Silicone Occlusive Treatment of Hypertrophic Scar in the Rabbit Model

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Background: *Hypertrophic scar formation at sites of healed cutaneous injury often produces functional and esthetic deficits. Treatments have been limited in part by a lack of understanding of scar etiology and the lack of animal models of hypertrophic scarring. Silicone dressing is reported to provide positive outcomes with respect to a reduction in scar hypertrophy and an improvement in color differences, although the exact mechanism is unknown.*

Objective: *We tested the effectiveness of silicone adhesive gel in the reduction of scar hypertrophy in an animal model of scarring.*

Methods: *Silicone adhesive gel was applied to scars in a rabbit ear model of hypertrophic scarring. Scarring in this model, which displays reduced hypertrophy in response to steroid injections and aging similar to that of human beings, was measured by the Scar Elevation Index (SEI), a ratio of the scar height over normal skin, in which readings greater than 1.0 represent a raised scar.*

Results: *SEIs were significantly reduced after 4-week applications of silicone gel (1.15 ± 0.15 vs 1.71 ± 0.33, respectively; $P < .001$) versus untreated scars. Nonsilicone control dressings did not alter SEIs in comparison with those found for controls. No histologic differences in scar cellularity, inflammation, or matrix organization were found between treatment groups; however, ultrastructural observation revealed numerous vacuoles in basal cells of control and nonsilicone-treated scars that were not found in unwounded skin or silicone gel–treated scars. The similarity in water vapor transmission rates for silicone gel and a nonsilicone dressing eliminated scar hydration as the sole mechanism of action of the silicone dressings.*

Conclusions: *Our findings with the rabbit model demonstrate the effectiveness of silicone gel for hypertrophic scar treatment and confirm the usefulness of this model for further study of the mechanism of occlusion.* (Aesthetic Surg J 2002;22:147-153.)

Hypertrophic scarring can occur after thermal injury, surgical incision, or other traumatic injury. These lesions are raised, pruritic erythematous scars that may widen, but which remain within the confines of the original wound. Histologically, hypertrophic scars appear hyperplastic and manifest a thickened epidermis, a dermis lacking dermal papillae, and the presence of collagen nodules in an abnormally increased vascular wound matrix.[1-2] The etiology of this abnormal scarring process is, for the most part, unknown.[3-5] Predisposing conditions appear to be related to prolonged inflammation, such as repeated trauma, continued irritation from foreign body inclusions, excessive wound tension, infection, or delayed reepithelialization.[6]

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There are multiple treatments for hypertrophic scars, but the most common first-line therapies have been pressure garments and silicone gel sheeting.[7-10] Steroid injections are often used to treat unresponsive scars.[11-14] Silicone gel was first used underneath pressure garments to achieve a better fit and was fortuitously noted to have a beneficial effect on hypertrophic burn scars.[15] Quinn et al[16] confirmed the beneficial effects in a larger uncontrolled study. Our group first established these benefits in a prospective randomized controlled trial in which the patient served as his or her own control in burn scars,[8] and then in the prevention and treatment of hypertrophic scars resulting from surgical incisions.[17] Since then, a number of authors have confirmed these findings through prospective randomized trials[9] and meta-analysis.[18]

Although silicone gel sheeting is now an established first-line therapy for hypertrophic scars, the basis for its mechanism of action has remained elusive. Previous investigators have excluded pressure, oxygen tension, temperature, and silicone absorption in the skin as possible mechanisms of action.[16,19,20] The generation of a charge difference at the skin surface (ie, static electricity) has also been proposed as a possible mechanism by which silicone sheeting affects scar resolution.[21] However, the most prevalent theory has focused on the ability of silicone occlusive dressings to increase hydration of the stratum corneum.[22] It has been suggested that silicone ointment may also be effective,[23] as well as occlusive oil without any silicone product.[24] The occlusion theory has gained significant support from an in vitro study of a keratinocyte fibroblast coculture system in which hydration by either water or silicone reduced fibroblast proliferation compared with no treatment.[25]

Our laboratory has developed a unique animal model of hypertrophic scarring in the rabbit ear that has been shown to be similar to the human condition in histologic and visual appearance with respect to larger wounds, response to steroids, and improvement in degree of scarring with advanced age.[26,27] Furthermore, the hypertrophy is more marked in wounds with delayed epithelialization. In this study, we used the rabbit ear hypertrophic scar model to compare the scar responses of adherent silicone gel sheeting with semiocclusive dressings, including microporous paper taping, which has been advocated as a treatment to improve scars.[28] We demonstrate the effectiveness of silicone gel sheeting and a lack of effectiveness of other forms of semiocclusive dressings, as well as report findings on transmission electron microscopy of changes in the basal epithelium and basement membrane, which yield new insights into the mechanism of action. The importance of understanding the mechanism of action lies in the potential to design new therapies that are more effective.

Material and Methods

Hypertrophic Scar Model

Twenty-five young adult New Zealand White female rabbits weighing between 2.5 and 3.5 kg were used in this study. The animals were handled according to procedures approved by the Northwestern University Animal Care and Use Committee. After the animals were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg), 4 wounds were created down to bare cartilage on the ventral surface of each ear by means of a 7-mm punch biopsy. A dissecting microscope was used to ensure removal of the epidermis, dermis, and perichondrium in each wound. Removal of the perichondrial layer delayed epithelialization of the 7-mm defect, which supports hypertrophic scar formation. Hemostasis was then obtained by applying pressure and each wound individually covered with polyurethane dressing (Tegaderm; 3M Health Care, St. Paul, MN). The wounds were kept covered until postoperative day 12 or until the entire wound appeared re-epithelialized on gross examination. Virtually all wounds were epithelialized by day 12, and 50% were epithelialized by day 7.

Treatment was begun on postoperative day 28. The occlusive materials tested in this study included the following: adhesive silicone gel (Cica-Care; Smith & Nephew, Largo, FL); Tegaderm; alternative polyurethane dressing (Op Site; Smith & Nephew); and a 2-layer paper strip (Steri-Strips; 3M). Five rabbits were treated with each type of occlusive material from day 28 through day 56, for a total of 4 weeks of occlusive treatment. Each rabbit had 4 wounds occluded; 4 wounds were left uncovered to serve as controls. This resulted in a total of 20 treated and 20 control wounds for each type of occlusive treatment, with each treated wound having a paired control on the same animal.

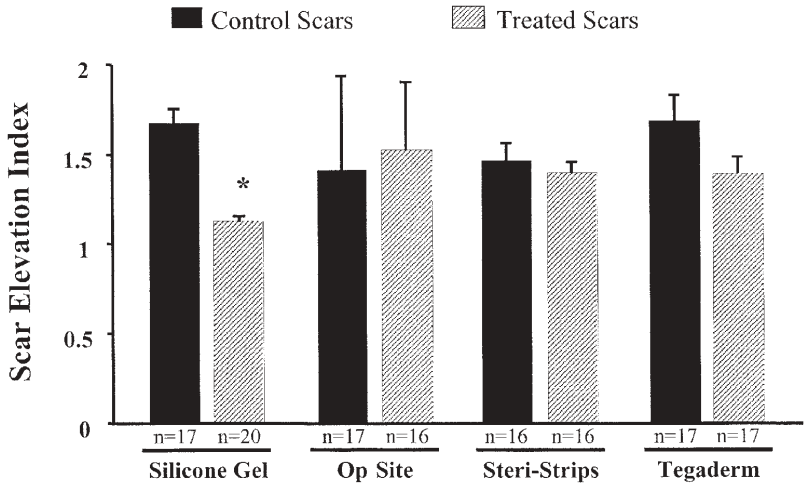

Figure 1. *Treatment of healed full-thickness wounds in the rabbit model. Treatment of hypertrophic scarring with adhesive silicone gel for 4 weeks (postwounding days 28 to 56) significantly reduced scar hypertrophy. Four-week treatment (postwounding days 28 to 56) of healed wounds with Op Site, Steri-Strips, or Tegaderm did not alter the scar elevation index (SEI) in the rabbit model of hypertrophic scarring. The SEI describes the height of the scar as a ratio to surrounding unwounded tissue. A ratio of 1 indicates no difference in the height of the wound area compared with unwounded skin. Treated and untreated wounds from each group were compared by means of the Student t-test. Resulting P values are .64, .51, and .11 for Op Site, Steri-Strips, and Tegaderm, respectively. *P < .001 versus control scar by means of the Student t-test for group comparison.*

Tissue Preparation

On postoperative day 56, all rabbits were sacrificed and the scars harvested. The scars were bisected through the point of maximum height of hypertrophic scar on palpation. An 0.5-cm margin of surrounding unwounded tissue was harvested with each wound. One half of each wound was fixed in 4% neutral-buffered formaldehyde, dehydrated, embedded in paraffin, cut in 4-m sections, and stained with the Masson trichrome stain. The remaining half of the wound was fixed in Karnovsky's fixative (5% glutaraldehyde, 4% formaldehyde, in 0.1 mol/L sodium cacodylate buffer, pH 7.4, at room temperature), postfixed with 1% OsO_4, dehydrated, infiltrated, embedded in Poly/bed 812 (PolySciences, Inc., Warrington, PA), and sectioned with a diamond knife for transmission electron microscopic examination.

Quantification Methods

The degree of hypertrophy of each scar was expressed as the Scar Elevation Index (SEI), whose measurements were based on those made from trichrome-stained tissue sections at 40×. This index is the ratio of total wound area tissue height to the area of normal tissue below the hypertrophic scar.[26,27] Each wound was measured twice by a blinded examiner using a calibrated eyepiece reticule. Overall dermal cellularity was quantified by counting cells per high-power field (100×). The dermis was further examined for collagen organization, vascularity, and inflammation in a semiquantitative manner. The parameters were rated on a scale of 1 to 4, with the higher value indicating a more disorganized collagen, greater vascularity, or higher numbers of inflammatory cells. The epidermis was evaluated for cellularity of the 4 upper layers and mitotic activity of the basal layer. The ultrastructure of the dermis and epidermis of scars from each treatment group was evaluated by transmission electron microscopy.

The water vapor transmission rate of all the products, with the exception of the 2-layer Steri-Strips, was measured by means of the international ASTM E96-95 Desiccant Method.[29] Briefly, the test specimen (or occlusive material, in this case) was sealed to the open mouth of a test dish containing a desiccant. The assembly was then placed in a sealed water bath to provide an atmosphere of constant temperature and humidity. Hourly weighings over an 8- to 12-hour period determined the rate of water vapor movement through the specimen into the desiccant and is described as grams per meter squared per hour.

Results

Degree of Hypertrophic Scarring

Hypertrophic scars occluded with silicone gel from postwounding day 28 to postwounding day 56 exhibited a

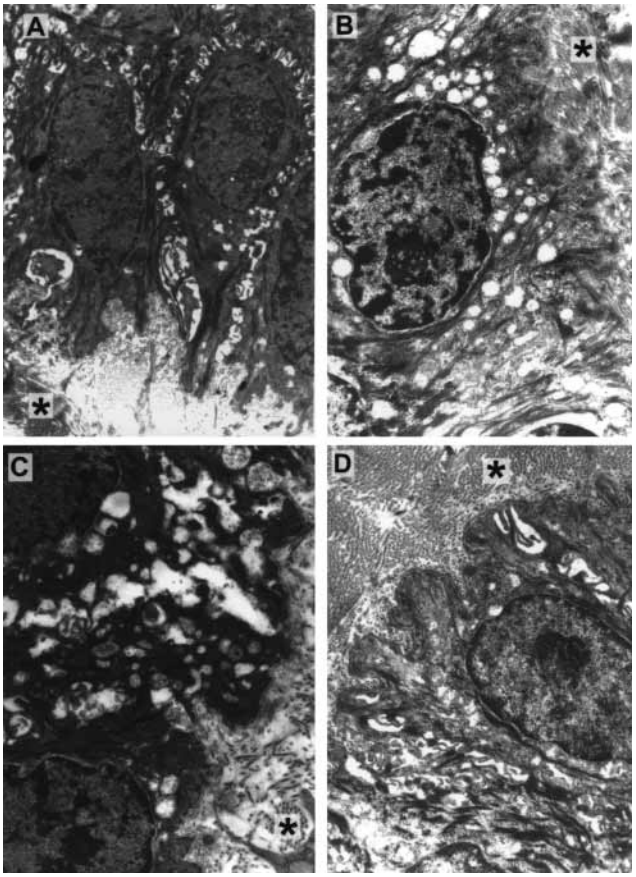

Figure 2. *Samples of unwounded skin, control (untreated) scars, and treated scars were prepared for ultrastructural analysis as described in "Material and Methods." Silicone gel treatment of scars resulted in an appearance similar to that found in unwounded skin, including reduction in the presence of basal cell vacuoles and more organized dermal collagen.* **A,** *Unwounded, control skin;* **B,** *untreated scar;* **C,** *Op Site–treated scar;* **D,** *Silicone gel–treated scar. Asterisk denotes dermal aspect of micrograph.*

significant decrease in measured SEI when compared with nonoccluded control scars ($P < .001$; Figure 1). Hypertrophic scars occluded for the same period of 28 days with Tegaderm, Op Site, or a 2-layer Steri-Strip dressing had no significant decrease in SEI when compared with nonoccluded control scars (Figure 1).

Dermal and Epidermal Histologic Examination

No significant difference was noted in overall dermal cellularity between occluded and nonoccluded control scars (data not shown). Examination of the dermal layer in the silicone gel–occluded groups revealed no significant difference in the following parameters rated in a semiquantitative fashion on a scale of 1 to 4: collagen fiber organization; dermal vascularity; dermal inflammation; and number of mast cells, lymphocytes, multilobulated cells, and eosinophils.

Semiquantitative examination of the layers of the epidermis for cellularity and mitotic activity revealed no difference between occluded and nonoccluded control scars.

Transmission Electron Microscopy Examination

The ultrastructural architecture of unwounded control tissue epidermis viewed by transmission electron microscopy included numerous complex interdigitated cellular margins with desmosomes, numerous hemidesmosomes, and distinct uniform basal lamina (Figure 2, *A*). Membrane-bound electron lucent cytoplasmic vesicles were infrequently noted in basal cells. Unwounded dermis displayed normal characteristics of thin skin, including reticular dermis of dense irregular connective tissue composed of fine, whorled collagen fibers.

By comparison, striking differences were noted in and between control (untreated) scars and silicone gel–occluded scars, especially in the basal cells. Basal cells of control scar and Op Site–treated scars contain numerous vacuoles generally oriented toward the basal lamina (Figure 2, *B* and *C*). By morphology, the vesicles did not appear to be endosomes, lysosomes, peroxisomes, or secretory in nature. The basal lamina is also less distinct than control skin. The untreated scar dermis is composed of loose, unorganized collagen compared to unwounded controls, particularly near the basal lamina. By contrast, the epidermis of silicone gel–treated scars contained few—although heavily flocculent—vesicles (Figure 2, *D*). Although irregular in appearance, the more distinct basal laminae of the silicone gel–treated scars were similar to unwounded skin. In addition, the dermis of the gel-treated scars appeared more similar in organization to unwounded skin at the ultrastructural level.

Water Vapor Transmission Rate of Materials

The measured water vapor transmission rates (WVTRs) of the various occlusive products were as follows: silicone gel, 5.2 ± 0.5 g/m^2/hr; Tegaderm, 27.0 ± 0.5 g/m^2/hr; Op Site, 4.3 ± 0.4 g/m^2/hr (Figure 3). The measurements were repeated 3 times for each occlusive product and only included with a linear regression value (r^2) greater than 0.85 for time (in hours) versus weight (grams). Of interest are the similar WVTRs for silicone gel and Op Site.

Discussion

Hypertrophic scarring is a difficult clinical problem with few treatment options. Silicone gel sheeting has been found to be partially effective both in the treatment and the prevention of hypertrophic scars secondary to burns, traumatic injuries, and surgery.[8,17,18] However, the basis of the effect of silicone gel is not known, and without animal models of hypertrophic scarring, the mechanism of action could not be systematically studied. We have previously used a rabbit model of hypertrophic scarring developed in our laboratory to demonstrate age-based differences in hypertrophic scarring,[27] and validated the model by its responsiveness to steroid injections.[26] The model has been further validated in this study by demonstration of scar reduction by silicone gel sheeting. We focused on the observation of many authors that the semiocclusive properties of silicone gel were responsible for its effects, and investigated other semiocclusive dressings.

Many investigators have attempted to elucidate the mechanism of action of silicone gel. Quinn et al[16] excluded the effects of pressure and temperature changes and changes in oxygenation, and, along with others,[20] excluded a chemical effect secondary to silicone absorption into the tissue. Quinn[19] measured the WVTR of silicone gel (4.5 g/m^2/hr) and found it to be half the reported rate of normal skin. The hypothesis that increased hydration of the stratum corneum secondary to the lower WVTR was examined by comparing silicone gel with a nonsilicone product with identical weight and WVTR because silicone gel was tested on human scars without evidence of efficacy.[19] However, in contrast to Quinn's[19] observations, Sawada and Sone[23,24] concluded that hydration and occlusion with a nonsilicone product or silicone oil induced a significant reduction in hypertrophic scar formation. However, both studies were uncontrolled, limiting their interpretation. A recent article documents a change in the hydration of the stratum corneum,[22] which is unique for topical silicone gel sheeting, and an in vitro coculture system of keratinocytes and fibroblasts demonstrates an inhibition of fibroblast proliferation from direct hydration.[25] This report supports the notion that the epidermal layer is capable of communicating and changing the behavior of the underlying dermis.

Our results from this study demonstrate that silicone occlusive therapy significantly reduces hypertrophic scarring in comparison with untreated control scars. The hypothesis that the sole effect of silicone gel sheeting is caused by its unique semiocclusive nature was explored. Dressings that provide absolute occlusion were not utilized because they lead to overhydration of the stratum corneum with maceration and inflammation.[30] The 2 polyurethane films tested, Op Site and Tegaderm, have different water-permeability properties. Tegaderm was found to have a high WVTR, whereas the WVTR of Op Site was very similar to that of silicone gel. Nevertheless, both Tegaderm and Op Site were ineffective in our model, demonstrating that the underlying mechanism is more complex than simple hydration. One explanation for this observation is that the rate of hydration of the stratum corneum is an important variable,[22] which may be different with silicone gel than with other semiocclusive dressings.

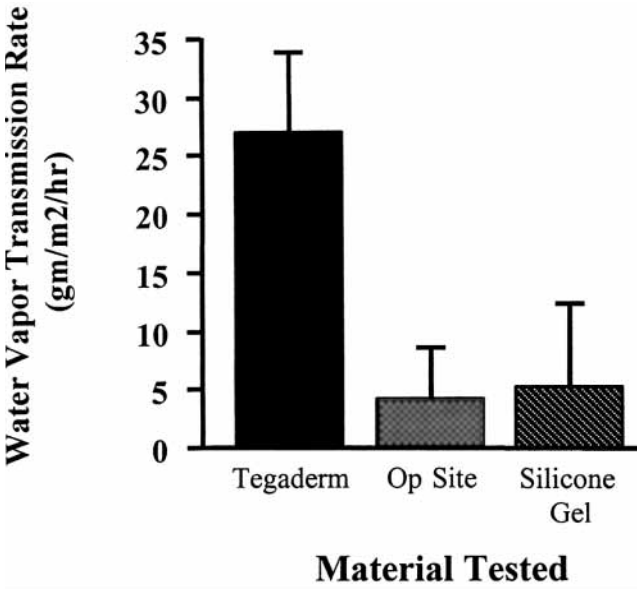

Figure 3. *Occlusive properties of the tested occlusive treatments were determined by measurement of the Water Vapor Transmission Rates (see "Material and Methods"). The similar transmission rates of Op Site and the adhesive silicone gel suggest that the scar-reducing property of the silicone gel is not dependent on the occlusive nature of the gel.*

Although detailed histologic analysis of treated and controlled scars failed to demonstrate a difference in the epidermal or dermal cellularity, vascularity, or matrix organization caused by silicone gel, electron microscopy showed significant effects in the basal epithelium and basement membrane in treated skin. The basal layer of silicone gel–treated scars appeared more like control (unwounded) epidermis than the epidermis from either untreated scars or scars treated with Op Site or Tegaderm. The untreated scars exhibited a large number

of intracellular vacuoles in the basal layer epidermal cells that are in contact with the basement membrane. In addition, the lamina lucida in the non-treated hypertrophic scars was noted to be quite irregular with frequent gaps. These abnormal findings were also present in the Op Site treated hypertrophic scars, which did not demonstrate a decrease in scar volume.

The presence of these vacuoles in the basal layer cells of the untreated hypertrophic scars and their absence in unwounded tissue suggest that they play a role in the development of excessive scar formation. Epidermal-dermal communication has been demonstrated in a variety of experimental systems. The presence of newly formed epidermis over granulation tissue induces apoptosis and reduction of inflammation in the underlying dermis.[27,31,32] The epidermis is a site of growth factor production, and treatment of healing wounds with keratinocyte growth factor, for which receptors exist only on epidermal cells, induces increases in the underlying granulation tissue.[33] One hypothesis is that the vacuoles contain growth factors or other soluble factors that cross the basement membrane and signal the dermis. As the epithelium of the healing scar matures, with maturation of the stratum corneum, the WVTR of skin would be expected to become lower. The in vitro study indicating an inhibition of fibroblast proliferation with hydration of the cocultured keratinocyte is consistent with these observations.

Conclusion

We have used our animal model of hypertrophic scarring to demonstrate the efficacy of adherent silicone gel sheeting in the reduction of hypertrophic scarring. Other semi-occlusive dressings, including a polyurethane film dressing with a similar WVTR, were ineffective, indicating that the effectiveness of silicone gel is not completely attributable to hydration of the stratum corneum.[22] The findings on electron microscopy are substantial and support epidermal signaling from immature epithelium as an important etiology for hypertrophic scarring. ■

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