Integra as a Dermal Replacement in a Meshed Composite Skin Graft in a Rat Model: A One-Step Operative Procedure

Chi-Sing Chu, MD, Albert T. McManus, PhD, Natalia P. Matylevich, PhD, Cleon W. Goodwin, MD, and Basil A. Pruitt, Jr., MD

Background: Current use of Integra, the collagen-based dermal analogue, requires a two-step grafting procedure to achieve wound closure with an “ultrathin” autograft.

Methods: A one-step operative procedure of meshed composite skin graft (MCSG) using Integra as a dermal template for a meshed split thickness autograft was developed in rats. The silicon layer of Integra was removed, the resulting dermal analogue was meshed (1:1.5), expanded, and placed on excised full thickness wound and covered with a meshed (1:1.5 or 1:6) split thickness autograft. Grafted wounds were dressed with BioBrane, Vaseline gauze, silver-impregnated nylon, or silver-nylon and direct current (SNDC). At scheduled intervals up to 3 months postgrafting, wounds were examined for epithelialization, collagen deposition and fibrosis, hair growth, and contraction. The results of wound closure and healing following the one-step procedure were compared with the outcome of the two-step grafting procedure where application of meshed Integra (step one) was followed in 14 days by removal of the silicon layer and application of the meshed autograft (step two).

Results: The one-step procedure applied to meshed autograft/Integra (1:1.5/1:1.5) composite graft accelerated wound closure by 6–19 days when compared with the two-step procedure. At 3 months postgrafting, the contraction of the healed wound dressed with SNDC, BioBrane, or Vaseline gauze was reduced by 13–16% following the one-step procedure compared with the two-step procedure (p < 0.05). The one-step procedure allowed the expansion of the autograft layer to 1:6 while achieving wound healing results similar to grafting with 1:1.5 meshed autograft layer using the two-step grafting procedure.

Conclusion: Single-step application of meshed, thin, split thickness autograft over meshed Integra-derived dermal substitute allows more rapid wound closure with less contraction and more efficient use of graft donor skin than can be obtained with the commonly used two-step grafting procedure.

J Trauma. 2002;52:122–129.

The current accepted procedure for wound closure, following excision of deep partial and full thickness burns, is the application of a split thickness cutaneous autograft obtained from an uninjured area of the body. In patients with extensive burns, the autografts are commonly meshed and expanded in ratios ranging from 1:1.5 to 1:4 to conserve the donor sites. The thickness and continuity of the dermal component of the autografts are major determinants that affect the functional and cosmetic outcome of the grafted wounds. Harvesting of thick dermis provides better healing outcome, but contributes to donor site morbidity and limits skin reharvesting. To overcome this limitation, materials which can replace dermis or orchestrate dermis regeneration have been developed. These include both natural and artificial dermal substitutes that serve as scaffolding for migrating dermal cells. Unfortunately, use of artificial dermal substitutes entails a delay, often of around 2 weeks, to allow for vascular ingrowths and fibroblast infiltration before they can be covered with split thickness autografts and requires two operative procedures.

Few successful attempts of one-stage application of composite grafts have been reported. Survival of the split thickness autograft applied over the dermal substitute, which was based on bovine, type I collagen and elastin-hydrolysate and scar elasticity, was evaluated in the treatment of clinical burns. Ultrathin split thickness autograft was applied over allogeneic or xenogeneic dermal substitute using a one-stage procedure in rats with full thickness skin injuries. Cryopreserved cellular and decellularized porcine allogeneic dermis, in conjunction with an overlying thin split thickness autograft, were compared in porcine full thickness wounds. All of these studies evaluated the usefulness of dermal substitutes and used conventional split thickness autograft as a control.

We have previously developed a single-stage procedure for application of meshed composite skin graft (MCSG) that utilizes a meshed split thickness autograft over a meshed viable dermal allograft that engrafts and persists without rejection when tested in two highly histoincompatible inbred...
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rat strains, the white female Lewis and the brown male ACI.9,10 In this model, the grafts were treated with silver-nylon (SN) or SN and direct electric current dressings. No immunosuppressive agents were used. This procedure provides permanent coverage of full thickness excision wounds with moderate or minimal contraction, and reduced or eliminated severe microbial contamination. Such grafting procedure permits the use of thin split thickness autografts meshed at 1:6 rather than the customary 1:1.5 or 1:4 ratios. In humans, the use of a cadaver allogeneic dermis is a major disadvantage due to the possible transmission of HIV, hepatitis B or C, or other pathogens. The ideal dermal analogue should be free of infection risk, readily available, and have a long shelf life. It should replace the dermal components of normal skin or serve as a scaffold for the formation of a neodermis, and exhibit near normal mechanical properties and cosmetic appearance. It should also be effective in promoting healing and expand as the patient grows. Integra comes close to fulfilling these requirements.5,11,12 It is produced from deantigenized cross-linked bovine collagen to which chondroitin sulfate is added.

In this study, using a rat model, we evaluated the closure and healing of full thickness skin defects following a single-step grafting with a “composite” graft of meshed Integra and a meshed cutaneous autograft and compared the results to the standard two-step grafting procedure. Four different wound dressings were used (SN, SN with direct current, BioBrane or Vaseline gauze with Trioptic ointment) to verify the effect of the operative procedure on graft healing.

MATERIALS AND METHODS

Experimental Animals

White female Lewis rats (Harlan, Houston, TX) weighing 220 ± 25 g were used as experimental animals. In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals, and to the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 85–23. The experimental protocol and animal care were approved by the animal care and use committee of our institute.

Experimental Model

Anesthesia

Before all surgical procedures, Buprenex (buprenorphine hydrochloride, Reckitt & Colman Products, Hull, England) was administered to the rats subcutaneously at a dose of 0.05 mg/kg of animal weight (the analgesic effect continues for 12 hours). Fifteen minutes later, anesthesia was induced with pentobarbital administered intraperitoneally at a dose of 38.0 mg/kg of animal weight.

Wound Dressings

The following wound dressings were used in the study: (1) Trioptic-P ointment (bacitracin zinc U.S.P. 400 units/g, neomycin sulfate U.S.P. 0.5% [equivalent to 3.5 mg/g of neomycin base], polymyxin B sulfate U.S.P. 10,000 units/g) (Opharmaderm, Altana, Inc., Melville, NY) applied as a thin layer over BioBrane wound dressing (Dow B. Hickam, Inc., New York, NY) (BBT); (2) Vaseline gauze with Trioptic-P ointment applied topically (VGT); (3) silver-impregnated nylon (SN); and (4) direct electric current (30 µA) applied through silver-nylon for 5 consecutive days as described in our previous studies (SNDC).13,14

The One-Step Grafting Procedure

Split thickness autografts (6 × 4 cm, 0.012 inch thickness) were harvested from the depilated dorsa of the rats and meshed 1:1.5 or 1:6 using a Zimmer Meshgraft II dermatome (Zimmer, Inc., Warsaw, IN). The residual dermal tissue and panniculus carnosus were excised to leave a 4 × 6 cm open wound. The silicon layer of the Integra (Integra Life Sciences Corp., Plainsboro, NJ) was peeled off; the remaining dermal analogue was meshed at a 1:1.5 ratio and was placed on the open wound surface (Fig. 1A). The autografts were proportionally expanded and placed over the Integra (Fig. 1B). Thereafter, the animals were randomly assigned to four groups and the grafts were dressed with one of the four experimental wound dressings described above (BBT, VGT, SN, or SNDC). A gauze and sponge covering was applied over the wound dressings and held in place with a tubular elastic net. The coverings were changed 5 or 7 days after initial application. The wound dressings were allowed to separate spontaneously.

The Two-Step Grafting Procedure

During the first step, a 4 × 4 cm area of skin, subcutaneous tissue, and panniculus carnosus was excised from the central depilated dorsum of the trunk. The Integra was meshed 1:1.5 using a Zimmer Meshgraft II dermatome, expanded and applied over the wound. The animals were randomly divided into four experimental groups and their wounds were dressed with one of four wound dressings listed above (BBT, VGT, SN, or SNDC). A gauze and sponge covering was applied over wound dressings and held in place with a tubular elastic net. The coverings were changed 5 or 7 days after initial application. The second grafting step was performed 14 days later. The animals were anesthetized as described previously and a 4 × 4 cm full thickness skin graft containing a thin panniculus carnosus was harvested from the shaved and depilated abdomen. The abdominal wound was sutured immediately after graft harvesting. The graft was placed on a smooth metallic plate with the epidermis side down and maintained under tension. The panniculus carnosus and part of the dermis were excised to obtain a partial thickness graft approximately 0.012 inch thick. The wound dressing was removed from the dorsal wound and the silicon layer of the Integra was easily detached. The autograft was meshed to 1:1.5 or 1:6 using a Zimmer Meshgraft II dermatome and applied over the Integra surface. Each wound was covered
with the same type of wound dressing as in the first step. A gauze and sponge covering was applied and maintained over the wound dressing in a fashion similar to the first step. The wound dressing was allowed to separate spontaneously.

**Postoperative Animal Care**

After the operation, all of the animals were kept in an animal intensive care chamber for a minimum of 48 hours. The temperature was maintained between 26 and 28°C and the humidity at approximately 50%. After being removed from the chamber, the animals were housed in individual cages for the rest of the study and fed regular rat chow and water ad libitum.

**Experimental Groups**

A pilot study was conducted to evaluate wound closure at 3 months postgrafting in three animals from 16 experimental groups (two types of grafting procedures [one-step or two-step], four types of wound dressings [SN, SNDC, BBT, or VGT], two autograft mesh expansion ratios [1:1.5 or 1:6]). All of the wounds grafted with a 1:6 meshed and expanded autograft following the two-step procedure failed to heal with complete wound closure within the 3-month period. These groups were discontinued from the study. Twelve groups enrolled in the study are listed in Table 1.

**Evaluation of Wound Healing**

**Gross Examination**

Five or six animals from each of the experimental groups were observed grossly during the first 3 months after grafting. Photographs of the wounds were taken following spontaneous wound dressing separation and at 30, 60, and 90 days postgrafting (PG). A ruler (cm) was included in the photo field. The following data were recorded: day postgrafting of complete epithelialization (less than 5% wound area occupied by granulation tissue), and wound size at 3 months PG (percentage of original excised wound, 4 cm in one-step and 4 × 4 cm in two-step grafted wounds). Measurements were preformed using planimetric analysis software Optimas 5.2.
RESULTS

One-Step Grafting

The procedure included harvesting split thickness graft followed by full thickness excision of 4 × 6 cm area of the dorsum. Integra (with silicon layer removed) was placed on the wound and covered with the split thickness autograft (meshed and expanded 1:1.5 or 1:6). In 1:1.5 meshed autografts dressed with SN (with and without current), the wound dressings spontaneously separated at 12–14 days PG and the grafted wounds showed complete (>95%) epithelialization (Table 2). Vaseline gauze and BioBrane dressings separated spontaneously at 21 days postgrafting; the wounds still had numerous small areas of granulation tissue. Complete epithelialization occurred within the subsequent 7 to 10 days (Table 2). At 3 months postgrafting, the healed wounds of the SNDC group had no contraction (100.8%, Fig. 2A), and the wounds in the SN, VGT, and BBT groups demonstrated mild contraction compared with original wound size (83.1%, 81.9%, and 84.9%, respectively, Table 1). When compared with grafts treated with the same dressing (SNDC, BBT, or VGT), contraction was less following the one-step procedure than with the two-step procedure (p < 0.05) (Table 1). Hair growth was denser in the animals treated with direct current and appeared to be denser following the one-step grafting than the two-step procedure (compare Fig. 2A and Fig. 3A).

In 1:6 meshed autograft groups, all of the wound dressings spontaneously detached from the graft between the 14th and 21st days PG. The grafted wounds had numerous small areas of granulation tissue. The epithelial cells had spread from the reticulum of the meshed autografts to increase the width of the reticular columns by threefold (Fig. 4A). The wounds epithelialization was completed during the following 2 to 3 weeks. As shown in Table 1, at 3 months PG the wound sizes were 84.3%, 63.3, 58.8%, or 63.1% of the original graft size in animals treated with SNDC, SN, VGT, or BBT, respectively. The wound contraction in the group treated with direct current was less than that of the other three dressing groups (p < 0.001). If treated with the silver-nylon dressing

### Table 2 The Time of Wound Closure in 1:1.5 Autograft/1:1.5 Integra Experimental Meshed Composite Skin Grafts (Mean ± SE, days)

<table>
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<tr>
<th>Grafting Procedure</th>
<th>Wound Dressing</th>
<th>SNDC</th>
<th>SN</th>
<th>BBT</th>
<th>VGT</th>
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<td>Two-step&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.6 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.5 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.1 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.7 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>One-step&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.6 ± 0.9</td>
<td>13.6 ± 0.6</td>
<td>29.0 ± 0.5</td>
<td>28.5 ± 0.6</td>
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<sup>a</sup> SNDC vs. SN, VGT, or BBT: p < 0.001.
<sup>b</sup> SNDC or SN vs. VGT or BBT: p < 0.001.
<sup>c</sup> Two-step vs. one-step: p < 0.001.

n, number of animals per group.

<sup>1</sup> SNDC vs. SN, VGT, or BBT: p < 0.001.
with direct current, the contraction of the 1:6 meshed autograft grafts following the one-step procedure was similar to the 1:1.5 meshed autografted wounds in the two-step procedure (Table 1). Hair growth was heaviest if SNDC was applied.

Histologic examination showed that at the fifth day PG, a few ink-filled capillaries were evident in the dermal analogue in all examined animals treated with direct current. By day 7 postgrafting, circulation had been reestablished in both layers of the graft in all wounds treated with direct current, and in the dermal analogue (but not autograft) of the grafts covered with SN, BBT, or VGT dressings. Fourteen days postgrafting, both the dermal analogue and the autograft layers of all experimental groups were completely vascularized. In wounds covered with 1:1.5 meshed autograft and treated with direct current, a four- to five-cell-thick epidermal layer was present, no white cell infiltration was evident, fibroblasts had penetrated into the dermal analogue, and recipient collagen was present. A few hair follicles, which apparently originated from the autograft, were evident in the Integra layer at 21 days PG. Histologic examination of tissue samples harvested from wounds covered with SN, BBT, or
VGT at the 21st day postgrafting revealed that the areas of granulation tissue were devoid of epidermis and were infiltrated with inflammatory cells.

At 3 months postgrafting, a few hair follicles had grown into the dermal analogue and had matured in all experimental groups (Fig. 2B and Fig. 4B). By that time, 50% of the dermal analogue layer was replaced by recipient collagen, and the grafted wound had the gross appearance of normal skin. Fibrotic tissue infiltration was not obvious in the SNDC group, and only mildly in the other groups, with no definite layer of subepidermal fibrosis evident. The tissue sections of the grafts revealed essentially normal skin morphology.

Two-Step Grafting

The procedure included full thickness skin excision of 4 × 4 cm area of the dorsum and placement of Integra in the first step and removal of silicon layer and application of meshed split thickness autograft in the second step. Following full thickness excision, the remaining skin area of the dorsal trunk was not large enough to be used for autograft harvesting; therefore, the autograft was harvested from the abdominal area. Due to the limited area of the abdominal skin and the need to avoid excessive tension on the sutures after donor wound closure, the size of the excised full thickness wound on the first step was limited to 4 × 4 cm, rather than 4 × 6 cm as done in the one-step operative procedure.

Following grafting, the silicon layer of the 1:1.5 meshed Integra could be easily detached at 14 days. The collagen layer of the Integra exhibited mature granulation tissue formation with a small amount of exudation (Fig. 5A). With the 1:1.5 meshed autograft applied in the second step of the procedure, the epithelialization rate exceeded 95% of total wound area on or about day 14 following autograft application if graft was treated with direct current, and at approximately 21 days postgrafting when SN, BBT, and VGT dressings were applied (Table 2). At 3 months PG, the sizes of the wounds were 83.3%, 80.6%, 73.6%, and 68.1% of the original graft size for the SNDC, SN, VGT, and BBT dressing groups, respectively, and wound contraction was significantly less in wounds covered with silver-nylon (p < 0.05) (Table 1 and Fig. 3A). Hair growth was denser if the grafts were treated with direct current, although the hair was unevenly distributed (Fig. 3A).

Microscopic evaluation demonstrated that vascularization of the Integra began at the lower surface with unevenly distributed ink-filled vessels registered in all of the examined animals of the SNDC group on day 4 following application of the Integra and on day 7 in the SN, BBT, and VG groups. At 14 days following application, the Integra was infiltrated with numerous dilated capillaries, white cells, fibroblasts, and collagen as shown in Figure 5B and C. Following application of the autograft, ink-filled vessels were noticed in the autograft on the fifth day after grafting in all animals of the SNDC group and on the seventh day in the SN, BBT, and VGT groups. At 3 months PG, the inflammatory process in the
Integra layer progressively decreased, fibroblast infiltration and fibrotic tissue formation occurred in both layers of the grafts but were greater in the Integra-derived dermal analogue, and recipient collagen deposition was noted throughout the dermal layer (Fig. 3).

DISCUSSION

Composite skin grafts are being developed to facilitate and improve burn wound coverage in patients with extensive burns and few donor sites. The collagen-containing dermal analogue may increase autograft take, accelerate maturation of the healing wound, and improve the function and appearance of the engrafted tissue. However, several limitations have been experienced with this approach. Use of composite skin grafts typically requires two separate procedures with an interval of 14 days between the two operations to allow for vascularization of the dermal layer.2-5 Until dermal substitutes are well vascularized, the composite skin grafts are more susceptible to microbial contamination and resultant graft failure. We have previously reported an experimental one-step meshed composite skin grafting procedure in which the allogeneic dermis not only can persist and survive on the excised wound bed, but can also support a meshed epidermal autograft and closure of the wound.3,10,15 However, human allografts are not always available and carry a risk of infection. The collagen-containing dermal analogue Integra, which is readily available, appears to be a reasonable substitute for the allogdenal component in our experimental MCSG.

As recommended by the manufacturer, the use of Integra for burn wound closure entailed a two-step operative procedure. In the study reported here, we show that a one-step procedure can successfully replace the two-step procedure. As compared with the two-step procedure, significant advantages of the one-step procedure include the following:

1. Earlier wound closure: When 1:1.5 mesh autografts were used in the one-step operative procedure, MCSG completely epithelialized within 13 to 29 days compared with 28 to 35 days following the two-step procedure.

2. Reduced wound contraction: When 1:1.5 mesh autografts were used in the two-step operative procedure, MCSG demonstrated mild contraction (68.1 to 83.3% of the original wound size) 3 months postgrafting compared with 81.9 to 100.8% if the grafts were applied in one step. In general, the wounds of the animals in the one-step procedure groups healed with better texture, and showed more hair growth.

3. Conservation of donor sites: The use of a 1:6 mesh ratio was associated with failure to heal by 3 months after grafting if the two-step grafting was employed. The one-step procedure permits use of meshed autografts with a sixfold expansion ratio.

4. Reduced risk of microbial contamination: The 14 days, which were required for Integra vascularization between the two grafting stages of the two-step procedure, were eliminated. Consequently, chance of microbial contamination of Integra-covered wounds associated with long-term wound exposure without epithelial coverage was reduced.

5. Enhanced vascularization of the autograft skin: Earlier application of meshed autograft over meshed Integra permitted the autograft to establish direct contact with the excised wound bed immediately after grafting, which allowed earlier revascularization of the autograft. This may be the key factor responsible for the improved healing noted with the one-step operative procedure as compared with the two-step operative procedure.

We used four wound dressings to verify the advantage of one-step vs. two-step grafting. Silver-nylon with direct current application proved to be the best wound dressing in this study. The silver-impregnated nylon fabric is sufficiently porous to...
permit drainage of exudate and transudate and the silver component serves as an antimicrobial barrier.\textsuperscript{13} We also demonstrated earlier that application of direct current accelerates graft revascularization and wound reepithelialization.\textsuperscript{10,14} In this study, the use of silver-nylon reduced wound contraction and accelerated wound reepithelialization when compared with traditional wound dressings (BioBrane, or Vaseline gauze).

**CONCLUSION**

A one-step application of a MCSG using the dermal analogue of Integra and a meshed split thickness autograft provided effective coverage of full thickness excised wounds. When compared with a two-step operative procedure, the one-step grafting was superior in terms of time of wound closure, postclosure wound contraction, and conservation of donor sites. These studies provide the basis for clinical evaluation of the one-step procedure in the treatment of full thickness burns.

**ACKNOWLEDGMENTS**

We wish to thank Mrs. J. Jamison, BS, Mr. A. Reyes, BS, and Mr. R. Coyle, BS, for their technical assistance.

**REFERENCES**