Hypertrophic scars are an abnormal physiological endpoint of cutaneous full-thickness wound healing. Hypertrophic scars are raised, erythematous scars that may widen, but do not extend beyond the original boundaries of the wound. They are frequently aesthetically displeasing and may cause pain, pruritus, contractures, and other functional impairments. Hypertrophic scars are known to result from excessive collagen deposition and occur most commonly in wounds with delayed epithelization. The precise mechanisms that lead to hypertrophic scar formation, however, are not well understood. Therefore, it is difficult to develop therapies to prevent and treat hypertrophic scars.

**Abstract:** Collagen synthesis and degradation are important in scar maturation. Collagenase has been used for debridement of wounds and treatment of Dupuytren’s disease but its effects on scar prevention are unknown. **Objective.** The objective of this study was to determine the effect of collagenase ointment on hypertrophic scar prevention by using a rabbit ear-scarring model. **Methods.** Four 7-mm, full-thickness dermal punches were made on the inner surface of each ear of 8 young adult New Zealand white rabbits. The wounds from one ear were treated with collagenase in petrolatum or petrolatum alone, while the wounds from the other ear of the same rabbits were not treated, serving as control. Tissues were collected at 28 days after surgery and histological analysis was performed. Total area of new scar and scar elevation index were determined. A paired two-tailed Student’s *t* test was performed to compare treated scars with their own controls and a non-paired Student’s *t* test was performed to compare collagenase-treated with petrolatum-treated scars. **Results.** Collagenase-treated wounds developed scars with less hypertrophy than untreated scars. The total area of new scar was smaller in collagenase-treated wounds than in untreated wounds (531 ± 32 vs. 617 ± 51; *P* = 0.03). The scar elevation index was lower in collagenase-treated wounds than in untreated wounds (2.08 ± 0.15 vs. 2.45 ± 0.20; *P* = 0.015). The adjusted scar elevation index was lower in collagenase-treated wounds than in petrolatum-treated wounds, and the difference showed a trend toward statistical significance (0.88 ± 0.04 vs. 1.03 ± 0.07; *P* = 0.10). **Conclusion.** Treatment with collagenase ointment reduces hypertrophic scar formation in a rabbit ear model.
Under normal physiologic conditions, a balance exists between collagen synthesis and collagen degradation in dermal tissues. Full-thickness wounds and thermal injuries can cause a disruption in this balance, which then leads to excessive collagen deposition. This excessive collagen deposition results in the formation of fibrotic scars, including hypertrophic scars. Hypertrophic scars are postulated to form in two phases. In the first phase, the rate of collagen synthesis is abnormally high and the rate of collagen degradation is abnormally low. In the second phase, the rates of both collagen synthesis and collagen degradation are normal. A therapy that could interrupt the initial phase might be effective in preventing hypertrophic scars.

Collagenase is a naturally occurring matrix metalloproteinase that digests type I collagen. It plays an important role in many biologic processes including collagen deposition and connective tissue remodeling, especially during the normal process of wound healing, and is thought to be involved in the formation of hypertrophic scars. Independent studies by Ghahary and Arakawa have indicated that there is a decreased expression of collagenase in hypertrophic scar fibroblasts. These findings suggest that reduced collagenase production and activity contribute to the excessive collagen deposition seen in hypertrophic scar. Clinically, collagenase intracord injection has been used to treat Dupuytren disease (an abnormal thickening of tissue beneath skin) in a phase III study, although surgery is still the current mainstay of treatment for the disease.

Collagenase ointment is a topical enzymatic debriding agent that contains clostridial collagenase, a bacterial collagenase that acts on native and denatured collagen in necrotic tissues. Collagenase has been used successfully to debride chronic ulcers and wounds and has been shown to expedite wound debridement and accelerate re-epithelialization. Although studies have demonstrated a more rapid debridement, as well as a shorter time to wound closure with the use of collagenase, the effect of collagenase on the resulting scar outcome has not been demonstrated. Collagenase ointment may be effective in preventing hypertrophic scarring. It may act to reduce scarring either by expediting debridement and allowing for faster re-epithelialization or by reducing collagen deposition.

A recent retrospective review found a decreased incidence of hypertrophic scar formation in partial-thickness burn wounds with the application of collagenase ointment. However, no prospective controlled studies have been conducted to investigate these findings further. The objective of this study was to determine the effect of collagenase ointment on the formation of hypertrophic scars by using a rabbit ear-scarring model.

**Key Points**

- Full-thickness dermal punches were made on the inner surface of each ear of 8 young adult New Zealand white rabbits. The wounds from one ear were treated with collagenase in petrolatum or petrolatum alone, while the wounds from the other ear of the same rabbits were not treated, serving as control.

**Materials and Methods**

**Animals.** Eight young adult New Zealand white rabbits (3–6 months, ~3 kg; Covance Research Products, Inc., Cumberland, VA) were acclimated, housed, and had access to food and water ad libitum under the conditions set forth in the Public Health Service Guide for the Care and Use of Laboratory Animals and the U.S. Department of Agriculture “Title 9-Animal Welfare Act” and its revisions. The study was conducted under an approved experimental protocol from the Northwestern University Animal Care and Use Committee.

Each rabbit was anesthetized with an intramuscular injection of ketamine (45 mg/kg) and xylazine (7 mg/kg). The ventral surface of both ears was depilated and prepared by rinsing the surgical site with a 3-minute Betadine rinse, followed by a 70% ethanol rinse, and then a final Betadine rinse. Four 7-mm, full-thickness dermal punch wounds were then made on the inner surface of each ear with a biopsy punch. For each wound, the epidermis, dermis, and the perichondrium were removed, and two nicks were made in the cartilage to allow identification of the lateral border of the scar edge. The result was 64 wounds were then divided into 32 pairs, each consisting of two wounds from the same rabbit. One wound from each pair was treated, while the other served as control. Collagenase in a petrolatum vehicle was the treatment in half of the pairs, and petrolatum was the treatment in the other half.

Collagenase ointment (Santyl®) and petrolatum were provided by Healthpoint Biotherapeutics, Ltd. (Fort Worth, TX). There was approximately 1.23 mg collagenase in 1 g of the petrolatum-based ointment. Approximately 0.05 mL of each treatment was applied topically to the treated wounds in each pair immediately following surgery and every 3 days until 14 days after the surgery. Tegaderm™ dressing (3M™, St. Paul, MN) was used to cover all the wounds and was maintained on each wound until
19 days after the surgery. The animals were monitored daily, and treatments and dressings were reapplied as needed. Treatments were applied immediately following surgery and every three days after dressing was removed and the wound was cleaned until 14 days after the surgery. If within 3 days a wound needed more ointment, then ointment was applied under the Tegaderm without removing it because frequent dressing changes adversely affect wound healing.

Digital photographs of the wounds were taken at weekly intervals. The tissues were collected 28 days after the surgery. One-half of each wound was harvested for histological analysis, while the other half of the wound was frozen at -80°C for future studies. Any scar with evidence of infection, desiccation, or necrosis was excluded from study.

**Histological analysis.** Each wound was considered a separate sample because the wounds healed independently and responded independently to treatments. The bisected scar tissue from each wound was fixed in 4% neutral-buffered formaldehyde, dehydrated, embedded in paraffin, sectioned into 4-μm sections, and stained with hematoxylin and eosin (H&E). The stained tissue sections were examined by light microscopy and were measured quantitatively with Nikon Imaging Software (Nikon Corporation, Tokyo, Japan). The observer of the histology was blinded as to the treatment for each wound, the total area of new scar (TA) and the scar elevation index (SEI) were determined. The TA of the scar tissue was measured between the two nicks in the cartilage that were made during the surgery. The SEI was used to express the degree of scar hypertrophy. This index represents the ratio of total area of new scar to the estimated area of normal underlying dermis. Because the dermal thickness varies over the rabbit ear, the height of the underlying dermis is determined by measuring the height of the dermis in nearby unwounded skin. An SEI of 1 indicates that the wound healed essentially flat; an increasingly larger SEI parallels an increasing quantity of new scar connective tissue. Finally, to be able to compare the collagenase-treated wounds to the petrolatum-treated wounds, an adjusted SEI was calculated for the collagenase- and petrolatum-treated wounds. These were determined by comparing the data for each wound with the data of its corresponding control. Since the collagenase-treated wounds and the petrolatum-treated wounds are not from the same animals, and there is a substantial variation between animals based on factors we cannot measure, such as stress of the animal, comparisons between animals (unpaired comparisons) are not valid without very large sample sizes. An adjusted SEI relative to their own controls are attained and then are compared with each other between the collagenase-treated wounds and the petrolatum-treated wounds.

**Statistical Analysis**

All wounds were created and harvested in a matched fashion and the data collected in a manner to allow paired analysis with each animal serving as its own control. Statistical analysis was done by using Microsoft Excel software for Windows. A paired two-tailed Student’s t test was performed to compare collagenase- or petrolatum-treated scars with their own controls of no treatment. A non-paired, two-tailed Student’s t test was performed to compare collagenase-treated scars with petrolatum-treated scars. A value of \( P < 0.05 \) was considered to be significant in all analyses.

**KeyPoints**

- There was no statistically significant difference between petrolatum-treated wounds and their own untreated controls in terms of either total new scar area (TA) or scar elevation index (SEI).
- Scars from collagenase-treated wounds appear less hypertrophic than scars from their control untreated wounds. The TA was smaller in the collagenase-treated wounds than in their own untreated controls.

**Results**

Sixty-four separate scars were created by making four punch wounds in each ear of eight rabbits. All 64 scars did not show any evidence of infection, desiccation, or necrosis and were included in the study. There were 16 scars for collagenase- or petrolatum-treated groups and their own control groups, respectively.

**Effect of petrolatum on scar formation.** First, the effect of collagenase’s vehicle petrolatum on scar formation was studied. Petrolatum was topically applied onto four wounds in one ear of four rabbits each, while four wounds in the other ear of the same rabbits were left untreated and served as controls. As shown in Figures 1 and 2, there was no statistically significant difference between petrolatum-treated wounds and their own untreated controls in terms of either TA (559 ± 54 μm² vs. 555 ± 46 μm²; \( P = 0.92 \)) or SEI (2.02 ± 0.18 vs. 2.03 ± 0.18; \( P = 0.95 \)). The results indicated that petrolatum has no effect on scar formation.

**Effect of collagenase ointment on scar formation.** Then, the effect of collagenase in petrolatum on
scar formation was evaluated. Collagenase in petrolatum was topically applied onto four wounds in one ear of four rabbits each, while four wounds in the other ear of the same rabbits were left untreated and served as controls. As shown in Figure 3, scars from collagenase-treated wounds appear less hypertrophic than scars from their control untreated wounds. As shown in Figures 4 and 5, the TA was smaller in the collagenase-treated wounds than in their own untreated controls (531 ± 32 µm² vs. 617 ± 51 µm²; \( P = 0.03 \), Figure 4), and the SEI was lower in the collagenase-treated wounds than in their own untreated controls (2.08 ± 0.15 vs. 2.45 ± 0.20; \( P = 0.015 \), Figure 5).

Comparison of the effects of collagenase ointment and petrolatum on scar formation. The adjusted SEI was lower in the collagenase-treated wounds than in the petrolatum-treated wounds (0.88 ± 0.04 vs. 1.03 ± 0.07; \( P = 0.10 \)) and the difference showed a trend toward statistical significance. It is possible that differences would be seen provided there was a larger sample size or if collagenase and petrolatum were applied in the same animals so they can compare to each other directly. Occlusion may have had some effect, but the authors do not think it explains the apparent effects seen with collagenase.
KEYPOINTS
- Multiple studies have demonstrated reduced collagenase expression in cultures of fibroblasts from hypertrophic scars. The addition of an exogenous collagenase could, therefore, exert its effect by increasing the overall level of collagenase present in the wound, thus interrupting the initial phase of collagen deposition that occurs in hypertrophic scar formation.
- Collagenase ointment may be more effective than petrolatum in reducing hypertrophic scar formation, but further studies with more animals will need to be done to demonstrate this at the \( P < 0.05 \) level.

Discussion
Collagenase ointment is an enzymatic agent that breaks down collagen. Although the mechanisms that lead to the alterations in collagen metabolism seen in hypertrophic scars are not well understood, it is reasonable to hypothesize that collagenase might be useful in the management of hypertrophic scars.

Very few studies have been done to investigate the effect of collagenase on hypertrophic scars. One study that examined the effect of intralesional collagenase on established hypertrophic and keloid scars showed no improvement in hypertrophic scars with intralesional collagenase injections. It also showed that treatment with intralesional collagenase had significant associated side effects such as pain, blistering, and ulceration. This study, however, is very limited because it was a study of seven patients, only two of whom had hypertrophic scars. Furthermore, it only investigates the effect of collagenase on established scars, and does not provide any information about the use of collagenase to prevent hypertrophic scars.

A recent retrospective study looked at scar outcomes in patients with partial-thickness burn injuries who received a routine wound management that consisted of either saline wet-to-dry dressings or collagenase ointment dressings. The main finding of this retrospective review was that only 9.2% of patients treated with collagenase ointment dressings developed scarring as compared to 20.4% of patients treated with saline wet-to-dry dressings. Although limited by its design, this study suggests that collagenase ointment is effective in preventing the formation of hypertrophic scars.

The present study demonstrated that collagenase ointment is effective in reducing hypertrophic scar formation in a rabbit ear-scarring model. The total new scar area (TA) is smaller and the scar elevation index (SEI) is lower in wounds that are treated with collagenase ointment than in wounds that are not treated. Also, the adjusted SEI is lower in wounds that are treated with collagenase ointment than in wounds that are treated with petrolatum. These results show a trend toward statistical significance. The authors suspect that collagenase ointment is more effective than petrolatum in the prevention of hypertrophic scars. Because of the individual variability between rabbits, a direct comparison between collagenase and petrolatum in different animals would require a larger sample size. Therefore, it is not surprising that the results show a trend toward, but do not reach, the level of statistical significance. Furthermore, although we did not address this in the present study, we suspect that topically applied collagenase is unlikely to have as many untoward effects as intralesional collagenase. Although a polyurethane film dressing is semiocclusive, and there is some effect on its own if it is used after re-epithelialization is complete, this is not the case for this study. In this study, both treatment and control groups are covered with a polyurethane film dressing just until re-epithelialization is complete.

The mechanisms by which collagenase exerts its effects are unknown. One hypothesis is that collagenase acts by expediting re-epithelialization and wound closure. Hypertrophic scars are known to occur more commonly in wounds with delayed epitheliazation. Garner hypothesized that the lack of an overlying epidermis results in excess collagen synthesis. Garner studied what happened when human keratinocytes were added to a human fibroblast culture and found a dose-dependent decrease in fibroblast collagen synthesis. While this study demonstrates that the keratinocytes have an effect, it does not clarify how the keratinocytes exert their effect. It may be that their presence alone has an effect or it may be that keratinocytes produce and secrete a substance that has an effect on the fibroblasts. Riley found collagenase can stimulate the proliferation and post-injury migration of keratinocytes in vitro and promote wound healing in vivo.

Another hypothesis is that by altering the balance between collagen synthesis and degradation, collagenase acts directly to decrease collagen deposition. Multiple studies have demonstrated reduced collagenase expression in cultures of fibroblasts from hypertrophic scars. The addition of an exogenous collagenase could, therefore, exert its effect by increasing the overall level of collagenase present in the wound, thus interrupting the initial phase of collagen deposition that occurs in hyper-
trophic scar formation. Further studies will need to be done to elucidate these mechanisms.

**Conclusion**

Collagenase ointment is effective in reducing hypertrophic scar formation in a rabbit ear-scarrying model when compared to no treatment. Collagenase ointment may be more effective than petrolatum in reducing hypertrophic scar formation, but further studies with more animals will be needed to be done to demonstrate this at the $P < 0.05$ level. There is a significant difference between the collagenase-treated and blank wounds, and a definite trend for differences between the collagenase-treated and vehicle-treated wounds. It is possible that with additional animals, comparing collagenase treated to petrolatum in the same animals would yield a significant difference.

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**References**