# Comparison of Mouse Matrix Metalloproteinase 13 Expression in Free-Electron Laser and Scalpel Incisions During Wound Healing

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**Collagenase-3 (matrix metalloproteinase 13, MMP-13) was employed as a surrogate marker to compare the characteristics of incisional wound repair after surgery with the free-electron laser at 6.1 μm and the scalpel. Using a transgenic mouse strain with the MMP-13 or the COL1A2 promoter driving luciferase expression, we observed MMP-13 and COL1A2 expression, tensile strength, macrophage infiltration, and wound histology for up to 62 d. The scalpel incisions showed higher tensile strength than free-electron laser wounds from days 10 to 22 postwounding, despite minimal collateral thermal damage. After 45 d healing was similar. Trichrome staining confirmed that the scalpel incisions had more dense collagen deposition than free-electron laser incisions up to 36 d postinjury, but at day 45 they became similar. MMP-13 expression was biphasic, with peak activities at days 15 and 37 after injury, whereas free-electron laser wounds showed greater luciferase activity than scalpel wounds. Peak COL1A2 activity preceded the MMP-13 maximum. MMP-13 expression localized predominantly to dermal fibroblasts near the epidermis at day 15, and in the region of the deep dermis, muscle, and fascia at day 37 postwounding. Migrating muscle cells, but not all skeletal muscle cells, also expressed MMP-13. Free-electron laser incisions contained more macrophages than scalpel wounds at days 2 and 7 postinjury, suggesting that free-electron laser irradiation exacerbated the inflammatory response and thereby stimulated MMP-13 expression. These results revealed that MMP-13 was involved in a series of coordinated events during wound healing, not only the long-term remodeling of wound connective tissue, but also skeletal muscle repair. MMP-13 activity *in vivo* may correlate with the extent of tissue damage.** ***Key words: collagenase/free-electron laser/luciferase/MMP-13/wound healing. J Invest Dermatol 121:926–932, 2003***

Laser techniques have been used in a variety of surgical applications and have proved to be a versatile and reliable tool. When the laser beam is focused, it can incise tissue in a fashion similar to a sharp blade but with improved hemostasis and controlled depth of injury without direct contact (Nemeth, 1993; Izzo *et al*, 2001). Lasers in general, however, have the disadvantage of causing a delay in wound healing compared with scalpel incisions due to collateral damage (Molgat *et al*, 1995). Currently, the $CO_2$ laser is the most commonly used laser for tissue ablation and incision. The incidental, collateral thermal damage associated with the laser is indisputable, however, attributable to the direct effects of thermal energy penetrating unintended tissues (Speyer *et al*, 1996; Bryant *et al*, 1998). Consequently, researchers have attempted to modify the pulse structure of the $CO_2$ laser to minimize this thermal injury and thus reduce wound-healing delays (Bryant *et al*, 1998). Some improvements have been gained, but the healing delays remain significant (Sanders and Reinisch, 2000).

The Vanderbilt free-electron laser (FEL) is a pulsed infrared laser that is tunable over a wavelength range from 2 to 9 μm (Bryant *et al*, 1998). This laser has been shown to ablate tissue efficiently with minimal collateral thermal damage in various tissues, in particular when tuned to a wavelength of 6.45 μm (peak absorption band for the amide II protein bond) (Edwards *et al*, 1994). At this wavelength, about 50% of the light is absorbed by the structural proteins and 50% of the light is absorbed by water. Previous studies (Peavy *et al*, 1999; Payne *et al*, 2001) have also demonstrated that the FEL beam with wavelengths in the 6.1 μm (amide I) to 6.45 μm (amide II) region, when delivered by a computer-controlled delivery system, showed clean, deep cortical bone ablations with minimal collateral thermal damage. Thus, the pulsed infrared energy produced by the FEL at specific wavelengths and targeted to specific dermal proteins rather than nonspecific water absorption could reduce lateral thermal damage. The $CO_2$ laser emits infrared light with a wavelength of 10.6 μm and is absorbed by tissue with high water content. The penetration depth in tissue depends on the amount of water in the tissue (Bryant *et al*, 1998). The water in target tissue absorbs this energy and is vaporized, resulting in thermal ablation. Furthermore, the $CO_2$ laser is essentially a continuous-wave laser, whereas the FEL is a pulsed laser with a 5 μs pulse length that allows for thermal dissipation. Theoretically, FEL incisions at appropriate wavelengths should produce less collateral tissue damage by tuning to wavelengths that target structural proteins in the tissue. Preliminary studies in the rat had suggested that incisional wound healing was superior in FEL-incised wounds (Davidson *et al*, 2001).

With these characteristics in mind, we compared the wound healing of incisions in mouse skin created by the FEL with a

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Abbreviation: FEL, free-electron laser; MMP, matrix metalloproteinase.

wavelength of 6.1 μm to incisions created by a conventional scalpel. We determined how the wound healing of FEL and scalpel incisions correlated with matrix metalloproteinase (MMP-13) expression by quantitative *in vivo* observation of MMP-13 and COL1A2 transcription in transgenic reporter mice during wound repair.

## MATERIALS AND METHODS

**Animal model** All the procedures using the animals were conducted in compliance with state and federal guidelines and approved by the Vanderbilt University Institutional Animal Care and Use Committee. In this study, the transgenic mice in which the MMP-13 promoter drives the luciferase reporter gene were made by our laboratory and have been described in detail previously (Wu *et al*, 2002). The collagen transgenic mice in which collagen (COL1A2) type I promoter drives luciferase and β-galactosidase gene were produced in the laboratory of B. Crombrugghe, MD Anderson Cancer Center, Houston, TX (Bou-Gharios *et al*, 1996).

**Scalpel and FEL wounds** Mice were anesthetized with isoflurane in an isoflurane vaporizer (Ohmeda, BOC Health Care, UK). After the mice were sedated, the dorsal fur was removed using a clipper. Two full-thickness longitudinal incisions, separated by 1.5 cm and each 1.0 cm in length, were made on the dorsal skin of each mouse. The first lesion was made using a standard #10 surgical scalpel. The second lesion was produced using the FEL. The laser was operated at a wavelength of 6.1 μm in 5 μs micropulses, with a pulse energy of 20 mJ and 30 Hz repetition rate (Izzo *et al*, 2001). The laser beam was delivered with a Computer Assisted Surgical Technique system (Reinisch *et al*, 1994). The pattern for the incisions was a straight line. The computer was programmed to create the incision by scanning the beam in a random, stepwise fashion until the line was complete. The pattern was repeated until the full-thickness wound was obtained. Finally, the wounds were closed using two 3–0 Ethilon (Ethicon, Somerville, NJ) sutures. Following the surgery, mice were given supplemental heat and allowed to recover fully in the animal care facility without any restrictions. At different time points, the transgenic mice were imaged for luciferase activity under anesthesia and returned to their cages, whereas the wild-type mice were euthanized and used to determine wound tensile strength.

**Tensile strength measurement** Wild-type male mice (C57/BL6) were used for a wound healing study. Tensiometry was performed on the skin incisions using the Instron 5542 tensiometer (Instron, Canton, MA) within 3 h after tissue harvest (Benn *et al*, 1996). All tensiometry was performed in a blinded fashion. The incision was harvested immediately after the animal was euthanized. A small portion of incision was placed in 4% paraformaldehyde and then embedded in paraffin, and the sections were used for immunohistochemistry. The rest of each tissue strip (approximately 0.5 cm of incision) was oriented in the jaws of the tensiometer perpendicular to the line of the incision. Peak breaking forces were measured and converted to tensile strength values (kilogram force per square centimeter) by dividing the breaking force by the cross-sectional area of the tissue that broke. The thickness of the specimen was assumed to be constant for all specimens. Data were reported as mean ± SD and compared using the Student's *t* test.

**Bioluminescence imaging in living animals** Luciferase images were taken as previously described (Wu *et al*, 2002). Briefly, mice were anesthetized by intraperitoneal injection of a mixture of ketamine and xylazine and were administered liquid luciferase substrate topically, luciferin (30 μL per wound, 16 mg per mL in dimethylsulfoxide (DMSO)), onto wounds and unwounded skin (Contag *et al*, 1997). The animals were placed in a light-tight imaging chamber after 5 min. A gray-scale, body-surface reference image (Sweeney *et al*, 1999) and a pseudocolor image representing light intensity were collected with the Hamamatsu Intensified Charged Coupled Device (ICCD) camera (Hamamatsu Photonics, Hamamatsu City, Japan). Gray-scale reference images and pseudocolor images were superimposed by using the image-processing software (Hamamatsu). Total, maximum, and average photon emissions from unwounded skin and wound sites within the images of each mouse were quantified with Argus50 software (Hamamatsu). Background values from unwounded skin that had been treated with luciferase/DMSO were subtracted from wound signals. The luciferase/DMSO solution did not produce local irritation.

***In situ* hybridization analysis** Mouse skin tissue was subjected to *in situ* hybridization using digoxigenin-labeled riboprobes as described previously (Hoff *et al*, 1999). Briefly, mouse skin was fixed in 4% paraformaldehyde and then embedded in paraffin. Sections were cut and deparaffinized in xylenes and hydrated through graded ethanols to phosphate-buffered saline (PBS). Tissue was fixed in 4% paraformaldehyde before and after treatment with proteinase K (Sigma, St Louis, MO; 20 μg per mL, in 100 mM Tris pH 8.0, 50 mM ethylenediamine tetraacetic acid (EDTA), 20 min at 37°C). Following acetylation with 0.5% acetic anhydride, tissue was dehydrated through graded ethanols and air-dried prior to hybridization. Tissue sections were hybridized at 55°C with digoxigenin-labeled mouse MMP-13 riboprobes (antisense and sense) in a humid chamber. Slides were washed in 50% formamide/2 × sodium citrate/chloride buffer at 65°C and 37°C, digested with RNase A (20 μg per mL RNase A; Clontech, Palo Alto, CA) in TNE buffer (10 mM Tris pH 7.5, 5 mM EDTA, 0.5 M NaCl) at 37°C for 30 min, and then washed in TNE at 55°C. The digoxigenin detection procedure was carried out using the GeniusTM3 kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instruction. The antidigoxigenin antibody was used at a 1:500 dilution, and the color reaction was allowed to proceed until development was determined to be sufficient. Slides were counterstained with 1% fast green FCF in distilled water for 1–2 min.

**Immunohistochemistry** Immunohistochemistry was performed as previously described (Uria *et al*, 1998). Briefly, sections (5 μm) were placed in PBS containing 3% hydrogen peroxide (vol/vol) to inhibit endogenous peroxidase activity. After blocking nonspecific protein binding with 5% goat serum in PBS, sections were incubated with diluted primary antibody, followed by biotin-conjugated secondary antibody. StreptAB-complex/horseradish peroxidase complex (Dako) was used to visualize the staining. Sections were counterstained with hematoxylin. For negative controls, the primary antibody was replaced by rat IgG (for macrophage) or rabbit IgG (for MMP-13). Rat monoclonal antibody raised against mouse macrophage (F4/80 antigen; Serotec, Oxford, UK) was used for macrophage detection, and rabbit polyclonal antibody against MMP-13 (Biomol Research Laboratory, Plymouth Meeting, PA) was used for mouse MMP-13 immunostaining. Secondary antibodies were biotin-conjugated goat antirat IgG and goat antirabbit IgG, respectively.

**Histologic study** Tissue sections were stained with Masson's trichrome staining to evaluate collagen deposition. All tissue sections were stained *en masse* to avoid variations in staining between specimens.

**Basic fibroblast growth factor (bFGF) administration *in vivo*** Six-week-old male transgenic mice were anesthetized, and the dorsal area was shaved. The solution of human recombinant bFGF (a gift from Scios) in PBS was mixed with a pluronic vehicle gel (Flow-gel Antiadhesion; Synechion, Dallas, TX) at 0°C. The mixture of 2 μg or 10 μg bFGF in 10 μL PBS and 50 μL pluronic gel was subcutaneously injected into intact skin of the dorsal area. The control was given as a mixture of water and pluronic vehicle. The luciferase image was taken at different time points using the ICCD camera.

## RESULTS

**Tensiometry** To compare the difference in wound healing between FEL and scalpel incisions, we measured the tensile strength of full-thickness wound skin from normal mice ($N = 5$) at days 10, 14, 22, 45, and 62 postwounding with a tensiometer. The incisions made with the FEL were about 56% as strong as those made with the scalpel at days 10, 14, and 22 after injury. At day 45 after wounding, the tensile strength difference between FEL and scalpel incisions was reduced. The average tensile strengths are plotted in **Fig 1**. Asterisks mark the tensile strengths of scalpel wounds that were significantly stronger than FEL incisions at the same time point ($^{*}p = 0.05$, $^{**}p = 0.01$).

**Visualization of MMP-13 and collagen promoter activity within FEL and scalpel wounds in transgenic mice** Transgenic male progeny 6–8 wk old (MMP-13 luciferase copy number ≈50, collagen luciferase copy number ≈20) were used to examine luciferase expression as a representation of MMP-13 promoter or collagen promoter activity during tissue repair. Wounds were imaged for bioluminescence at different time points after injury. Quantitation of bioluminescent signals was

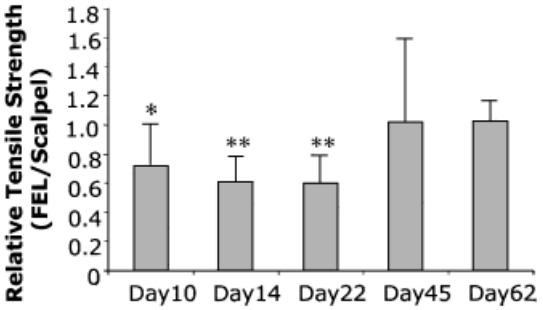


**Figure 1. Relative tensile strengths of healing incisions *versus* time.** Values at each time point represent the mean ratios of tensile strength of paired FEL and scalpel wounds in individual, wild-type animals at each time point ($N = 5$). Asterisks indicate a significant difference in tensile strength between FEL and scalpel wounds at that time point ($^{*}p < 0.05$, $^{**}p < 0.01$). At days 10, 14, and 22, the FEL wounds show lower tensile strength than scalpel incisions. At days 45 and 62 tensile strength was not significantly different between the two lesions.

achieved by integrating the signal over time (3 min) and reporting the data as total photon counts. As shown in **Fig 2**(***A***), the MMP-13 promoter was dynamically active during wound healing. Quantification of bioluminescence (photon counts) indicated that the MMP-13/Luc signal showed an initial peak of activity at day 15 after wounding. Surprisingly, there was a second phase of gene activation with peak expression at day 37 after injury. The FEL incisions showed greater bioluminescent luciferase activity than scalpel wounds during wound healing, but the expression kinetics of MMP-13 promoter activity were parallel in FEL and scalpel wounds. Among the individual mice with FEL and scalpel wounds ($N = 15$), there was a very similar pattern of luciferase expression. As shown in **Fig 2**(***B***), there was only one expression peak in type I collagen (COL1A2) transgenic mice during wound healing, and the peak expression of the COL1A2 promoter was at 10 d after wounding; at 14 d postinjury the promoter activity decreased dramatically and almost no signal could be seen at 18 d after wounding. Thus, collagen expression declined during the elevation of MMP-13 activity. Unwounded skin in transgenic animals and wounds in nontransgenic animals did not produce a bioluminescent signal. The tail, snout, and paw areas, however, appeared to show high constitutive MMP-13 activity when systemic luciferin was used (see **Fig 7**, later).

***In situ* hybridization** To understand the temporal and spatial distribution of MMP-13 mRNA in the transgenic mice after injury, *in situ* hybridization was performed. Mice were sacrificed at different time points after wounding, and the skin was fixed, embedded, and sectioned. Digoxigenin-labeled riboprobes for MMP-13 were hybridized to sections of mouse skin. The result (**Fig 3*A***) showed that MMP-13 transcripts were present in dermal fibroblasts of dermis adjacent to the epidermis, but not in the keratinocytes, at day 15 after wounding. Little signal was detected near the subdermal musculature of the panniculus carnosus, and no signal was detected in the muscle (data not shown). At day 37 after wounding (**Fig 3*B***), MMP-13 mRNA was predominantly detected in fibroblasts that associated with deep dermis, muscle, and fascia. In addition, some signal was also observed within the muscle of the panniculus carnosus (**Fig 3*C, D***), particularly in those cells migrating toward the wound center. Only selected muscle cells were positive for MMP-13. The patterns of both MMP-13 and luciferase (data not shown) expression in FEL and scalpel wounds were indistinguishable. No signal was detected in adjacent, serial sections that were reacted with sense probe (data not shown).

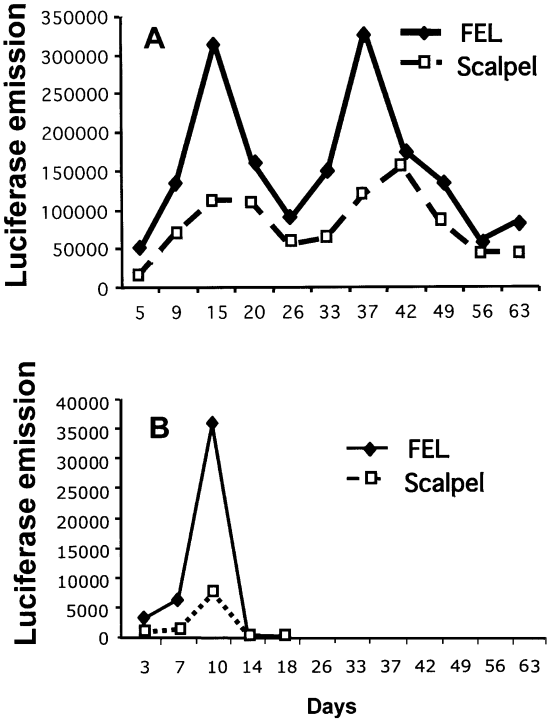


**Figure 2. Patterns of MMP-13 promoter activity within FEL and scalpel wounds in transgenic mice.** The signals of luciferase bioluminescence from the FEL and scalpel wound area of a representative mouse were quantified, and the total photon counts were plotted with respect to days after injury. The signals of luciferase from FEL and scalpel wounds showed a similar temporal pattern of promoter activity. (*A*) MMP-13 promoter activity in MMP-13 transgenic mice. There were two phases of MMP-13 promoter activity: the first peak was at around 15 d, and the second peak was at 37 d after injury. FEL incisions showed higher luciferase activity than scalpel incisions during wound healing. (*B*) Collagen promoter activity in COL1A2 transgenic mice. The peak collagen promoter activity was at 10 d after injury, and the signal decreased dramatically at 14 d after injury. After 18 d, almost no signal was detected. Data from additional animals are available (supplementary figure).

**MMP-13 expression in FEL wounds** To confirm that the MMP-13 expression during the wound repair process was correlated with the MMP-13 mRNA transcription, we did immunostaining for MMP-13 with FEL wound tissues. As shown in **Fig 4**(***A, B***), at day 15 after wounding there was more extensive MMP-13 expression near the epidermis than the middle of the dermis. At day 37 (**Fig 4*C, D***), most of the MMP-13-positive cells were located in the deep dermis.

**Histologic studies for collagen** To assess collagen deposition, the wound areas were sampled at different time points after laser irradiation and scalpel incision and fixed in 4% paraformaldehyde. Tissue sections were stained with Masson's trichrome to contrast collagen. As shown in **Fig 5**, at days 10 and 36 after wounding, the scalpel incisions appeared to have much more collagen organization in the wound site than did the FEL wound, suggesting that the scalpel incisions had healed more rapidly. By day 45 after injury, the collagen density in FEL and scalpel incisions appeared similar (**Fig 5*E, F***), suggesting that the physical strength of FEL and scalpel wounds would be comparable. Consistent with previous observations (Edwards *et al*, 1994), there was essentially no evidence of collateral thermal damage at the margins of the FEL wounds at any of the time points.

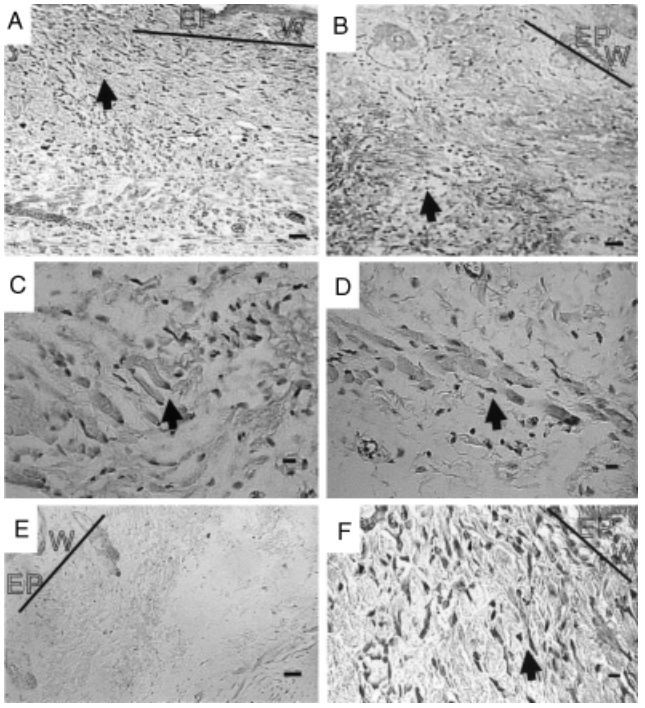


**Figure 3. Localization of MMP-13 mRNA in FEL wounds from transgenic mice.** Sections of mouse skin at 15 d and 37 d after injury were hybridized with digoxigenin-labeled antisense riboprobes derived from mouse MMP-13 cDNA and counterstained with fast green. EP, epidermis; W, wound site. (*A*) At 15 d during the first expression peak of MMP-13 promoter activity, MMP-13 mRNA was localized to the dermal fibroblasts near the epidermis (*arrow*). (*B*) At 37 d, during the second peak of MMP-13 promoter activity, *in situ* hybridization showed the bulk of MMP-13 expression had shifted to the deep dermis, muscle, and fascia. *Panels C* and *D* illustrate regions of the regenerating panniculus carnosus that expressed MMP-13 mRNA at day 37 after injury. MMP-13 signals were detected on cells on the tip of migrating muscle as well as muscle cells behind the leading edge (*panel D*). *Panel E* is the negative control. *Panel F* is a high magnification view of the upper dermis in *panel A*. A spindle-shaped fibroblastic cell with positive signal is indicated by the *arrow*. *Scale bars*: *A*, *B*, *E*, 50 μm; *C*, *D*, *F*, 10 μm.

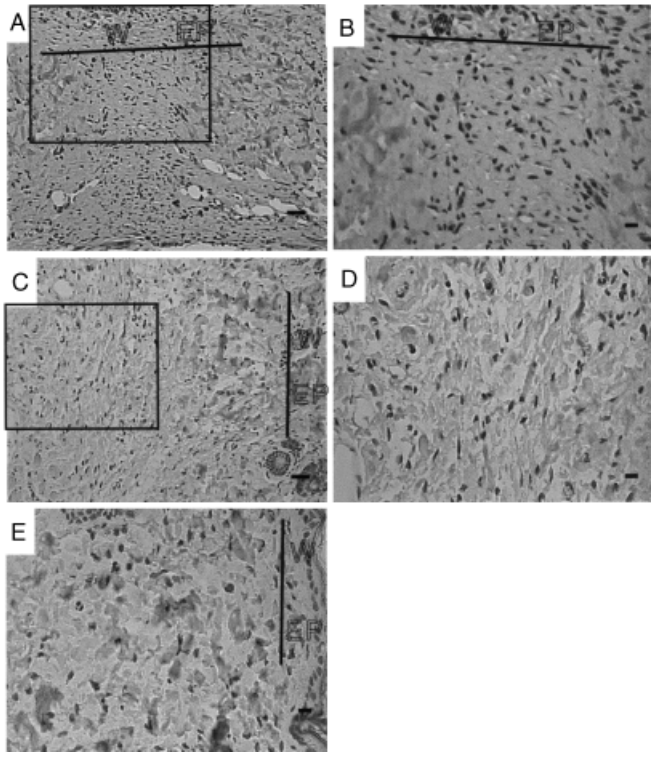


**Figure 4. Immunostaining of MMP-13 in the FEL wound.** Sections of FEL incisional tissues at day 15 (*A*, *B*) and day 37 (*C*, *D*) postinjury were stained with polyclonal anti-MMP-13 antibody. In the IgG control (*E*), there was no detectable labeling for MMP-13. At day 15 (*A*), there was a greater extent of MMP-13 expression near the epidermis. At 37 d (*C*, shown at higher magnification in *D*), most MMP-13 expression was located in the deep dermis. *Scale bars*: *A*, *C*, 50 μm; *B*, *D*, 10 μm.

**Inflammatory response to incisions** Tissue sections from FEL and scalpel incisions at days 2 and 7 postwounding were immunostained for macrophages to estimate the inflammatory response after FEL and scalpel incision. As shown in **Fig 6**, macrophages were more abundant at day 7 than day 2 after injury in both types of wounds, and more macrophages were present in FEL incisions than scalpel incisions at both time points.

**MMP-13 stimulation by a growth factor** To confirm that the higher MMP-13 promoter activity in FEL wounds could be stimulated at least in part by growth factors from inflammatory cells such as macrophages, we injected bFGF subcutaneously into the intact dorsal skin in transgenic mice ($N = 6$) and imaged the luciferase bioluminescence on successive days. As shown in **Fig 7**(***A***), at day 2 after injection we did not detect a difference between control and bFGF injection sites; however, by day 3 after injection (**Fig 7*B***), the signals in bFGF injection sites were much higher than day 2 and control. At day 4 (**Fig 7*C***), the signals at injection sites became weaker than day 3.

## DISCUSSION

Currently, the $CO_2$ laser is the most commonly used laser for tissue ablation and incision, and it has been proved to be a reliable tool for a variety of surgical procedures. The thermal damage produced by the laser to the tissue ablation area, however, leads to a delay in wound healing (Bryant *et al*, 1998). A variety of techniques have been studied or developed to minimize this thermal damage to surrounding tissues, including the use of a reduced spot size, microspot micromanipulators for precise beam delivery, computer-controlled beam delivery, local cooling, and modification to the energy pulse duration and repetition rate (Sanders and Reinisch, 2000). The FEL has been shown to produce less thermal damage than standard shuttered continuous-wavelength $CO_2$ lasers. Because the micropulsed infrared energy emitted by the FEL is targeted to specific dermal proteins rather than nonspecific water absorption, it can reduce collateral thermal damage (Edwards *et al*, 1994). This may permit more rapid wound repair.

Wound repair is a succession of overlapping biochemical and cellular events that result, in the best of circumstances, in the restoration of both the structural and functional integrity of the damaged tissue (Davidson and Benn, 1996). Unencumbered, these wound repair processes follow a specific time sequence and can be temporally categorized into three major groups: inflammation, tissue formation, and tissue remodeling (Clark and Henson, 1996).

The MMP are a family of structurally related zinc-dependent neutral proteinases. They play important roles in various normal physiologic situations such as embryonic development, angiogenesis, parturition, ovulation, and remodeling of scar tissue, and pathologic conditions such as rheumatoid arthritis, tumor cell invasion, and metastasis (Henney *et al*, 1991; Stetler-Stevenson *et al*, 1993; Mao *et al*, 1999). Collagenase-3 (MMP-13) is the predominant collagenase in the mouse (Balbin *et al*, 2000; 2001). It is a member of both the MMP family of enzymes and the collagenase subfamily of MMP, which includes three members (i.e., MMP-1, MMP-8, and MMP-13) with an overall sequence

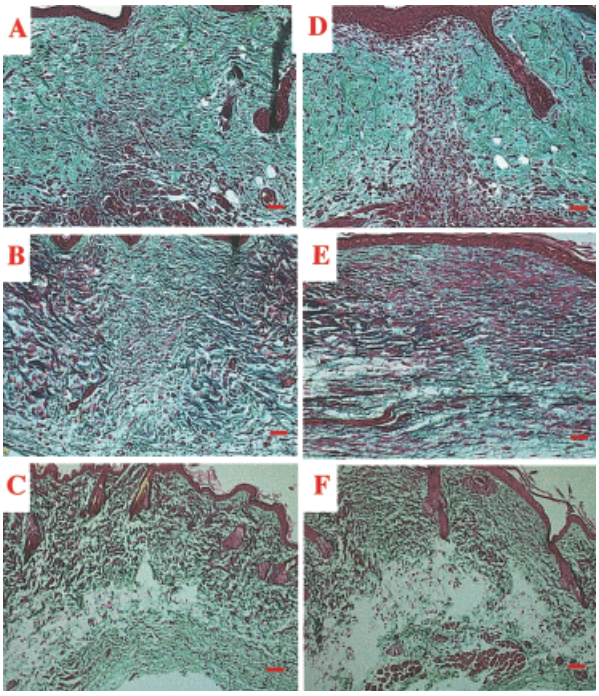


**Figure 5. Connective tissue organization in laser and surgical incisions.** Sections of scalpel (*A*, *B*, *C*) and FEL (*D*, *E*, *F*) incisional wounds from day 10 (*A*, *D*), 36 (*B*, *E*), and 45 (*C*, *F*) postinjury were stained with Masson's trichrome. Scalpel incisions show more intense collagen staining and lower cellularity than FEL wounds at days 10, 36, and 45, but by day 45 the amount of collagen appeared similar between FEL and scalpel incisions (*C*, *F*). *Scale bars*: 100 μm.

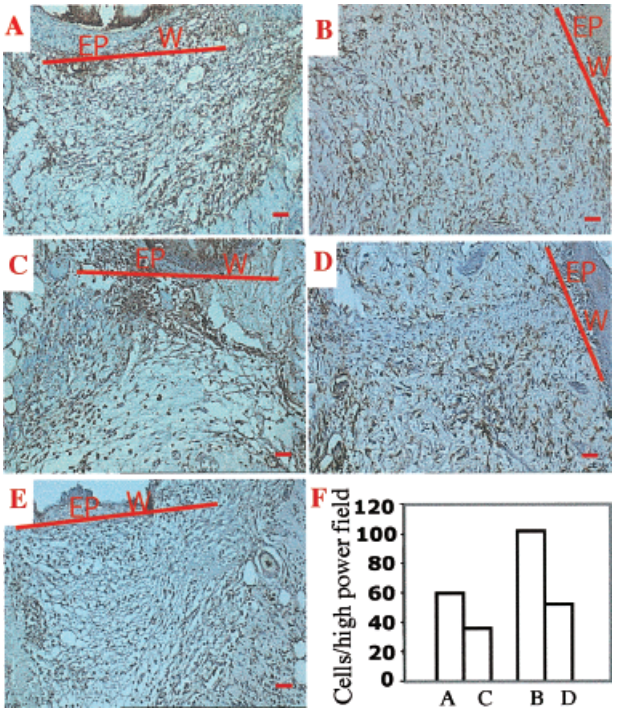


**Figure 6. Detection of macrophages in FEL and scalpel wounds.** Sections of FEL (*A*, *B*) and scalpel (*C*, *D*) incisional tissues at day 2 (*A*, *C*) and day 7 (*B*, *D*) postinjury were stained with monoclonal antimacrophage antibody. In the IgG control (*E*), there was no detectable labeling for macrophage. There were more macrophages in FEL (*A*, *B*) than scalpel (*C*, *D*) wound areas at the same time point. *Panel F* shows the mean macrophage numbers per high power field ($N = 10$) for the FEL and scalpel wounds at days 2 and 7. *Scale bars*: 100 μm.

homology of 55%. They are the principal neutral proteinases capable of degrading native fibrillar collagens in the extracellular space (Ravanti *et al*, 1999a; 1999b). Biochemical characterization of recombinant human MMP-13 has shown that this enzyme cleaves type II collagen about 6-fold more effectively than types I and III collagens, and it displays a 40-fold stronger gelatinase activity than MMP-1 or MMP-8 (Knauper *et al*, 1996). MMP-13 is expressed in situations in which either rapid or effective remodeling (Johansson *et al*, 1997; Stahle-Backdahl *et al*, 1997) or excessive degradation of collagenous extracellular matrices takes place (Mitchell *et al*, 1996; Reboul *et al*, 1996).

Collagenase expression has been strongly implicated in not only matrix remodeling but epithelial migration (Madlener, 1998). MMP-13 is expressed by normal and transformed murine keratinocytes (Netzel-Arnett *et al*, 2002; Warmka *et al*, 2002), and MMP-13 expression is further amplified by inflammatory cytokines and growth factors (Netzel-Arnett *et al*, 2002). In human epidermis, MMP-1 is prominently expressed at the leading edge of migrating epidermis (Pilcher *et al*, 1997), and collagenase has similarly been localized in murine excisional wounds during the first few days of wound closure (Madlener, 1998). In human wounds, MMP-13 expression is reported to occur only in the connective tissue of chronic wounds (Vaalamo *et al*, 1997). Our studies used an incisional model that minimizes the requirement for epidermal resurfacing, and it was clear that the connective tissue compartment was the dominant source of MMP-13 expression during the middle and late phases of wound repair. Low level, early expression of MMP-13 in the epidermis could not be excluded with our data.

To study the role of mouse MMP-13 gene in cutaneous wound healing, we have generated a transgenic mouse line in which the MMP-13 promoter directs the expression of luciferase reporter gene with the same temporal and spatial pattern as the endogenous MMP-13 gene and contains information essential for directing

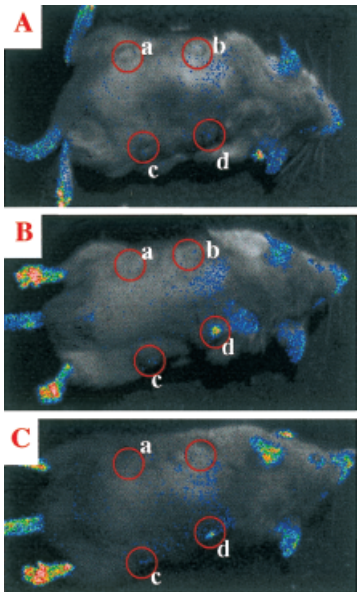


**Figure 7. MMP-13 stimulation by bFGF in transgenic mice.** A mixture of 2 μg (*c*) or 10 μg (*d*) of bFGF in 10 μL PBS and 50 μL pluronic gel were subcutaneously injected into intact dorsal skin. The controls (*a*, *b*) were given with a mixture of water and pluronic gel. At 2 d after injection (*A*), there was no detectable signal in the bFGF injection sites. At 3 d after injection (*B*), bFGF injection sites (*c*, *d*) showed higher signals than controls (*a*, *b*), and at 4 d (*C*) the signals were weaker than day 3. There was a high level of constitutive MMP-13 promoter activity in the tail, snout, and paws.

tissue-specific expression. In this study we compared the wound healing of FEL and scalpel incisions and tried to correlate wound healing with MMP-13 expression by visualizing luciferase activity in transgenic mice.

To compare the wound healing of FEL and scalpel incisions, we determined the tensile strengths of FEL and scalpel wounds. Preliminary studies in the rat showed that FEL incisions had greater strength than surgical incisions at 6.1 and 6.45 μm, whereas the $CO_2$ laser was slightly inferior to the scalpel (Davidson *et al*, 2001). In contrast, the results in the thinner skin of the mouse showed that the tensile strength of scalpel incision increased more rapidly than that of the FEL incision at days 10, 14, and 22 after injury with selected parameters (6.1 μm, 30 Hz, 20 mJ). This difference suggested that the FEL surgery resulted in some alteration to the adjacent tissue and caused a delay in wound healing. The response of cutaneous tissue to FEL radiation may vary substantially as a function of species, skin thickness, and laser operating parameters. It is well known that the conventional laser as used in cutaneous surgery will produce direct effects of thermal energy penetrating to adjacent tissues. Thermally denatured connective tissue may be a barrier to reorganization of the wound site by newly formed granulation tissue (Bryant *et al*, 1998). The delay in wound healing might also be explained by an increased burden of tissue debris whose degradation requires the prolonged activation of inflammatory cells. There was no histologic evidence of thermal denaturation, however, and wounds incised with the FEL at 6.1 μm showed little thermocoagulation of dermal vasculature. There were more macrophages in the provisional matrix or granulation tissue of FEL incisions at days 2 and 7 after injury, suggesting that the FEL incision had a prolonged inflammatory process. It is possible that a greater extent of macrophage activation induced higher MMP-13 expression by fibroblasts in granulation tissue due to cytokine release. Although the identity of this inducing factor is not certain, cytokines such as tumor necrosis factor α, interleukin-1, epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor can stimulate MMP-13 expression in cultured fibroblasts (Borden *et al*, 1996; Ravanti *et al*, 1999a), and macrophages can secrete such signal molecules (Vaes *et al*, 1980; Dayer *et al*, 1985). As shown in **Fig 7**, the induction of MMP-13 by bFGF in our transgenic mice provided direct evidence that at least one growth factor, bFGF, can stimulate cutaneous MMP-13 expression *in vivo.* bFGF is a chemoattractant for numerous cell types, a potent mitogen for angiogenesis, and an activator of wound contraction. The induction of MMP-13 expression at the wound site may amplify collagen degradation and reduce collagen deposition during the early phase of wound healing, thus reducing the physical strength of the wound. Although collagen gene expression was higher in FEL wounds, collagen deposition was reduced. Exuberant collagen deposition is not always beneficial. The reduced collagen deposition during the early phase of wound healing after FEL surgery may decrease the possibility of hypertrophic scarring and may be beneficial. Hypertrophic scar and keloids, which may cause severe functional and cosmetic problems, are characterized by an overabundant extracellular matrix, including dermal collagen (Niessen *et al*, 2001).

It was evident that luciferase/MMP-13 expression in FEL incisions was higher than in scalpel incisions through most of the repair process, indicating a more potent, aggressive fibroblast response. As fibroblasts migrate into the wound, they participate in collagen remodeling, a process that is dependent on both continued collagen synthesis and collagen catabolism. The synthesis and assembly of collagen fibers is required for the gradual gain in tensile strength with progressive formation of larger collagen bundles and an alteration in intermolecular crosslinks (Clark and Henson, 1996). The arrangement and distribution of collagen fibers in the wound/scar, however, changes dramatically during the course of wound healing. The degradation of wound collagen is controlled by a variety of collagenases, including MMP-13 (Stanton *et al*, 2002). This finding suggests a more intense tissue remodeling in FEL incisions; however, the expression kinetics of FEL and scalpel incisions were parallel. We suggest that MMP-13 expression, indicated by luciferase in our transgenic mice, may be correlated to somewhat elevated tissue damage. This was supported by the fact that there was an inverse correlation between the tensile strength of wound skin and luciferase activity at the early phase of wound healing. MMP-13 promoter activity as visualized by luciferase bioluminescence appears to be a valuable surrogate marker for the extent of wound remodeling, and it can be used to investigate the effects of laser radiation and laser-induced collateral thermal damage. It was clear that there were two MMP-13 expression peaks during wound healing, and the MMP-13 expression by fibroblasts was located to different regions of the wound at different stages of the repair process. During the early peak, MMP-13 mRNA localized to dermal fibroblasts near the epidermis, whereas at day 37 the expression of MMP-13 was located near the deep dermis, muscle, and fascia. This observation shows that healing of mouse skin is a biphasic process, with two distinct remodeling events. Immunostaining for MMP-13 showed that MMP-13 expression was correlated with the MMP-13 mRNA transcription. Our previous studies showed that the most prevalent expression of luciferase and MMP-13 was in the dermal fibroblasts at the wound margin at the early phase of wound repair (Wu *et al*, 2002). The expression of MMP-13 by fibroblasts in the surrounding dermis suggested that this form of collagenase may facilitate the migration of these cells into the wound area. Normal fibroblasts are embedded in a fibrillar collagen-rich matrix. During acute injury, fibroblast invasion into granulation tissue is likely to involve the production of matrix-degrading proteinases (Giannelli *et al*, 1997), permitting release of these cells from local sites within the tissue. Neovascularization of wounds occurs simultaneously with collagen degradation and fibroplasia by capillary buds sprouting from blood vessels adjacent to the wound and extending into the wound space. The process of capillary formation (angiogenesis) has been studied in the usually avascular cornea, and the capillary endothelial cells grown *in vitro* release plasminogen activator and collagenase in response to angiogenic stimuli (Kalebic *et al*, 1983). As MMP-13 was expressed by fibroblasts near the epidermis, MMP-13 may also contribute to re-epithelialization. Although the epithelial cells did not express MMP-13, collagenase is required for the migration of keratinocytes (Pilcher *et al*, 1997). The second peak of expression suggests that MMP-13 is also involved in long-term collagen remodeling and skeletal muscle repair. It appeared that regenerating muscle fibers migrating towards the center of the wound were active in the second phase of MMP-13 expression. This implies that MMP-13 may also participate in muscle repair during long-term scar remodeling.

In summary, we have compared the wound healing of FEL and scalpel incisions and visualized the MMP-13 promoter activity during FEL and scalpel incisional wounds *in vivo.* The FEL-induced lesions at a wavelength of 6.1 μm showed more luciferase activity than scalpel wounds, and the tensile strength of FEL incisions was weaker than scalpel incisions at the early phase of wound healing. This suggests that the luciferase/MMP-13 activity in our transgenic mice may correlate with the extent of damage to the wound, and luciferase activity may be a valuable indicator of the extent of wound remodeling that can be used to investigate the effect of laser radiation and collateral thermal damage. MMP-13 was involved in a series of coordinated events during wound healing. We speculate that the elevated MMP-13 expression in FEL incisions may be beneficial for better collagen deposition, remodeling, and scar reduction.

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