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.

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 handle-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 periocular 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 or 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 RT 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 housekeeping genes. The fold up- or down-regulation was analyzed by individual pairwise comparisons at each subsequent time point as relevant to baseline.
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 individual patient. The dependent variable was the expression of each of the following molecular markers: β2-microglobulin, hypoxanthine phosphoribosyltransferase-1, ribosomal protein L13a, glyceradehyde-3-phosphate dehydrogenase, and β2-actin. There was no statistically significant difference in the expression of these markers in the 9 patients.

Laser resurfacing is clearly efficacious in producing cosmetic improvements in patients’ skin. Healing after CO2 laser resurfacing appears to adhere to the well-established phases of wound healing. 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.

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. In the first study of its kind, Orringer et al demonstrated several findings. First, primary cytokines interleukin 1β 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 al proposed the schema that the proinflammatory cytokines (interleukin 1β 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.

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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. 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. 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-
Clinical changes seen with fractional CO2 laser therapy are consistent with the biocellular effects. These are very similar to those seen with fully ablative CO2 laser resurfacing. 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.

### Table. Matrix Metalloproteinase (MMP) Gene Expressions

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>GenBank Accession No.</th>
<th>Description</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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<tr>
<td>MMP1</td>
<td>NM_002421</td>
<td>Interstitial collagenase</td>
<td>1.54</td>
<td>10.12a</td>
<td>10.83</td>
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<td>MMP2</td>
<td>NM_004530</td>
<td>Gelatinase A</td>
<td>-1.01</td>
<td>1.43</td>
<td>1.23</td>
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<td>MMP3</td>
<td>NM_002422</td>
<td>Stromelysin 1, progelatinase</td>
<td>1.30</td>
<td>3.12</td>
<td>6.83</td>
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<tr>
<td>MMP7</td>
<td>NM_002423</td>
<td>Matrilysin, uterine</td>
<td>-2.14</td>
<td>-1.67</td>
<td>1.48</td>
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<td>MMP8</td>
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<td>Neutrophil collagenase</td>
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<td>MMP9</td>
<td>NM_004994</td>
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<td>3.49a</td>
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<td>1.87a</td>
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<td>MMP10</td>
<td>NM_002425</td>
<td>Stromelysin 2</td>
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<td>10.71a</td>
<td>2.63</td>
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<td>MMP11</td>
<td>NM_005940</td>
<td>Stromelysin 3</td>
<td>1.18</td>
<td>3.43a</td>
<td>2.81a</td>
<td>3.67a</td>
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<tr>
<td>MMP12</td>
<td>NM_002426</td>
<td>Macrophage elastase</td>
<td>-3.31</td>
<td>1.15</td>
<td>-2.56</td>
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</tr>
<tr>
<td>MMP13</td>
<td>NM_002427</td>
<td>Collagenase 3</td>
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<td>6.73a</td>
<td>8.21a</td>
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<td>NM_004985</td>
<td>Matrix metalloproteinase 14</td>
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<td>1.24</td>
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<td>MMP15</td>
<td>NM_002428</td>
<td>Matrix metalloproteinase 15</td>
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<td>MMP16</td>
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<td>Matrix metalloproteinase 16</td>
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aP < .05 vs baseline (before the treatment) by paired t test.

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


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