The Effect of Fibrin Sealant on the Healing of Laser-resurfaced Skin

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Background: Fibrin sealant is an effective hemostatic agent and a useful tissue sealant. Studies have also suggested that fibrin sealant may accelerate the normal wound-healing process. Objective: This study was designed to ascertain whether fibrin sealant would enhance wound healing after CO$_2$ laser resurfacing in a guinea pig model. Methods: The CO$_2$ laser was used to create equal areas of skin resurfacing on both sides of 14 Dunkin Hartley guinea pigs. Fibrin sealant was applied to the treatment side, whereas bacitracin was applied to the control side. Biopsies of these areas were performed on days 1, 3, 7, and 10. A histologic evaluation was performed with the use of a grading scale that compared acute and chronic inflammation, granulation tissue, collagen deposition, and epidermal regeneration. Results: The wounds treated with fibrin sealant demonstrated a statistically significant reduction in the degree of acute and chronic inflammation as well as collagen deposition. At day 7, fibrin sealant was noted to enhance neovascularization and result in a slight delay in reepithelialization. All wounds were completely reepithelialized at day 10. No wound infections or other complications were noted as a result of the application of fibrin sealant. Conclusions: Although wound healing was not accelerated, the application of fibrin sealant after CO$_2$ laser resurfacing diminished the acute and chronic inflammatory response, enhanced neovascularization, and reduced collagen accumulation. Further research is needed to assess whether the effects of fibrin sealant noted in this study result in improved cosmetic healing after CO$_2$ laser resurfacing. (Aesthetic Surg J 2001;21:509-517.)

Commercial fibrin sealants have been available in Europe since the 1970s; the US Food and Drug Administration (FDA) recently approved 2 solvent-detergent viral-deactivated forms of fibrin sealant. Although specific indications for the use of fibrin sealant have been slow to evolve, it has been used successfully in various clinical
settings, including induction of hemostasis, tissue adhesion, and as an adjunct in wound healing.\textsuperscript{1-10} The adhesive and hemostatic properties of the fibrin sealant may favorably augment wound healing by stimulating growth of fibroblasts and inducing angiogenesis.\textsuperscript{7,11-13} In vivo, fibrin is crucial in wound healing as both a hemostatic barrier and also as a chemoattractant for migrating leukocytes and fibroblasts.\textsuperscript{13}

In this study, we sought to determine whether the application of fibrin sealant after CO\textsubscript{2} laser resurfacing would enhance and possibly accelerate the normal wound-healing process in an animal model.

Materials and Methods

To replicate the wound-healing characteristics of human skin, Dunkin Hartley guinea pigs were chosen. Previous research has demonstrated that the epidermal and dermal characteristics of these animals closely resemble those of humans.\textsuperscript{14} Fourteen guinea pigs were anesthetized by metaphane inhalation, and the skin was shaved and cleaned with povidone-iodine. Baseline punch biopsies (1 to 2 mm) were taken. Deepithelialization with the Coherent Carbon Dioxide Ultra Pulse Laser 5000c (Coherent, Inc., Santa Clara, CA) was performed on two 4-cm squares on both the right and left side of the animal. Two passes were performed on each area with a power setting of 50 W, pulse at 200/s, and fluence, 250 mJ, resulting in complete epidermal desquamation and thermal injury to the superficial dermis. The lower power setting of 50 W used for this experiment induced a thermal injury similar in depth to the injury obtained with a power setting of 60 W in human skin.

After this procedure, one wound (right side) on each guinea pig was dressed with bacitracin as a control. The other (left side) was treated with fibrin sealant (Figure 1). The fibrin sealant used for this experiment contained 30 g/L of fibrinogen and 200 U/mL of porcine thrombin.

Punch biopsies of the healing skin from both the control and fibrin sealant–treated areas were taken from the edge of the wound on postprocedure days 1, 3, 7, and 10. Biopsies were placed in a 10% buffered-formalin solution, set in paraffin, and sectioned. The sectioned slides were then stained with either hematoxylin and eosin or trichrome solution. A numbering system was used to provide a blinded histologic evaluation. A pathologist then graded the slides. A scale was devised to grade the depth of thermal damage, degree of acute and chronic inflammation, amount of granulation tissue, collagen deposition, and extent of epidermal regeneration (Table 1). The data for each group were collected and compared at each time point for the histologic features listed above.

Results

Mean scores for the biopsy specimens are listed in Table 2. The data were analyzed with the paired 2-sample t test for means to determine statistically significant differences. A P value of <.05 was used to determine statistically significant differences between groups.

Baseline biopsies to assess the depth of thermal damage confirmed that uniform thermal damage of more than...
one half the dermis (grade 2) was achieved in both the control and treatment groups.

Biopsies taken on day 1 revealed a significant diminution of the acute inflammatory response \((P = .007)\) in wounds treated with fibrin sealant (Figure 2). No chronic inflammation, granulation tissue, or collagen deposition was noted in this initial biopsy. All specimens had complete epidermal loss with no regeneration.

A similar reduction in the acute inflammatory response in the fibrin sealant group was noted on biopsies taken on the third day after laser resurfacing \((P = .009)\). These specimens also revealed no chronic inflammation, granulation tissue, or collagen formation. However, by day 3,
a partial regeneration of the epidermis was noted in both groups. No statistical difference was determined, as the devised scale only scored wounds with complete reepithelialization.

Analysis of specimens taken on day 7 revealed the degree of acute inflammation decreased in both groups, with the difference between specimens just reaching statistical significance ($P = .04$) (Figure 3). The fibrin sealant treatment group also demonstrated a significant reduction in the degree of chronic inflammation ($P = .006$) and collagen formation ($P = .009$). The amount of granulation tissue measured by the degree of neovascularization was also increased in the fibrin sealant group ($P = .02$). A slight delay in complete epidermal regeneration was noted in the fibrin sealant–treated group ($P = .04$). Of note, a thick crust of fibrin sealant was present on the surface of the wounds during this stage of wound healing.

Both groups demonstrated minimal acute inflammation at day 10. Nevertheless, in contrast to previous specimens, the fibrin sealant–treated group had a slightly higher degree of acute inflammation that was statistically significant ($P = .02$). The reduction in collagen deposition seen in the fibrin sealant group at day 7 was even more pronounced at day 10 ($P = .001$) (Figure 4). By day 10, the treatment and control groups demonstrated a similar degree of chronic inflammation, granulation tissue, and epidermal regeneration, with complete reepithelialization noted in all specimens (all with $P > .05$). All wounds had shed the fibrin sealant crust covering by day 10. No wound infection or other complications occurred in any of the 14 guinea pigs.

**Discussion**

This study was designed to determine the effect of fibrin sealant on wound healing after CO$_2$ laser resurfacing with the use of a guinea pig model. Fibrin sealant is composed of variable concentrations of thrombin and fibrinogen, with or without added factors such as factor XIII and aprotinin. Thrombin, as a protease, activates fibrinogen to form fibrin monomers that polymerize to become an unstable fibrin clot.$^{13}$ Thrombin also acti-
vates factor XIII, which then catalyzes cross-linking between the fi-brin molecules, increasing the strength of the clot and providing protection from degradation. In this way, fibrin sealant mimics the last step of the coagulation cascade.13

The CO2 laser is well documented as a method for skin resurfacing to reduce facial skin rhytids and superficial defects.15-17 Its laser has a high-energy short-duration pulse that effectively ablates the tissue with virtually no eschar formation and minimal damage to the surrounding tissue. The degree of initial residual thermal damage is directly proportional to the duration of laser application and the beam density.15-17 Histologic examination of specimens reveals that skin depth ablation with the CO2 laser is dose dependent.15-17 The mechanism of the skin resurfacing involves complete epidermal desquamation and creation of a superficial thermal injury to the dermis. The final appearance of laser-resurfaced skin largely depends on the events that occur during the inflammatory and maturation/remodeling phases of wound healing.

Complications of the CO2 laser include delayed wound healing, skin discoloration, hypertrophic scarring, and full-thickness skin necrosis.18,19 These complications are often related to the depth of thermal injury and may occasionally result from a prolonged inflammatory phase of wound healing. An assortment of occlusive dressings or biomembranes have demonstrated varied results in regards to improved wound healing or relief of postoperative pain.20 Although our results did not demonstrate an acceleration of the overall wound-healing process, the application of fibrin sealant did result in significant differences during several phases of wound healing, including the degree of acute and chronic inflammation as well as collagen deposition.

Acute and chronic inflammation

Tissue injury initiates a cascade of both local and systemic cell signals, including cytokines, growth factors, hemostatic factors, and platelet-derived factors. These signals in turn stimulate or inhibit a variety of cellular responses involved in normal wound healing, many of

Figure 3. Slides comparing chronic inflammation on postoperative day 7. A, Right side (control). B, Left side (fibrin sealant). (Hematoxylin and eosin staining, original magnification x40.)
which are just beginning to be understood.21-27 Some degree of inflammation appears necessary to protect a wound from infection, rid a wound of dead tissue, and create an environment in which collagen formation, angiogenesis, and the final phases of wound healing can occur.21-27 The precise amount of inflammation needed to accomplish these essential components of wound healing remains to be determined.

It has also been shown that excessive tissue damage and poor wound healing can result from an abnormal or prolonged inflammatory phase of wound healing. An abnormal inflammatory response appears to be at least partially responsible for hypertrophic or keloid scarring, whereas a continued or exaggerated inflammatory response, in combination with excess collagen formation and decreased collagen degradation, ultimately results in pathologic scar formation.21,25

In addition, studies of the characteristics of “scarless” fetal surgery have documented a significantly reduced inflammatory response when compared with normal adult wound healing.28-32 Liechty et al 29 recently used models of “scarless” fetal wound healing to show that an amplified inflammatory phase resulted in scar formation similar to that seen in adult wound healing.

The exact mechanism for the diminished acute and chronic inflammatory response after the application of fibrin sealant in the current experiment is unclear but is likely multifactorial. Previous studies have shown that high concentrations of fibrinogen and thrombin can inhibit the activity of neutrophils and macrophages in vivo.33-35 However, the concentrations of these components in the fibrin sealant used in this experiment were significantly lower than those shown to inhibit inflammatory cells.33-35

Fibrin sealant forms a protective barrier and functions much like the fibrin-rich hemostatic clot normally deposited in a healing wound. This natural covering may help attenuate the inflammatory response by immediately shielding the wound from further exposure to the environment. Moreover, fibrin sealant resembles the extracellular matrix produced during the early phases of wound healing. The immediate applica-

Figure 4. Slides comparing collagen formation on postoperative day 10. A, Right side (control). B, Left side (fibrin sealant). (Trichrome staining, original magnification ×40.)
tion of fibrin sealant may provide a “pseudomatrix” framework that is ideally suited for the adherence of inflammatory and proliferative cells. This may allow the bypassing of much of the inflammatory phase during which time the matrix would normally be created. The hemostatic properties of fibrin sealant may also halt the continued efflux of blood and serum from exposed dermal capillaries into the surrounding tissues, thereby limiting the extent of tissue injury and subsequently reducing the inflammatory response.

In contrast to our results demonstrating a significant diminution in the chronic inflammatory response after the application of fibrin sealant, Scardino et al,9 using human and bovine fibrin sealant applied to open wounds in a dog model, noted a mild increase in chronic inflammation at days 5 and 30 compared with control wounds. The reason for this increased chronic inflammation is unknown; however, this response may be attributable to an immunologic reaction to the human or bovine fibrin sealant in the canine model used for the experiment.

In the guinea pig model chosen for the current experiment, we did, however, observe increased acute inflammation at day 10 compared with control wounds. We believe this mild increase in acute inflammation is related to the loss of the protective layer of fibrin sealant, which occurred during this time period. The shedding of the fibrin sealant layer once again exposed the wound surface to the environment, which may have induced a mild inflammatory response. Nevertheless, the very small degree of acute inflammation did not appear to cause a significant alteration in this phase of wound healing. The attenuated inflammatory response noted in wounds treated with fibrin sealant did not result in increased infectious complications, nor did it cause a significant delay overall wound healing.

**Granulation tissue and collagen deposition**

The application of fibrin sealant resulted in a significant increase in the amount of granulation tissue. The enhanced neovascularization noted in the fibrin sealant–treated wounds is a necessary component of rapid and efficient wound healing. This finding is agreement with previous reports demonstrating that fibrin induces angiogenesis.11 Additional capillaries supply much needed oxygen and nutrients to support the metabolic demands of a healing wound.

Previous studies have demonstrated conflicting results regarding the effect of fibrin sealant on collagen deposition.12,36 The current study revealed a highly significant reduction in collagen accumulation at day 7, with an even more pronounced difference between treatment and control wounds on postoperative day 10. Collagen formation is necessary for adequate wound healing. However, it is the quality and alignment of the collagen that determine the strength of a healing wound and the cosmetic appearance of the scar tissue.21,25,28,29

The continuation of the wound-healing process is regulated by the cells involved in the initial inflammatory response; therefore, the decrease in collagen production may be a direct result of the diminished inflammatory response noted in the fibrin sealant–treated group.23

A modest decrease in collagen deposition may actually benefit wound healing by minimizing scar formation and wound contracture. This has been suggested by several studies of “scarless” fetal wound healing and in studies of pathologic wound healing states, such as hypertrophic and keloid scar formation. Other fetal wound-healing reports have demonstrated that reduced collagen accumulation results in a more normal dermal architecture.21,25,28,29

**Reepithelialization**

Previous studies have documented an acceleration of reepithelialization and enhanced keratinocyte cell migration with more rapid healing after occlusion of laser-resurfaced wounds.20 Although reepithelialization was complete in all wounds by postoperative day 10, a statistically significant reduction in complete reepithelialization in the fibrin sealant–treated wounds was demonstrated at postoperative day 7. We believe that the delay in reepithelialization was the result of a mechanical effect caused by a crust formed over the surface of the fibrin sealant–treated wounds, preventing the migration of keratinocytes across the entire surface of the wound. By postoperative day 10, the fibrin sealant crust was shed in all treatment wounds, removing this barrier to keratinocyte migration. Other studies investigating the effect of fibrin sealant on wound healing have suggested a similar mechanism for a delay in reepithelialization.36

Complete reepithelialization was the only measure used in the current study to compare accelerated wound heal-
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Future applications

The potential benefits of fibrin sealant in wound healing may be realized through future studies that use fibrin sealant as a carrier of long-acting local anesthetics, growth factors, or antibiotics. Much like other occlusive dressings, fibrin sealant should result in diminished postoperative pain. With the addition of long-acting local anesthetics, fibrin sealant may provide significant long-term pain relief after CO2 laser resurfacing.

The effects of various growth factors are just beginning to be understood. By using the differential properties of these hormones, it may be possible to accelerate wound healing or overcome defects in wound healing, such as hypertrophic and keloid scarring.

Studies have demonstrated that fibrin sealant allows for improved survival of skin grafts in infected fields. The addition of antibiotics to fibrin sealant may also improve healing of other infected wounds.

Conclusion

The results of this early series demonstrate several significant alterations in the wound-healing process after the application of fibrin sealant. These include a reduction in acute and chronic inflammation, a lessening of collagen accumulation, and improved angiogenesis. By reducing some of the potentially harmful consequences of the inflammatory response and limiting collagen deposition, we believe that the application of fibrin sealant may result in an improved cosmetic appearance of CO2 laser-resurfaced skin. Nevertheless, long-term animal and human studies are needed to assess any overall beneficial effect of fibrin sealant on wound healing after CO2 laser resurfacing.

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