

Long-Term Evaluation of Collagen and Elastin Following Infrared (1100 to 1800 nm) Irradiation

Yohei Tanaka MD,^{a,b} Kiyoshi Matsuo MD PhD,^a Shunsuke Yuzuriha MD PhD^a

^aDepartment of Plastic and Reconstructive Surgery, Shinshu University School of Medicine, Matsumoto, Japan

^bClinica Tanaka Plastic and Reconstructive Surgery and Anti-Aging Center, Matsumoto, Japan

ABSTRACT

Background: Infrared irradiation stimulates collagen production, but histological differences in its long-term effects on type I versus type III collagen and elastin in human tissue are unclear.

Objective: To investigate the effects of infrared irradiation.

Methods and Materials: In vivo human tissues in sun-protected and sun-exposed areas were irradiated with infrared. Histological samples were analyzed, and visual changes were assessed up to 90 days post-treatment.

Results: Infrared irradiation provided long-term increases in collagen and elastin levels on post-irradiation days 30, 60 and 90 compared to controls. Significant increases in type I collagen persisted until 30 and 60 days, and in sun-protected and exposed skin biopsies, respectively. Significant increases in type III collagen and elastin persisted until 90 days in both sun-protected and sun-exposed skin biopsies.

Conclusion: Infrared irradiation provides safe and effective long-term stimulation of collagen I and III and elastin, which is beneficial for improving skin laxity and wrinkles.

INTRODUCTION

Skin aging is a complex biological phenomenon affecting different components of skin, and consists of photoaging and intrinsic aging.¹⁻³ Skin aging is commonly associated with skin wrinkling, sagging and laxity. Non-ablative lasers work by thermally stimulating dermal collagen remodeling.⁴ Thermal damage denatures the collagen and encourages the generation of new collagen, resulting in tighter skin.⁵⁻⁹

Infrared (IR) irradiation is also thought to induce the generation of collagen and, thus, result in tighter skin.⁹ IR lasers produce clearly measurable elevations in markers of collagen production.¹⁰ IR irradiation increases the amounts of collagen and elastin in human dermal fibroblasts, and can result in clinical improvement of skin texture.¹¹ IR irradiation at 10 or 20 J/cm² increases the amounts of both collagen and elastin in all layers of the dermis without denaturing the collagen in human skin.¹² The authors previously reported that IR irradiation induces preferential elevation of type I collagen without a long-term increase in type III collagen. The increases in collagen production by IR irradiation were estimated to fade gradually over three-to-four months in rat tissue.¹³

The authors hypothesized that IR irradiation also induces long-term proliferation of collagen and elastin in human tissue. To verify this hypothesis, the authors investigated changes over time by histological investigation after IR irradiation.

MATERIALS & METHODS

Serial skin biopsies were taken from two study volunteers with Fitzpatrick skin type III. Biopsies of sun-protected skin were taken

from the thigh a 33-year-old Japanese man, and biopsies of sun-exposed skin were taken from the face of a 30-year-old Japanese woman. Neither subject had a history of any type of skin disease or any cosmetic procedures affecting the treatment areas within the last five years. Control biopsies received neither treatment nor irradiation. Treatments consisted of two passes of IR irradiation at 36 J/cm². All biopsies were taken at least 1 cm from previous biopsies to ensure wound healing would not affect adjacent biopsy sites. The study participants received complete information on the treatment and signed informed consent forms. No topical pre-treatment was used, and the post-treatment skin care regimen consisted of a gentle cleanser and sunblock.

IR irradiation was performed using a Titan infrared handpiece (Cutera, Brisbane, CA) that emits a spectrum of infrared from 1,100 to 1,800 nm to safely heat water in the tissue without absorption by melanin. IR heats the dermis without causing epidermal damage by cooling the skin (pre, parallel and post) during treatment through a temperature-controlled sapphire window.¹⁴

Fluence ranges for the study were determined by clinical experience with Titan and prior published efficacy ranges. Typical Titan treatment parameters range from 22 to 34 J/cm². These match with the range of 20–40 J/cm² reported by Esparza to be the effective fluence range for Titan therapy.¹⁴ The authors reported previously that IR irradiation at 20 J/cm² is sufficient to induce histological changes in the epidermis, but that higher energies have a greater response, and are preferable for dermal effects, such as skin tightening.¹³ Therefore, the authors performed irradiation at 36 J/cm² in this study, which was the highest energy that could be used without topical anesthesia.

All patients gave informed consent to participation in the study after reading the experimental protocol and being advised about the risks of treatment.

Histological Investigation

Ten skin specimens (five from each patient) were obtained for microscopic investigation. Biopsies were taken pre-irradiation as a control and on post-irradiation days 30, 60 and 90 (P30, P60 and P90, respectively). The specimens were fixed in 20% neutral buffered formalin and processed for paraffin embedding. Specimens were then serially sectioned in the sagittal plane (3–4 μm thick), and evaluated by hematoxylin and eosin, immunohistochemical and Victoria Blue staining. Collagen staining was performed with purified anti-human collagen type I and III polyclonal antibodies (Novotec, Lyon, France) at a dilution of 1:500 for type I and 1:1000 for type III. Immunohistochemical staining was performed with the EnVision detection system, peroxidase/DAB+ (K5007; Dako Denmark A/S, Glostrup, Denmark). The sections were examined and photographed under an Olympus BX51 microscope (Olympus, Tokyo, Japan) equipped with a digital camera system (DP50; Olympus) under 200 \times magnification. The sections were examined and photographed with a digital camera system and processed with Adobe Photoshop (Adobe, San Jose, CA, U.S.A.).

To calculate the area of the stained regions in the dermis, an optimized color threshold was applied to each image to distinguish between the stained areas and background. The proportion of the selected color was calculated as a percentage of the total area and used as a measure of collagen and elastin density. Collagen and elastin synthesis were scanned and quantified in five representative fields per section. The scores of the five fields were then averaged to obtain a final score for each section.

The data of collagen and elastin are presented as the means plus or minus standard deviation. Statistical analyses of results were performed by paired t-test for comparisons between groups at each time point. A confidence level of 95% ($P < 0.05$) was used to determine statistical significance.

RESULTS

Histological investigation exhibited changes over time as shown in Figure 1 (sun-protected skin) and Figure 2 (sun-exposed skin). Results of measurements of Types I and III collagen and elastin are shown in Tables 1 and 2 and Figure 3. In the control tissue, there were low densities of types I and III collagen and elastin in both sun-protected and sun-exposed skin. Biopsies from irradiated skin showed that IR irradiation increased the amounts of collagen and elastin on P30, P60 and P90 compared to the control (Figures 1 and 2). Investigation of the percentages of collagen and elastin stimulation in sun-protected skin biopsies revealed that a significant increase in type I collagen persisted until P30, and significant increases in type III collagen

FIGURE 1. Collagen and elastin staining histology 200 \times magnification of sun-protected skin. Twelve images: Images from top to bottom show type I collagen, type III collagen, and elastin, and from left to right show control, 30 days post-irradiation (P30), 60 days post-irradiation (P60), and 90 days post-irradiation (P90).

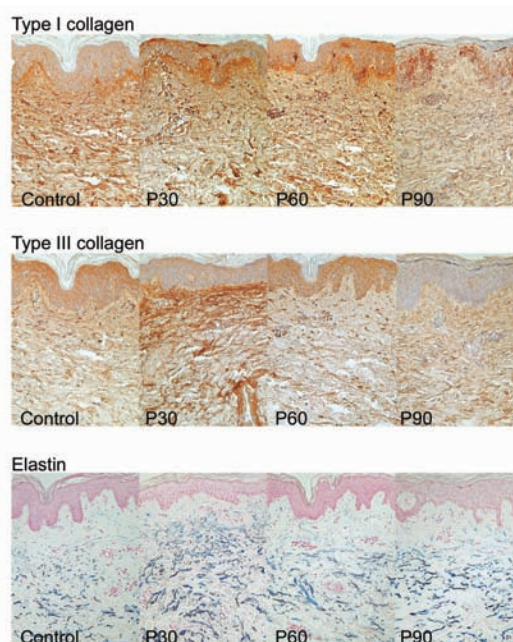
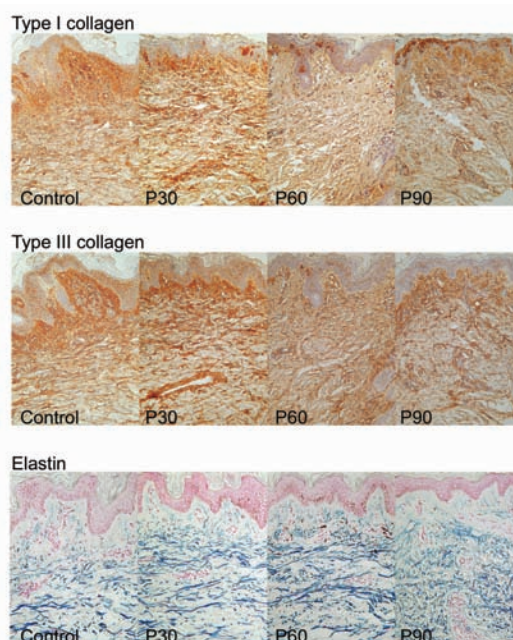


FIGURE 2. Collagen and elastin staining histology 200 \times magnification of sun-exposed skin. Twelve images: Images from top to bottom show type I collagen, type III collagen, and elastin, and from left to right show control, 30 days post-irradiation (P30), 60 days post-irradiation (P60), and 90 days post-irradiation (P90).



and elastin persisted until P90. In sun-exposed skin biopsies, a significant increase in type I collagen persisted until P60, and significant increases in type III collagen and elastin persisted until P90. Tables 1 and 2 show the significance of changes at each measurement relative to the control. No complications were observed throughout this study.

DISCUSSION

The safety and efficacy of modern lasers are attributable to the work of Anderson and Parrish.^{15,16} Utilizing a wavelength range of 1,100–1,800 nm and contact cooling, this infrared IR device is able to target water without targeting melanin or hemoglobin, allowing safe treatment of the deep dermis without risk to the basement membrane. Previously, we reported that IR irradiation provides safe, consistent long-term stimulation of type I collagen with only short-term stimulation of the more rigid type III collagen. This is preferential for improvement of skin laxity and wrinkles while seeking smoother more youthful skin.¹³

The energy produced by the Titan infrared handpiece penetrates into the skin and is absorbed primarily by water within the dermis, resulting in heating throughout the skin.^{8,14} This absorption increases the temperature, causing the release of inflammatory chemical mediators that stimulate the collagen healing process. These inflammatory mediators released from vascular endothelial cells induce new dermal collagen production by fibroblasts.¹⁷ Dermal injury after a single near-infrared irradiation treatment indicates that near-infrared irradiation affects various aspects of the healing process, including the degree of inflammation, formation and organization of the collagen, neovascularization, and epithelization.¹⁸

The connective tissue of the skin is composed mostly of collagen and elastin. Collagen makes up 70–80% of the dry weight of the skin and gives the dermis its mechanical and structural integrity. Elastin is a minor component of the dermis, but it has an important

function in providing the elasticity of the skin. These conclusions are supported by the accelerated aging and sagging of the skin seen in several hereditary disorders involving collagen or elastin deficiency.^{2,19-21} Both collagen and elastin are turned over slowly in tissues, and are therefore susceptible to age-related changes.²²

Approximately 60% of the total weight of the dermis is water, which is retained largely as a result of the water-binding capacity of the glycosaminoglycan/proteoglycan complexes, such as hyaluronic acid. They play a critical role by providing hydration.² To protect the subcutaneous tissues against natural IR, humans have acquired a defense mechanism in which hemoglobin and fluid absorb IR through blood vessel expansion and the skin turning

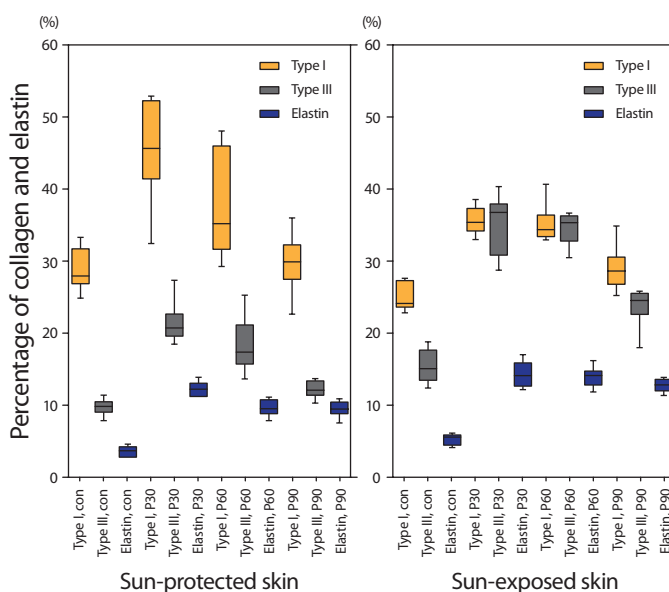


FIGURE 3. Graphs show the percentages of type I collagen (yellow), type III collagen (gray), and elastin (blue). Left: the results of sun-protected skin; right: the results of sun-exposed skin.

TABLE 1.

The Results of Sun-Protected Skin

Sun-Protected Skin	Control	P30	P60	P90
Type I collagen (%)	29.035±3.300	45.571±8.130*	38.166±8.192	29.804±4.818
Type III collagen (%)	9.725±1.314	21.586±3.387*	18.544±4.316*	12.206±1.327*
Elastin (%)	3.717±0.681	12.295±1.112*	9.708±1.244*	9.571±1.293*

*= significant change compared with control, $P<0.05$.

TABLE 2.

The Results of Sun-Exposed Skin

Sun-Protected Skin	Control	P30	P60	P90
Type I collagen (%)	25.195±2.191	35.763±2.151*	35.404±3.09*	29.093±3.538
Type III collagen (%)	15.579±2.546	35.048±4.724*	34.558±2.45*	23.704±3.25*
Elastin (%)	5.31±0.831	14.411±1.967*	13.992±1.608*	12.858±1.028*

*= significant change compared with control, $P<0.05$.

red or forming foam if necessary. This is because IR is strongly absorbed by hemoglobin and fluids. However, using this device due to cooling the surface during irradiation, IR is not absorbed in the superficial tissue. Thus, the dermis tends to increase the amount of fluid by inducing increases in collagen, elastin, and water-binding protein to protect the body against IR.

The loss of normal elastic fiber functions is the common age-associated feature of both photoaging and intrinsic aging processes.^{1,23} Actinically damaged skin is characterized by accumulation of elastotic material, while in sun-protected areas the number of elastic fibers is decreased.¹⁹ As an increase in relatively thin elastic fibers without irregular elastic fibers, such as solar elastosis, was evaluated in this study, this IR irradiation can achieve skin rejuvenation.

Some authors reported that clinical improvement following dermabrasion of photo-aged skin is correlated with synthesis of collagen I.^{24,25} Orringer et al.²⁶ reported that both types I and III collagen are important in dermal proliferation after ablative laser resurfacing. Levels of types I and III procollagen remain elevated for at least 6 months after CO₂ laser therapy.²⁶ The skin of elderly subjects shows an increase in type III collagen in normally sun-protected skin, and a marked increase in type III collagen in sun-exposed sites compared with sun-protected sites.^{27,28} In the present study, the skin samples were obtained from study participants in the fourth decade, and the same results were observed.

To evaluate how long the effects of IR irradiation last, the authors investigated biopsies taken on each of control, P30, P60 and P90. Investigation of collagen and elastin stimulation percentages in sun-protected skin biopsies indicated that a significant increase in type I collagen persisted until P30, and significant increases in type III collagen and elastin persisted until P90. In sun-exposed skin biopsies, a significant increase in type I collagen persisted until P60, and significant increases in type III collagen and elastin persisted until P90. These results support the conclusion that IR irradiation provides long-term stimulation of collagen I and III and elastin, and it is efficient for skin-rejuvenation.

The authors previously reported that type I collagen stimulation persisted for 90 days in rat tissue, while type III collagen stimulation showed no statistical significance by 45 days after IR irradiation. In rat tissue, the proliferation stage lasts for one month, and the maturation stage begins at day 45. In human tissue, the proliferation stage is thought to last for about one-to-three months, and the maturation stage begins at around two-to-three months. Thus, the human healing period is about two-to-three times longer than that in rats. The effects of IR irradiation on type I collagen were estimated to fade gradually over three-to-four months in rat tissue, extrapolating to roughly one year in human tissue.¹³ Collagen remodeling in humans is believed to continue for six-to-twelve months after stimulation of fibroblasts by trauma.¹⁷ Type

I and type III procollagen levels remain elevated for at least six months after CO₂ laser therapy.²⁶ However, Nelson et al. reported a significant increase in fibroblast staining for procollagen I at three weeks after dermabrasion indicating induction of collagen synthesis, and by 12 weeks collagen synthesis was returning toward the normal level.²⁴ The period of stimulated collagen synthesis would depend on various factors, such as skin condition and the extent of treatment. In this study, the effects of IR irradiation on collagen and elastin appeared to fade gradually over two-to-three months, and were not as significant as in the authors' previous study in rat tissue. Histological changes were more remarkable in amelanotic rat skin as compared to pigmented human skin, as melanin absorbs many photons and avoids photon-induced activation of mitochondria.¹² More output or additional passes of this IR irradiation may be useful to address these longer-term effects and significant differences. Further studies are needed under various conditions and over longer periods. However, these findings would not contradict the clinical impression that IR therapy is recommended once every few months.

Orringer et al. reported that the levels of tropoelastin and fibrillin 1, which are major components of elastic fibers, were still rising 28 days after CO₂ treatment, and tropoelastin mRNA levels remained elevated after six months.²⁶ The effects of IR irradiation on elastin persisted during this investigation, which was much longer than the effects on collagen. Due to this long-term stimulation of elastin, resiliency and elasticity of the skin after IR therapy would persist for a long time, which should be investigated further in future studies.

It should be noted that this was a preliminary study based on fairly small number of skin biopsies. In this study, the number of biopsies was small because of practical limitations in the number of serial biopsy specimens that could be obtained from the face. While there may be a subtle influence of taking serial biopsies near previous biopsy sites, there is no evidence that a distance of 1 cm from previous biopsies would be insufficient to ensure that wound healing would not affect adjacent biopsy sites. However, the authors cannot exclude the possibility that UV and IR exposure in everyday life may affect the changes demonstrated in this study. Further studies in this area are warranted in larger numbers of patients and with longer post-treatment periods, to evaluate variations in treatment parameters and correlation with UV exposure. These studies will enable practitioners to develop procedures capable of achieving maximum results while providing the greatest margin of safety.

The authors hope that this study will contribute to the design of more efficacious treatments for achieving skin rejuvenation.

CONCLUSION

The results of this study indicated that IR irradiation provides long-term stimulation of the production of collagens I and

III and elastin. Further studies in larger numbers of patients and with longer post-treatment periods are needed, as well as investigation of the variations in treatment parameters and correlations with UV exposure. These studies will enable practitioners to develop procedures to achieve maximum results while providing the greatest margin of safety. Infrared from 1,100 to 1,800 nm provides safe and effective long-term stimulation of collagens type I and III and elastin.

DISCLOSURES

The authors of this paper state that they have no conflicts of interest to declare.

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ADDRESS FOR CORRESPONDENCE

Yohei Tanaka, MD

Department of Plastic and Reconstructive Surgery
Shinshu University School of Medicine
Asahi 3-1-1
390-8621 Matsumoto
Nagano, Japan

Phone: +81-263-37-2833
Fax: +81-263-37-1920
E-mail: yoheimd@yahoo.co.jp