Laser Induced Collagen Remodeling: A Comparative Study In Vivo on Mouse Model

Huaxu Liu, MD, PhD,[1,2] Yongyan Dang, PhD,[3] Zhan Wang, MD, PhD,[1] Xinyu Chai, PhD,[1] and Qiushi Ren, PhD[1]*

[1]*Institute for Laser Medicine and Bio-Photonics, Department of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China*

[2]*Shandong Provincial Institute of Dermatology and Venereology, Shandong Academy of Medical Science, Shandong 250022, China*

[3]*School of Life Science, East China Normal University, Shanghai 200240, China*

Background and Objective: Many lasers have claimed the clinical efficacy on skin rejuvenation. In this study, the mechanisms of laser induced collagen remodeling were explored systematically on a Kunming (KM) mouse model in vivo by comparing the different non-ablative laser effects using four different laser treatment modalities.

Materials and Methods: The dorsal skin of KM mice was exposed by depilation before the laser treatments. Four laser treatment modalities were used: the 595-nm pulsed dye laser (PDL) (10 ms), 1,320-nm neodymium–yttrium–aluminum garnet (Nd:YAG) laser (0.35 ms), 1,064-nm Nd:YAG laser with Q-switched (5 ns), and long-pulsed (0.3 ms) mode. Each modality exposed one side of the mouse dorsal skin leaving the other side as the contralateral control. Then skin histology, fibroblast number, and the genesis of collagen type I and III were studied by comparing the treatment site and control site at 1 hour, 1 day, 1 week, 3 weeks, 4 weeks, and 8 weeks after laser treatment. Hydroxyproline content of the skin tissue was measured 4 weeks and 8 weeks after laser exposure.

Results: All laser treatments led to marked improvements in dermal layer thickness and collagen fiber density, and the increase in fibroblast number and hydroxyproline content compared with their own controls. Collagen synthesis and remodeling induced by the Q-switched 1,064-nm laser was most effective 4 weeks after treatment, while there was no significant difference among the other three modalities. Among the new collagen genesis after the different laser treatments, collagen type III increased sharply after the Q-switched 1,064-nm laser treatment whereas more collagen type I was elicited by the other laser treatment modalities.

Conclusions: The efficacy of photo-mechanical effects in promoting more effectively the synthesis of collagen type III, whereas the photo-thermal effect favored more the formation of collagen type I. Lasers Surg. Med. 40:13–19, 2008. © 2008 Wiley-Liss, Inc.

Key words: collagen remodeling; laser; mouse model

INTRODUCTION

In recent years, non-ablative skin rejuvenation by various laser treatment modalities has achieved growing popularity due to their minimal down-time and good clinical efficacy. Goldberg first reported the effect of non-ablative laser skin resurfacing with the Q-switched 1,064-nm neodymium–yttrium–aluminum garnet (Nd:YAG) laser in 1997 [1]. They found that the Q-switched 1,064-nm Nd:YAG laser provides satisfactory clinical results with no post-operative morbidity in the treatment of periocular and perioral rhytides. Later Goldberg performed a series of clinical studies with histologic examinations to prove the collagen remodeling effects of Q-switched 1,064-nm Nd:YAG laser [2,3]. The 1,320-nm Nd:YAG laser with a dynamic surface cooling device was the first laser system specifically designed for the purpose of non-ablative skin rejuvenation [4–6]. Trelles et al. [5] investigated the histological changes in human skin 6 weeks after 8 treatments with the 1,320-nm Nd:YAG laser and observed that there is an increase in the number and density of collagen fibers, indicating some compaction in the remodeling process, less interfibrillary space, and good linear orientation of the fibers parallel to the dermoepidermal junction. The pulsed dye laser (PDL) is commonly used to treat port wine stains, hemangiomas, telangiectasias, and other vascular anomalies [7,8]. Studies using this laser have also noted post-treatment improvement in atrophic striae distensa, hypertrophic scars, and rhytides [9]. Several studies have confirmed the findings that PDL treatment of sun-damaged skin can lead to improvements in wrinkles with histological evidence [10–15]. Long pulsed 1,064-nm Nd:YAG laser with its low scattering coefficient and weak absorption by water and melanin has also been shown to improve the appearance of coarse wrinkles and fine lines and to reduce skin laxity [16–20]. Rapid sequences of long 1,064-nm pulses (0.3 ms) enable a large

Abbreviations Used: KM, Kunming; PDL, pulsed dye laser; DCD, dynamic cooling device; Nd:YAG, neodymium–yttrium–aluminum garnet.

*Correspondence to: Qiushi Ren, PhD, Institute for Laser Medicine and Bio-Photonics, Department of Biomedical Engineering, Shanghai Jiao Tong University, Room 406 Life-Science Building 2, 800 Dong-Chuan Road, Shanghai 200240, China.
E-mail: renqsh@sjtu.edu.cn

Accepted 25 October 2007
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20587

area to be covered quickly by multiple passes, causing diffused general heating of the upper dermis and thus stimulating a wound healing response and collagen remodeling. Although the efficacy of the different laser treatment modalities varies from one group to another, the reported histological findings indicate new collagen formation after the laser treatments.

The effectiveness of collagen remodeling induced by laser skin treatment as reported above can be attributed to the unique physical properties of laser technology. By wavelength selection, the laser can effectively target the specific chromospheres, such as melanin, oxygen–hemoglobin, and water, and defines the penetration depth, which affects the extent of the laser treatment in the skin. The laser pulse duration determines the mechanism of laser-tissue interactions (photo-thermal vs. photo-mechanical effect), which may result in different repairing mechanisms once the local damage is induced by the laser treatment. The laser energy density affects the degree of specific laser effect set by the laser pulse duration. The optimal combination of those parameters may lead to the most effective collagen remodeling while minimizing side-effect during the non-ablative laser skin rejuvenation.

In this paper, we conduct a comparative in vivo study on KunMing (KM) mouse model to evaluate the collagen remodeling process induced by the different non-ablative laser treatment parameters: namely, the 595-nm PDL (10 ms), 1,320-nm Nd:YAG laser (0.35 ms), 1,064-nm Nd:YAG lasers with long-pulsed (0.3 ms), and Q-switched (5 ns).

MATERIALS AND METHODS

Animal Preparations

This study was approved by Shanghai Jiao Tong University Institutional Review Board for Animal Study. One hundred standard breed KM mice were provided by the Experimental Animal Production and Supply Center of Shanghai Institutes for Biological Science (Shanghai, China). These KM mice were 8 weeks old. They were fed standardized and kept in a constant temperature and humidity environment with a 12-hour light/dark cycle. After 48 hours, the mice were shaved on their back and then denuded with depilatory cream ($(HSCH_2COO)_2Ca$,α-bisbololws, monoglyceryl ester, SIMP hair depressant, Liangfu^{TM}, Bingwang Biomedical Company, Shanghai, China).

Laser Irradiation

Experimental animals were anesthetized with Chloral Hydrate 1 ml/kg intraperitoneally. First, the dorsal area was divided into two 2×2-cm grids by a marker pen. The left grid was for laser irradiation leaving the right one as the respective control. Fifty mice were treated with 595-nm PDL (Vbeam, Candela Corporation, Wayland, MA, USA) and 1,320-nm Nd:YAG laser (CoolTouch II, Roseville, CA), and the other 50 mice were intervened with Q-switched (Medlite IV, Continuum Biomedical Inc, Livermore, CA, USA) and long-pulsed 1,064-nm Nd:YAG laser (Coolglide Vantage, Altus Medical Inc, CA, USA).

The treatment fluence level for each laser was determined as 80% of the minimal fluence level that induced erythema effect on the treated skin with that laser. These treatment parameters for each laser modality are shown in Table 1. Each mouse received three passes of laser treatment with 10% overlap.

Histological Evaluation

Skin samples of eight mice for each laser modality were taken at 1 hour, 1 day, 1 week, 3 weeks, 4 weeks, and 8 weeks, fixed in 10% formalin and then embedded in paraffin. Totally samples of 96 mice were taken and the remaining four mice were used as standby. Four samples were taken from each mouse (two laser intervened area and two control area). The samples were then sectioned to slices of 5 μm thickness and stained with hematoxylin/eosin to evaluate dermal changes and Van Gieson stain to assess the amount and organization of collagen fibers. Histopathologic analysis was performed and photographs were obtained with an Olympus light microscope.

On the slides stained with H&E, fibroblast number was counted. The cell nucleus of fibroblast was stained blue. An experienced pathologist counted the number of fibroblast in the microscopic field at a magnification of 400×. Three fields were chosen at random in each section. Eight sections were counted in each experimental group. The mean value was calculated and represented as the average number of fibroblasts.

Measurement of Type I and III Collagen

Sections at 1 week, 3 weeks, 4 weeks, and 8 weeks were stained with sirius red (Sigma, Germany) at room temperature for 1 hour. After that, they were hydrated by a series of concentrations of ethanol, then cleared with xylene, and mounted with the neutral resin. A polarization microscope (XPT-7, Cany Precision Instruments Co., Shanghai, China) was used to distinguish collagen type I from collagen type III. Under the polarized microscope, the collagen fibers of type I showed red while the collagen fibers of type III were

TABLE 1. Laser Parameters

Lasers (nm)	Fluence (J/cm^2)	Pulse Width	Spot (mm)	Cooling	Epidermal Temperature
595	12	10 ms	7	25 ms	—
QS 1064	2.5	5 ns	6	—	—
LP 1064	30	0.3 ms	6		
1320	22	350 μs	10	25 ms	40–42°C

green. The increase in percentages of collagen type I and III at 8 weeks were evaluated by two histologists who were unaware of the laser modalities.

Quantitative Analysis of Hydroxyproline

Skin samples at 4 weeks and 8 weeks were taken for hydroxyproline content assay. The samples were delipidized with a blade and weighed for 100 mg. A 2% skin homogenate was prepared from a physiological saline solution. Hydroxyproline concentration was measured with the chemical colorimetry method by commercial detection kit (A030 Hydroxyproline Detection Kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical Analysis

All experimental data were analyzed using SPSS 11.5 software, and the figures were made via SAS V8.2. Both of the software were commercially available. Statistical analysis was performed with the paired t-test analysis for each laser modality between the laser irradiated skin and the control. Then, one-way AVONA test was done to compare the difference between those different laser modalities. Data were expressed as mean $\pm$ standard deviation, and difference in measured values was considered to be statistically significant when $P<0.05$.

RESULTS

Histological Changes Induced by Four Laser Treatment Modalities

Histological observations demonstrate that: 1 hour after the Q-switched Nd:YAG laser irradiation, thin vessels were dilated with extravasations of red blood cells followed by a mild chronic inflammatory infiltration. Vessel dilation and edema were observed in the irradiated areas of the 595-nm laser while obvious denaturization of collagen fibers was noted for the 1,320-nm laser. For the 1,064-nm long pulsed laser, only slight dermal wound was observed after irradiation. However, the epidermis was intact irrespective of laser wavelength. After 1 day, neutrophils and monocytes appeared in the extravascular dermis. Their appearance is followed somewhat later by lymphocytes, which appear with great numbers at 1 week after treatment. After 1 week the inflammatory reactions associated with fibroblastic proliferation disappeared. Meanwhile, the epidermis became even and its thickness increased markedly. After 3 weeks, collagen fibers began to increase, ranging from disorganized collagen with reduced affinity for stain to new collagen formation with an improvement in the organization of collagen fibrils. After 4 weeks, the increases in collagen and fibroblasts were quite remarkable, associated with thicker epidermis. Figure 1 showed that a significant increase in fibroblasts was noted in the areas of laser irradiation compared to their respective controls at 4 weeks. It was found that the fibroblast number increased by $37.0 \pm 5.2\%$ in the 595-nm laser-treated group, $35.5 \pm / -4.5\%$ in the 1,320-nm laser-treated group, $31.4 \pm 4.6\%$ in the 1,064-nm long pulse laser-treated group, and $58.0 \pm 5.1\%$ in the Q-switched Nd:YAG laser-treated group.

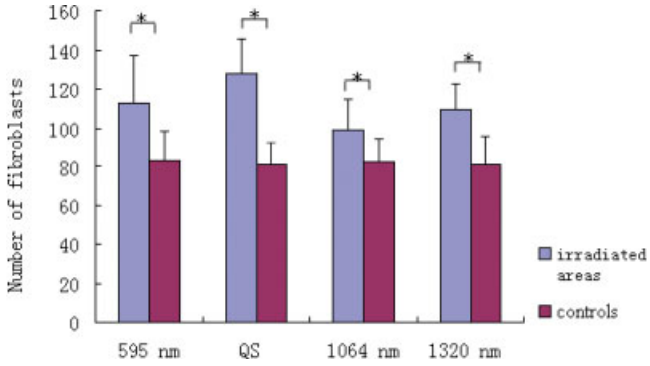

Fig. 1. The number of fibroblasts at 4 weeks after the four laser irradiation. *$P < 0.05$. [Figure can be viewed in color online via www.interscience.wiley.com.]

Obviously, the largest increase in fibroblast number was for the Q-switched Nd:YAG laser treatment at 4 weeks post-irradiation ($P < 0.05$). Moreover, following the quick increase at the fourth week of post-operative treatment, the number of fibroblast maintained similar increase level up to 8 weeks in the histological improvement when compared with controls, whereas the epidermal thickness at 8 weeks returned to the normal level. The histological examination at 4 weeks and 8 weeks revealed that collagen fibers increased markedly after laser irradiation, ranging from disorganization and reduced affinity for the stain to parallel well-oriented fibers with enhanced staining (Fig. 2). Additionally, the Q-switched Nd:YAG laser induced more histological improvement, consistent with the fibroblast count described above (Fig. 2).

Fibroblast Proliferation Accounted for the Increase in Hydroxyproline Content

As shown above, fibroblasts proliferated markedly following laser irradiation. In order to examine whether fibroblast proliferation is related to the increase in collagen, we used a biochemical method to quantify hydroxyproline content in the skin. Biochemical analysis revealed that the level of hydroxyproline elevated in the sites of laser irradiation, compared to non-irradiated skin. At 4 weeks, the mean values of hydroxyproline content in the irradiated areas were 0.72 ± 0.09 μg/mg (the 595-nm laser), 0.68 ± 0.07 μg/mg (the 1,320-nm laser), 0.64 ± 0.07 μg/mg (the 1,064-nm long pulse laser), and 0.82 ± 0.05 μg/mg (the Q-switched Nd:YAG laser). By contrast, their controls were 0.33 ± 0.02, 0.32 ± 0.01, 0.31 ± 0.02, and 0.33 ± 0.01 μg/mg respectively. Hydroxyproline content increased approximately one fold for the four lasers within 4 weeks and remained elevated at 8 weeks after laser irradiation.

Figure 3 illustrated the correlations between fibroblast number and hydroxyproline content at 4 weeks. The amount of hydroxyproline content showed a marked increase in relation to the fibroblast proliferation. The positive correlation between hydroxyproline level and fibroblast number was highly significant ($r = 0.892$).

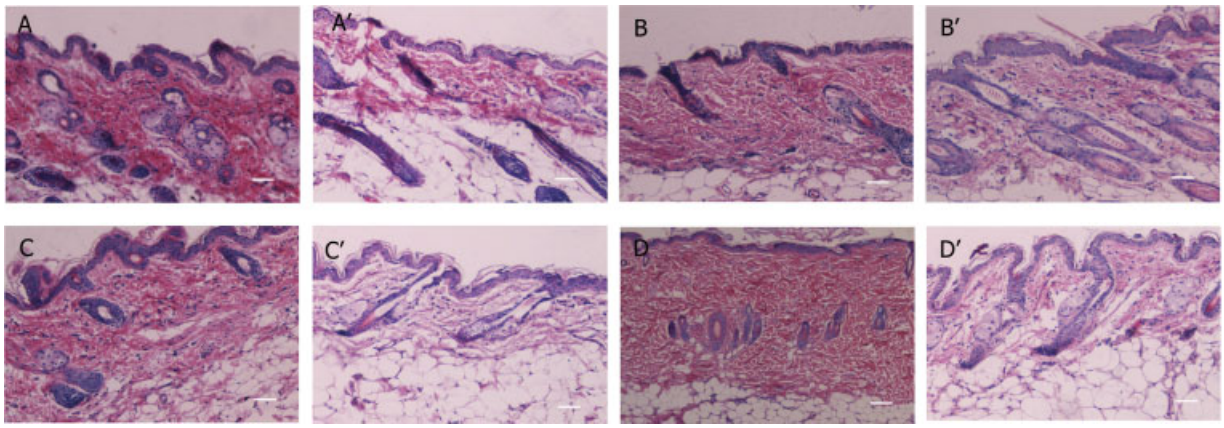

Fig. 2. Hematoxylin and eosin stain of mouse skin taken at 4 weeks after the four laser treatment modalities. Collagen fibers in the irradiated sites showed a marked increase in the amount and a significant improvement in the organization with the high affinity for the stain; (**A**) the 595-nm pulsed dye laser (PDL), (**B**) 1,320-nm neodymium–yttrium–aluminum garnet (Nd:YAG) laser, (**C**) 1,064-nm long-pulsed Nd:YAG lasers, (**D**) 1,064-nm Q-switched laser; **A′**, **B′**, **C′**, and **D′** were their respective controls. Scale bar = 100 μm. [Figure can be viewed in color online via www.interscience.wiley.com.]

The Increase in Collagen Type I and III by Four Laser Treatment Modalities

We compared the effects of the four laser irradiation paradigms on type I and III collagen synthesis. Eight weeks after laser treatments, increase in collagen type I and III were respectively observed with the 595-nm ($52.7 \pm 5.6\%$ vs. $24.4 \pm 2.8\%$), 1,320-nm ($48.9 \pm 3.7\%$ vs. $22.5 \pm 4.2\%$), 1,064-nm long-pulsed ($46.4 \pm 4.6\%$ vs. $15.9 \pm 2.9\%$), and Q-switched ($45.1 \pm 6.1\%$ vs. $50.3 \pm 5.4\%$) lasers (Figs. 4 and 5). Figure 5 presented the relative percent increase in collagen type I and III after the four laser irradiation. Obvious increase in collagen type III was observed in the irradiated sites of the Q-switched Nd:YAG laser compared to the other lasers ($P<0.05$), while there was no significant differences among the other three laser modalities.

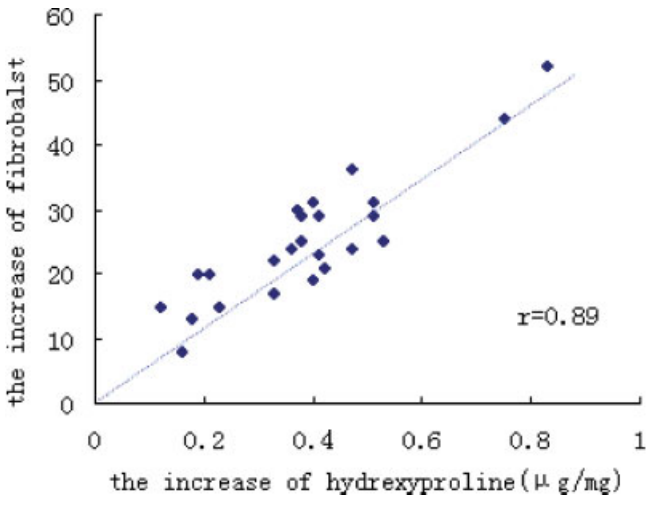

Fig. 3. Correlation between the increase in hydroxyproline content and fibroblast proliferation.

DISCUSSION

As Goldberg suggested, non-ablative skin rejuvenation is based on the theory of delivering a useful packet of photothermal damage to the upper dermis under a cooled epidermis, thereby initiating the wound healing process under the biological protection of an intact epidermis and achieving the required collagen synthesis and remodeling [3]. Based on the theory of selective photothermolysis [21], the four lasers we applied in this study target different chromospheres. 595-nm PDL emits yellow light, which selectively targets oxyhemoglobin. However, it is also strongly scattered by the epidermis of the skin, resulting in limited penetration. Thus, the effect of 595-nm laser was thermal and relatively defined in the superficial layer of the skin. The 1,320-nm Nd:YAG laser was designed to thermally injure the dermis while protecting the epidermis with its surface cooling mechanism. By cooling the epidermis, the epidermal chromophores are effectively shielded from injury by the incident light. The relatively long 1,320-nm wavelength achieves excellent penetration into the papillary and midreticular dermis where it is non-specifically absorbed by dermal water [22]. The large scattering coefficient of the 1,320-nm Nd:YAG laser causes the thermal energy to disperse laterally within the dermis, inducing a large volume of dermal injury relative to the beam size [23]. For the long-pulsed 1,064-nm laser, since the main target is protein with water as a secondary target, the heating effect in the target tissue absorbing the 1,064-nm beam is non-specific [20]. In addition, because of the scattering effect of tissue at this wavelength, the area of

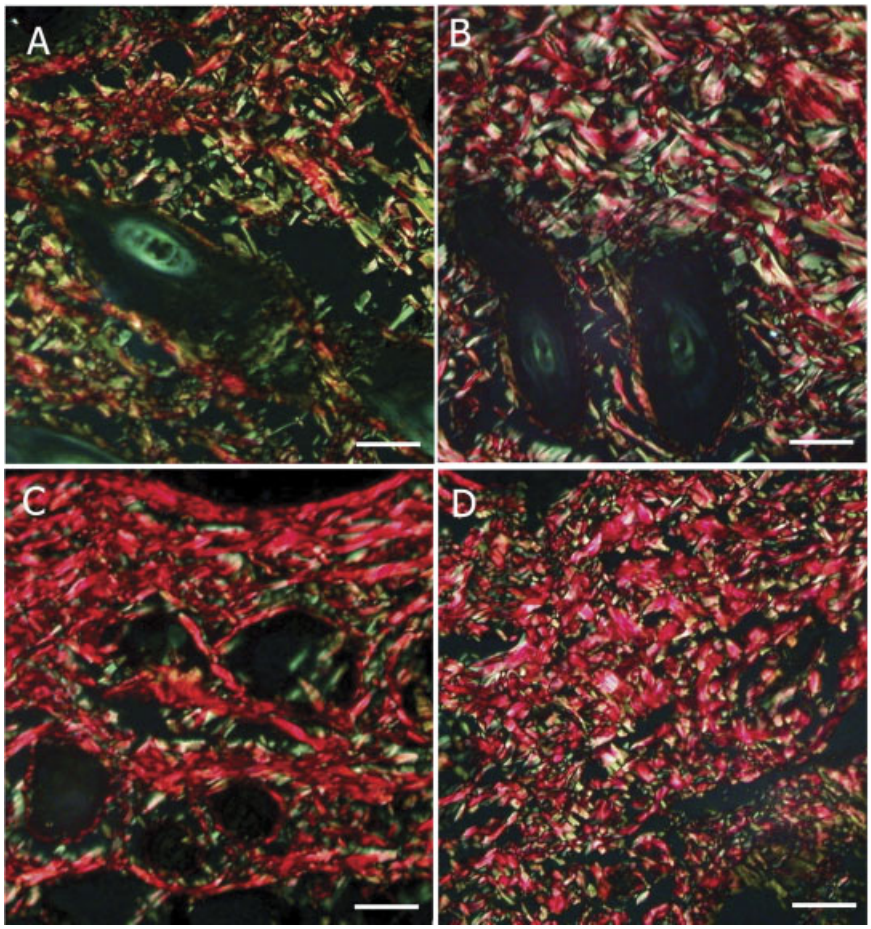

Fig. 4. The Q-switched 1,064-nm laser caused more increase of collagen type III (**A**) (green) while the 595-nm pulsed dye, long pulsed 1,064-nm, and 1,320-nm Nd:YAG lasers induced more increase in collagen type I (red) (**B**, **C**, **D**). Sirius Red stain, scale bar = 100 μm. [Figure can be viewed in color online via www.interscience.wiley.com.]

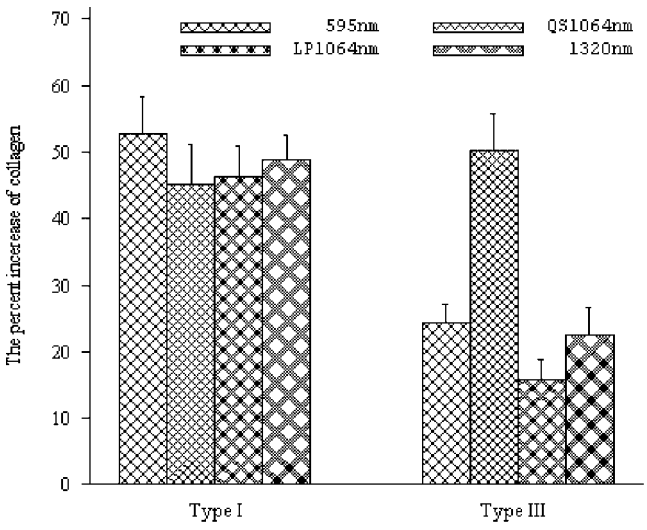

Fig. 5. The relative increase in percentages of collagen type I and III ranked by two blind histologists at 8 weeks after the four laser irradiation.

the highest photon density, and thus the photo-thermal effect, is not at the surface of the tissue, but some 1–2 mm below the surface [20]. The Q-switched 1,064-nm Nd:YAG laser is characterized by very short pulse width, which produces the dermal damage mainly by the photo-mechanical effect; moreover, the Q-switched laser has less water absorption and lower scattering, which results in a relatively deeper dermal wound [3].

In our study, we compared the effects of dermal remodeling with these four laser modalities, and found that, although all the lasers produced a significant improvement when compared with their respective controls, there does exist marked differences in tissue response within these lasers. The 1,064-nm Q-switched Nd:YAG laser caused more histological improvement, fibroblast proliferation, and collagen synthesis than the other three lasers in our study (Figs. 1 and 2). By contrast, collagen synthesis and remodeling induced by the 1,064-nm 1ong pulsed laser was less than the other three lasers. Therefore, the 1,064-nm Q-switched Nd:YAG laser was most effective for KM mice skin collagen remodeling among the four lasers. These findings indicated that collagen remodeling

after laser irradiation was related to laser-tissue effects. Within the set of parameters tested, the photo-mechanical effect proved most efficacious in collagen remodeling.

When skin samples were examined, the Q-switched 1,064-nm laser irradiation caused thin vessel dilation, dermal edema, and subsequent inflammatory changes. Following these changes, collagen fibers began to increase. Therefore, the likely mechanism by which the 1,064-nm Q-switched laser non-ablative treatment improved skin morphology was due to inflammatory reactions. For the 595-nm laser, we observed disruption of vessels and extravasation of red blood cells, so the 595-nm laser was mediated through vascular hemoglobin absorption leading to endothelial disruption, cytokine activation, and subsequent collagen remodeling. For the long-pulsed 1,064-nm laser treatment areas, only slight dermal wound and inflammatory changes were observed; meanwhile, fibroblast number and collagen content were less than the 595-nm and the Q-switched 1,064-nm lasers. To some extent, more dermal damage correlated with more new collagen syntheses. For the 1,320-nm laser, obvious denaturization of collagen fibers was noted. Based on the past studies demonstrating the relation between the mechanical stress on fibroblast and collagen synthesis [24,25], collagen remodeling induced by the 1,320-nm laser might be mainly dependent on the contraction of collagen fibers.

Fibroblasts play a crucial role in mediating wound healing responses, ranging from their synthesis and remodeling of extracellular matrix to the production of growth factors [26]. Since hydroxyproline is a major component of collagen and rarely found outside collagen, it can be used to measure the content of collagen. In this study, we applied fibroblast number analysis and hydroxyproline measurement in addition to making general histological observations and measuring the dermal thickness. After 2 months, we found that both fibroblast number and hydroxyproline content increased markedly in the laser-treated areas when compared with respective controls. This indicated laser irradiations could strongly stimulate collagen synthesis that was crucial to wrinkle removal. Additionally, there was a positive correlation between hydroxyproline level and fibroblast number (Fig. 3), which suggests that fibroblast proliferation accounted for the increase in hydroxyproline content.

Dermal matrix in adult skin is composed of collagen type I (80–85%) and type III (10–15%), in addition to glycosaminoglycans and elastin fibers [27,28]. Reduction of fibrillar (type I and III) collagen is a characteristic feature of chronologically aged skin and is enhanced by photodamage treatments [29]. Collagen types I and III are synthesized by dermal fibroblasts as α procollagens. Type III collagen is the dominant isotype found in foetal skin during the early stages of development. However, during childhood and early adult life collagen type I becomes the dominant collagen of the skin [30]. During the aging process, collagen type III is gradually replaced by collagen type I [31]. Therefore, collagen type III was demonstrated to be very important in skin collagen remodeling. In our study, not only collagen type I but also collagen type III increased markedly after the four laser treatments. Moreover, the differences between the four laser treatments were significant. The Q-switched 1,064-nm laser resulted in more synthesis of collagen type III than that of the other lasers, while for the other three laser modalities, the increase in collagen type I overpasses markedly collagen type III. Correlating their different laser-tissue effects and our findings, we demonstrated photo-mechanical effect using a Q-switched Nd:YAG laser promotes more effectively the synthesis of collagen type III, whereas the photo-thermal effect favored more the formation of collagen type I. As demonstrated in our study that the Q-switched 1,064-nm Nd:YAG laser promotes the most synthesis of collagen type III, which is the predominant collagen in youth skin, our results suggest that the Q-switched 1,064-nm Nd:YAG laser treatment modality may be more effective in increasing the elasticity of treated skin. Recent published results [32,33] demonstrated that Q-switched 1,064-nm Nd:YAG laser leads to significant quantitative improvements in skin topography in patients with mild to moderate atrophic acne scars, and showed a greater response compared with the 1,320-nm laser system, which could be explained by our experimental findings.

Although the 1,064-nm Q-switched and long-pulsed Nd:YAG lasers with the have the same wavelength, their different action mechanisms lead to different effects of collagen remodeling responses. Since the long pulsed 1,064-nm laser, just like the 595-nm PDL and 1,320-nm Nd:YAG laser, created dermal damage by photo-thermal effects while the Q-switched 1,064-nm laser produces photo-mechanical effects, it is clear that collagen synthesis and remodeling are indeed related to the mechanisms of laser-tissue interactions.

Simultaneously, we observed that the collagen began to increase 3 weeks after laser treatments and the increasing trend could persist for 5 weeks reaching its greatest level of effect in 4 weeks, a result similar to human clinical trials [19]. Maintenance treatments may be required to achieve lasting effects. Further studies are needed to observe how long the newly created collagen will last.

In summary, our results demonstrated a high correlation between collagen remodeling and laser-tissue effects. We observed that all the four laser irradiation modalities could promote collagen synthesis and remodeling of KM mice skin, and that fibroblast proliferation correlated with collagen synthesis. We also demonstrated that photo-mechanical effect promotes more effectively the synthesis of collagen type III, whereas the photo-thermal effect favored more the formation of collagen type I.

Since it has been documented previously that clinical improvement is not always directly consistent with observed histological changes [34], similar comparative studies in human clinical settings are needed to confirm the results achieved in our in vivo experiments.

ACKNOWLEDGMENTS

Many thanks to Shanghai Kang-Ao Plastic and Laser Surgery Hospital for providing laser devices to Yuxiu Liu

and Jinsheng Zhang for the help with histological technique. This research is supported by the National Basic Research Program of China (973 Program, 2005CB724302), National Science Fund for Distinguished Young Scholars from The National Natural Science Foundation of China (60588101), and the Shanghai Commission of Science and Technology (05DZ22318, 05DZ22325, 04DZ05114).

References

1. Goldberg DJ, Whitworth J. Laser skin resurfacing with the Q-switched Nd:YAG laser. Dermatol Surg 1997;23(10):903–906.
2. Goldberg DJ. Q-switched Nd:YAG laser: Rhytid improvement by non-ablative dermal remodeling. J Cut Laser Ther 2000;2:157–160.
3. Goldberg DJ, Silapunt S. Histologic evaluation of a Q-switched Nd:YAG laser in the non-ablative treatment of wrinkles. Dermatol Surg 2001;27:744–746.
4. Lask GL, Lee PK, Seyfadeh M, et al. Nonablative laser treatment of facial rhytids. SPIE Proc 1997;2970:338–349.
5. Trelles MA, Allones I, Luna R. Facial rejuvenation with a nonablative 1320 nm Nd:YAG laser: A preliminary clinical and histologic evaluation. Dermatol Surg 2001;27:111–116.
6. Levy JL, Trelles M, Lagarde JM, Borrel MT, Mordon S. Treatment of wrinkles with the non-ablative 1,320-nm Nd:YAG laser. Ann Plast Surg 2001;47(5):482–488.
7. Alster TS, Kurban AK, Grove GL, Tan OT. Alteration of argon laser induced scars by the pulsed dye laser. Lasers Surg Med 1993;13:368–373.
8. Alster TS. Improvement of erythematous and hypertrophic scars by the 585 nm flashlamp-pumped pulsed dye laser. Ann Plastic Surg 1994;32:186–190.
9. Alster TS, William CM. Treatment of keloid sternotomy scars with 585 nm flashlamp-pumped pulsed dye laser. Lancet 1995;345:1198–1200.
10. Zelickson BD, Kilmer SL, Bernstein E, Chotzen VA, Dock J, Mehregan D, Coles C. Pulsed dye laser therapy for sun damaged skin. Lasers Surg Med 1995;25(3):229–236.
11. Zelickson B, Kist D. Effect of pulsed dye laser and intense pulsed light source on the dermal extracellular matrix remodeling. Lasers Surg Med 2000;12 (Suppl):17.
12. Tanghetti EA, Sherr EA, Alvarado SL. Multipass treatment of photodamage using the pulse dye laser. Dermatol Surg 2003;29:686–691.
13. Dahiya R, Lam S, Williams EF. A systematic histologic analysis of nonablative laser therapy in a porcine model using the pulse-dye laser. Arch Facial Plast Surg 2003;3:518–523.
14. Goldberg DJ, Sarradet D, Hussain M, Krishtul A, Phelps R. Clinical, histologic and ultrastructural changes after nonablative treatment with a 595-nm flashlamp-pumped pulsed dye laser: Comparison of varying settings. Dermatol Surg 2004;30:979–982.
15. Hsu TS, Zelickson B, Dover JS, Kilmer S, Burns J, Hruza G. Multicenter study of the safety and efficacy of a 585 nm pulsed-dye laser for the non-ablative treatment of facial rhytides. Dermatol Surg 2005;31(1):1–9.
16. Rogachefsky AS, Becker K, Weiss G, Goldberg DJ. Evaluation of a long-pulsed Nd:YAG laser at different parameters: An analysis of both fluence and pulse duration. Dermatol Surg 2002;28:932–935.
17. Dayan S, Damrose JF, Bhattacharyya TK, Mobley SR, Patel MK, O'Grady K, Mandrea S. Histological evaluations following 1,064-nm Nd:YAG laser resurfacing. Lasers Surg Med 2003;33(2):126–131.
18. Dayan S, Vartanian AJ, Menaker G. Nonablative laser resurfacing using a long pulse 1064-nm Nd:YAG laser. Arch Facial Plast Surg 2003;5:310–315.
19. Schmults CD, Phelps R, Goldberg DJ. Nonablative facial remodeling: Erythema reduction and histologic evidence of new collagen formation using a 300-microsecond 1064-nm Nd:YAG laser. Arch Dermatol 2004;140(11):1373–1376.
20. Trelles MA, Alvarez X, Martin-Vazquez MJ, Trelles O, Velez M, Levy JL, Allones I. Assessment of the efficacy of nonablative long-pulsed 1064-nm Nd:YAG laser treatment of wrinkles compared at 2, 4, and 6 months. Facial Plast Surg 2005;21(2):145–153.
21. Anderson RR, Parrish JA. Selective photothermolysis: Precise microsurgery by selective absorption of pulsed radiation. Science 1983;220:524–527.
22. Fatemi A, Weiss MA, Weiss RA. Short-term histologic effects of nonablative resurfacing: Results with a dynamically cooled millisecond-domain 1320 nm Nd:YAG laser. Dermatol Surg 2002;28:172–176.
23. Sadick N, Schecter AK. Utilization of the 1320-nm Nd:YAG laser for the reduction of photoaging of the hands. Dermatol Surg 2004;30(8):1140–1144.
24. Fluck M, Giraud M-N, Tunc V, Chiquet M. Tensile stress-dependent collagen XII and fibronectin production by fibroblasts requires separate pathways. Biochim Biophys Acta. 2003;1593:239–248.
25. Varani J, Schuger L, Dame MK, Leonard C, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ. Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin. J Invest Dermatol 2004;122: 1471–1479.
26. Cook H, Davies KJ, Harding KG, Thomas DW. Defective extracellular matrix reorganization by chronic wound fibroblasts is associated with alterations in TIMP-1, TIMP-2, and MMP-2 activity. J Invest Dermatol 2000;115(2):225–233.
27. Moseley R, Stewart JE, Stephens P, Waddington RJ, Thomas DW. Extracellular matrix metabolites as potential biomarkers of disease activity in wound fluid: Lessons learned from other inflammatory diseases? Br J Dermatol 2004; 150(3):401–413.
28. Baumann L, Kaufman J, Saghari S. Collagen fillers. Dermatol Ther 2006;19(3):134–140.
29. Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ. Decreased collagen production in chronologically aged skin: Roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol 2006;168(6):1861–1868.
30. Lovell CR, Smolenski KA, Duance VC, Light ND, Young S, Dyson M. Type I and III collagen content and fibre distribution in normal human skin during ageing. Br J Dermatol 1987;117(4):419–428.
31. Fernandes D. Minimally invasive percutaneous collagen induction. J Oral Maxillofac Surg Clin North Am 2005;17: 51–63.
32. Friedman PM, Jih MH, Skover GR, Payonk GS, Kimyai-Asadi A, Geronemus RG. Treatment of atrophic facial acne scars with the 1064-nm Q-switched Nd:YAG laser: Six-month follow-up study. Arch Dermatol 2004;140(11):1337–1341.
33. Yaghmai D, Garden JM, Bakus AD, Massa MC. Comparison of a 1,064 nm laser and a 1,320 nm laser for the nonablative treatment of acne scars. Dermatol Surg 2005;31(8 Pt 1):903–909.
34. Alam M, Hsu TS, Dover JS, Wrone DA, Arndt KA. Nonablative laser and light treatments: Histology and tissue effects-a review. Lasers Surg Med 2003;33:30–39.