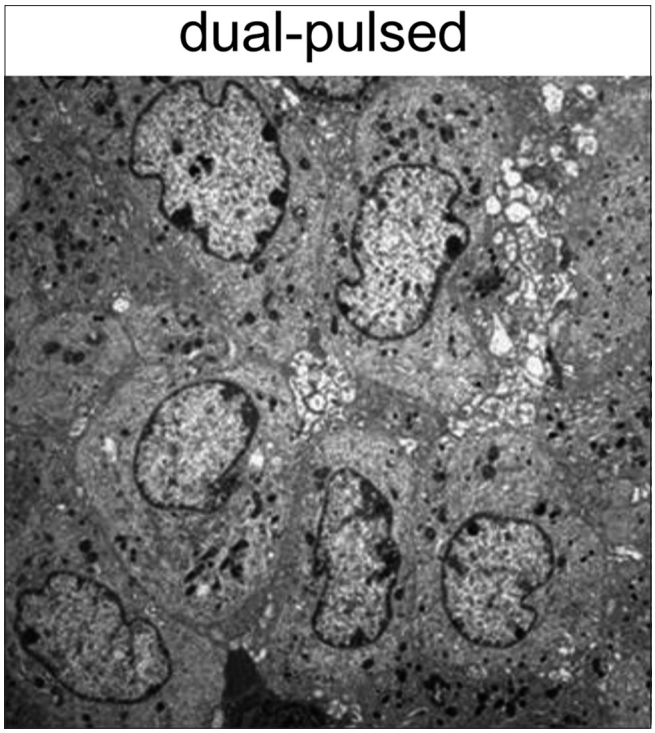

Figure 4c: Inter-cellular vacuolar spaces with melanosome disruption were seen in cultured Melan-A cells one hour post-irradiation with the dual-pulsed mode (×3000 magnification)

into the concept of strict "subcellular selective photothermolysis", that is the selective elimination of pigment, while simultaneously minimizing the adverse events caused by cellular damage and adjacent inflammatory events.[3] Although the small sample size does not allow generalization of our findings, we propose that the dual-pulsed mode of the 1064 nm QS Nd:YAG laser may be more suitable than the conventional mode for those who are prone to post-inflammatory hyperpigmentation, sensitive skin, or melasma in darker skin types.

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Conflicts of interest

There are no conflicts of interest.

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