

Molecular Effects of Fractional Carbon Dioxide Laser Resurfacing on Photodamaged Human Skin

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Objective: To elucidate the sequential changes in protein expression that play a role in the clinically beneficial results seen with fractional carbon dioxide (CO₂) laser resurfacing of the face and neck.

Methods: Nine healthy volunteers were recruited for participation from the senior author's facial plastic surgery practice. After informed consent was obtained, each volunteer underwent a 2-mm punch biopsy from a discrete area of infra-auricular neck skin prior to laser treatment. Patients then immediately underwent laser resurfacing of photodamaged face and neck skin at a minimal dose (30 W for 0.1 second) with the Pixel Perfect fractional CO₂ laser. On completion of the treatment, another biopsy specimen was taken adjacent to the first site. Additional biopsy specimens were subsequently taken from adjacent skin at 2 of 3 time points (day 7, day 14, or day 21). RNA was extracted from the specimens, and

reverse transcriptase–polymerase chain reaction and protein microarray analysis were performed. Comparisons were then made between time points using pairwise comparison testing.

Results: We found statistically significant changes in the gene expression of several matrix metalloproteinases (MMPs). The data demonstrate a consistent up-regulation of MMPs 1, 3, 9, and 13, all of which have been previously reported for fully ablative CO₂ laser resurfacing. There was also a statistically significant increase in MMP-10 and MMP-11 levels in this data set.

Conclusion: This study suggests that the molecular mechanisms of action are similar for both fractional and fully ablative CO₂ laser resurfacing.

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WITH AGING, PREDICTABLE changes occur to the facial skin. Rhytids, mottled pigmentation, and erythema are common complaints of patients in pursuit of facial rejuvenation. Histologically, the neat dermal and epidermal architecture is lost. The epidermis is thickened, the dermis appears hypercellular, and increased vascularity is present. The rate of collagen synthesis and lifespan of dermal fibroblasts are both reduced. The result is an overall decrease in normal collagen.

Treatment of aging skin can be accomplished to varying degrees by different lasers.^{1,2} In 1983, the concept of selective photothermolysis was introduced, offering a mechanism for the delivery of laser energy in brief pulses to spare the surrounding tissues from heat-induced damage.³ Pulsed carbon dioxide (CO₂) lasers have been shown to vaporize a surface layer of epidermal cells and cause coagulation necrosis of an un-

derlying cell layer. These lasers act further to denature extracellular proteins in a subjacent zone and cause nonfatal damage to cells in a deeper zone.⁴ The entire epidermis and varying thicknesses of the dermis are removed in this process. Although extremely effective for repairing damaged skin, the adverse effects of a pulsed CO₂ laser include edema, erythema, burning, crusting, pigmentary changes, acne flares, herpes simplex virus infection, scars, milia formation, and dermatitis.⁵

Orringer et al⁶ were the first to study the molecular alterations that occurred after CO₂ laser resurfacing. From their results with this fully ablative laser, the authors asserted that specific proinflammatory cytokines induce the expression of various matrix metalloproteinases (MMPs), which break down and remove collagen. This allows for replacement with new, well-organized collagen bundles.⁶

While Orringer and colleagues⁶ elucidated the molecular pathways involved in CO₂ laser resurfacing, the concept of frac-

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Figure 1. A 46-year-old woman with periorcular fine lines. before (A) and 3 weeks after (B) treatment with fractional carbon dioxide laser resurfacing.



Figure 2. A 48-year-old woman with periorcular fine lines before (A), immediately after (B), and 4 weeks after (C) treatment with fractional carbon dioxide laser resurfacing.

tional photothermolysis was being developed.⁷ In fractional photothermolysis, columns of thermal damage occur in lieu of homogeneous tissue ablation. These microscopic treatment zones result in spatially confined thermal damage. The neighboring areas of normal tissue allow for rapid repopulation of these damaged columns, and re-epithelialization is usually complete within 24 to 48 hours.

In recent years, there has been a transition to fractional laser treatments by both practitioners and laser manufacturers. Our experience with the fractional CO₂ laser (Pixel Perfect Omnifit; Alma Lasers Ltd, Buffalo Grove, Illinois) has placed it at the forefront of our armamentarium in the treatment of aging and photodamaged skin. We have seen consistently positive clinical effects on fine lines and skin laxity (**Figure 1** and **Figure 2**). We found, with an anonymous study of our patient population, that patient satisfaction is higher with this method of rejuvenation than with smaller wavelength fractional lasers, and the decreased downtime relative to fully ablative lasers has made the fractional CO₂ laser a desirable option for both patients and physicians in the pursuit of facial rejuvenation (M.J.R. and G.S.K, unpublished data, August 2008).

While the molecular pathways involved in skin healing have been well studied, few studies have focused their attention on explaining the changes that occur after laser skin resurfacing. Despite its documented clinical efficacy and widespread use, there is a dearth of knowledge regarding the molecular effects of treating aging skin with the traditional or fractional CO₂ laser. Our goal was to understand the sequential changes in protein expression and the molecular pathways involved in the favorable clinical results achieved with skin rejuvenation from fractional CO₂ laser resurfacing.

METHODS

Nine healthy volunteers were recruited for participation from the senior author's (G.S.K.) facial plastic surgery practice. Patients were offered inclusion in the study if they were older than 40 years, had visible evidence of photodamaged skin, and had Fitzpatrick skin grade of I to III. Patients were excluded for any of the following: systemic dermatologic conditions (ie, eczema), uncontrolled diabetes, autoimmune disorders, and pigmentary disorders. The first 9 patients interested in participating in the study all met the inclusion criteria.

Informed consent was obtained from all patients regarding risk, benefits, and alternatives to treatment. Anesthetic ointment (lidocaine, 7% , and benzocaine, 23%) was applied generously to each patient's face and neck and allowed to take effect for at least 30 minutes. Excess ointment was removed. Volunteers were laid supine on the treatment table, and 1 mL of lidocaine, 1%, with 1:100 000 epinephrine was injected subcutaneously at the planned infra-auricular biopsy site. To serve as a control, a 2-mm punch biopsy was taken. Patients immediately underwent laser resurfacing of photodamaged face and neck skin with CO₂ laser using the Pixel CO₂ OMNIFIT hand-piece attachment (Alma Lasers Ltd). One pass was made over the entire face and neck using a power of 30 W and a pulse width of 0.1 milliseconds. An additional pass was made in the perioral and periorcular regions, depending on the patient's needs. On completion of the treatment, another biopsy specimen was taken adjacent to the first site from the infra-auricular neck skin. Two simple, interrupted 6-0 fast-absorbing gut sutures were placed to close the punch sites (**Figure 3**).

Biopsy specimens were subsequently taken from adjacent skin at 2 of 3 additional time points (day 7, day 14, or day 21) for a total of 4 specimens from each patient. All specimens were stored at -80°C in RNA stabilizing solution (SABiosciences, Frederick, Maryland) for later RNA extraction.

The expression of human extracellular matrix and adhesion molecules was detected by the SYBR green-based real-time reverse transcriptase-polymerase chain reaction (RT-PCR) technique. Total RNA extraction from the skin specimen was performed by RNeasy Plus Mini Kit (Qiagen, Valencia, California). The total RNA was reverse transcribed using the first-strand complementary DNA synthesis kit (SABiosciences). The Human Extracellular Matrix and Adhesion Molecules RT² Profiler PCR Array System (SABiosciences) was then used according to the manufacturer's instructions to assess for the gene expression of 84 genes important for cell-cell and cell-matrix interactions. Data generated from the arrays were analyzed by Excel-based analysis template (SABiosciences). Gene expression was calculated from the number of cycles by using standard curves, and the results were normalized to the house-keeping genes. The fold up- or down-regulation was analyzed by individual pairwise comparisons at each subsequent time point as relevant to baseline.

RESULTS

Findings from statistical analysis of our data demonstrate significant changes in gene expression of various proteins at the different time points of the study. At day zero, there were no statistically significant changes in MMP expression. However, there was a trend toward an immediate 3-fold decrease in the expression of MMP-12 (-3.31 fold; $P=.10$) and 2-fold decrease in the expression of MMP-7 (-2.14 fold; $P=.18$). Seven days after treatment, there were statistically significant increases ($P<.05$) in the following MMPs: MMP-1 (10.12 fold), MMP-9 (3.49 fold), MMP-10 (10.71 fold), MMP-11 (3.43 fold), and MMP-13 (6.73 fold). There was also a more than 3-fold increase in MMP-3, though this did not appear to be statistically significant in our data set (**Table**). Fourteen days after treatment, there was continued up-regulation of MMPs 1, 3, 9, 10, 11, and 13. However, only MMP-11 (2.81 fold) and MMP-13 (8.21 fold) showed statistical significance. At 21 days after treatment, there were persistently elevated levels of MMPs 9, 10, and 11, which were statistically significant (Table).

Collectively, MMP-1, MMP-3, and MMP-13 levels peak near day 14 before down-regulating, while MMP-9, MMP-10, and MMP-11 levels remained elevated beyond 21 days after treatment. MMP-12 appears to be down-regulated immediately following CO₂ laser treatment and to remain so for an extended period.

A 1-way analysis of variance was conducted to determine whether there were interpatient differences in expression of these markers at the various time points. The independent variable was the expression of each of the following molecular markers: β_2 -microglobulin, hypoxanthine phosphoribosyltransferase-1, ribosomal protein L13a, glyceraldehyde-3-phosphate dehydrogenase, and β_2 -actin. There was no statistically significant difference in the expression of these markers in the 9 patients.

COMMENT

Laser resurfacing is clearly efficacious in producing cosmetic improvements in patients' skin.⁵ Healing after CO₂ laser resurfacing appears to adhere to the well-established phases of wound healing.⁸ The literature suggest a combination of collagen denaturation and contraction, physical ablation of photodamaged tissue, and neocollagenesis as the most likely mechanism(s) of action.^{6,9,10}

Histologic studies demonstrate that similar collagen changes occur with the fractional vs fully ablative CO₂ lasers, but the data on the molecular effects of treatment are sparse.^{7,11} In the first study of its kind, Orringer et al⁶ used RT-PCR to elucidate the molecular changes that occurred after laser resurfacing with a fully ablative CO₂ laser (Ultrapulse; Coherent Inc, Santa Clara, California). Carbon dioxide laser treatments were performed on 28 patients at 60 W for 2 passes.⁶ Punch biopsy specimens were taken at baseline and then at 5 additional time points. The messenger RNA was extracted from these specimens, and samples were analyzed for expression of a limited panel of genes.

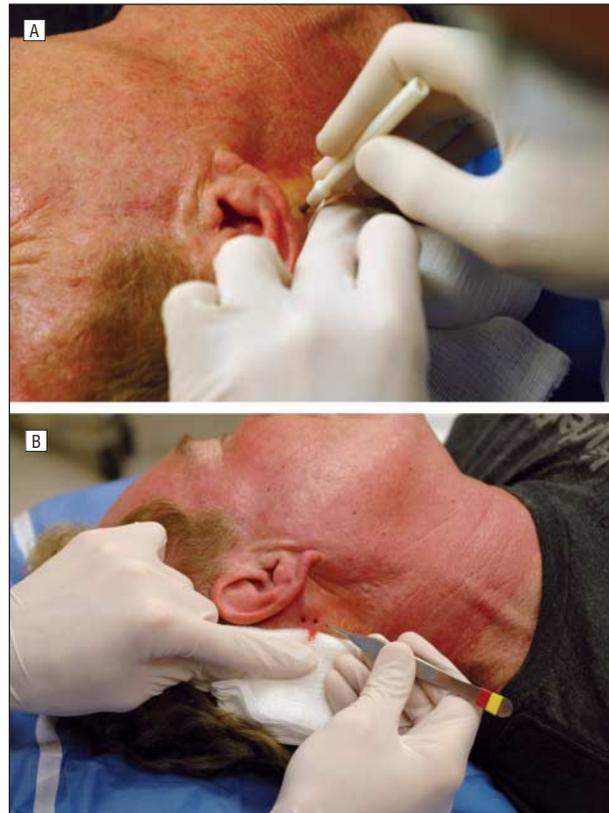


Figure 3. Biopsy from infra-auricular neck skin prior to (A) and after (B) fractional carbon dioxide laser resurfacing.

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The results from the landmark study by Orringer et al⁶ demonstrated several findings. First, primary cytokines interleukin 1 β and tumor necrosis factor are rapidly induced after laser resurfacing. MMP-1 and MMP-3 levels were found to rise on day 3 and peak on day 7. MMP-9 level was found to rise on day 3 and levels were found to stay elevated for at least 28 days. MMP-13 was also up-regulated and was found to peak at day 14.

From their results, Orringer et al⁶ proposed the schema that the proinflammatory cytokines (interleukin 1 β and tumor necrosis factor) induce the expression of MMPs, which has been previously supported in the literature. The MMPs break down the collagen, which requires both a collagenase (MMP-1 and MMP-3) and gelatinase (MMP-9). The continued degradation of collagen fragments explains the persistently elevated levels of MMP-9. According to this theory, the degradation and removal of the photodamaged collagen allows for replacement by new, well-organized collagen bundles. The authors' results also showed MMP-13 to be up-regulated and to peak at day 14.⁶

MMP-13 has been shown to have a role in collagen remodeling and healing of ulcers and fetal wounds, but it is not overexpressed in normally healing adult skin wounds.¹² In a molecular study of skin healing after incisions with laser vs scalpel, MMP-13 demonstrated a significant increase in a biphasic manner at 2 and 6 weeks.¹³ The same pattern for MMP-13 has been seen for human skin treated with radiofrequency generated to a temperature of 72°C, which indicates the possibility of a temperature-dependent pathway for induction of this par-

Table. Matrix Metalloproteinase (MMP) Gene Expressions

Gene Symbol	GenBank Accession No.	Description	Fold Up- or Down-Regulation			
			Day 0	Day 7	Day 14	Day 21
MMP1	NM_002421	Interstitial collagenase	1.54	10.12 ^a	10.83	2.57
MMP2	NM_004530	Gelatinase A	-1.01	1.43	1.23	1.25
MMP3	NM_002422	Stromelysin 1, progelatinase	1.30	3.12	6.83	2.65
MMP7	NM_002423	Matrilysin, uterine	-2.14	-1.67	1.48	-4.26
MMP8	NM_002424	Neutrophil collagenase	1.01	1.15	-1.22	2.70
MMP9	NM_004994	Gelatinase B	-1.25	3.49 ^a	2.21	1.87 ^a
MMP10	NM_002425	Stromelysin 2	-1.29	10.71 ^a	2.63	5.69 ^a
MMP11	NM_005940	Stromelysin 3	1.18	3.43 ^a	2.81 ^a	3.67 ^a
MMP12	NM_002426	Macrophage elastase	-3.31	1.15	-2.56	-3.48
MMP13	NM_002427	Collagenase 3	1.34	6.73 ^a	8.21 ^a	1.44
MMP14	NM_004995	Matrix metalloproteinase 14	-1.06	1.24	1.06	1.13
MMP15	NM_002428	Matrix metalloproteinase 15	-1.36	-1.10	-1.01	1.25
MMP16	NM_005941	Matrix metalloproteinase 16	-1.20	-1.13	-1.28	1.17

^a $P < .05$ vs baseline (before the treatment) by paired *t* test.

ticular protein.¹⁴ In an in vitro study of wound contracture in human skin fibroblasts, viral vector-induced overexpression of MMP-13 resulted in a marked and dose-dependent increase in collagen contraction compared with controls.¹⁵ Cumulatively, the data support our hypothesis that MMP-13 is a key modulator of collagen reorganization and may be responsible for the ordered structure of the newly formed collagen in the papillary and reticular dermis after CO₂ laser resurfacing.

In our study, MMPs 1, 3, 9, and 13 were all up-regulated in a manner consistent with these prior studies. Our data also demonstrate an up-regulation of 2 additional MMPs, MMP-10 and MMP-11. The ability of MMP-10 to superactivate procollagenases suggests that it plays a key role in the pathway of collagen degradation found in arthritis.^{16,17} Unlike cartilage, which has limited ability to regenerate, skin rejuvenation likely benefits from the collagenase function of this enzyme as an important part of the process of collagen removal and replacement.

MMP-11, also known as stromelysin-3, appears to act at the epithelial-stromal interface of remodeling tissues. It is the first MMP known to exhibit antiapoptotic function, encouraging some degree of cell survival in an otherwise degrading environment.¹⁸ The up-regulation of MMP-11 found in this study is consistent with what is known about its role in epithelial homeostasis in healing wounds.

While comparisons cannot be drawn from this study about the degree of collagen reorganization in fractional vs fully ablative CO₂ laser resurfacing, the data from our experiment demonstrate that the molecular pathways are very similar. Given these findings, fractional CO₂ laser resurfacing appears to be a promising technique for limiting recovery and potential adverse effects, while still providing effective rejuvenation of aging facial skin.

In conclusion, based on the results of this preliminary study, the molecular mechanisms of action are similar for both fractional and fully ablative CO₂ laser resurfacing. These biocellular effects are consistent with the clinical changes seen with fractional CO₂ laser therapy.

Matrix metalloproteinases 1, 3, 9, and 13 appear to play a key role in the denaturation, degradation, and reorganization of collagen seen in the dermis after CO₂ laser therapy. Therapies targeted to enhance the expression of these proteins in conjunction with the fractional CO₂ laser may serve to further improve the treatment possibilities for aging skin.

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Author Contributions: Dr Hokugo had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Reilly, Cohen, and Keller. *Acquisition of data:* Reilly and Keller. *Analysis and interpretation of data:* Reilly, Hokugo, and Keller. *Drafting of the manuscript:* Reilly, Cohen, and Keller. *Critical revision of the manuscript for important intellectual content:* Reilly, Hokugo, and Keller. *Statistical analysis:* Reilly and Hokugo. *Obtained funding:* Reilly, Hokugo, and Keller. *Administrative, technical, and material support:* Reilly, Cohen, and Keller. *Study supervision:* Reilly and Keller.

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