Objectives: To develop an animal model to investigate the survival of split-thickness skin grafts (STSGs) on bone without periosteum, to compare STSG attachment to bone with and without periosteum, and to determine the effect of fibrin glue on STSG attachment to bone.

Design: Prospective laboratory study.

Setting: University laboratory.

Subjects: Sprague-Dawley rats.

Main Outcome Measure: Percentage of survival of the STSGs at 2 weeks determined independently by the authors and a third, blinded head and neck surgeon.

Results: In experiment 1, which included 40 rats, the sutured STSGs showed an average survival rate of 38% when attached to bone with periosteum, 6% when attached to bare bone, and 10% when attached to bare bone using fibrin glue. The poor survival rate was thought to be attributable to the animals scratching at their bolster dressings. In experiment 2, 18 animals underwent a posteriorly based U-shaped flap of skin and subcutaneous tissue. The grafts were placed and isolated from the overlying flap with a biosynthetic wound dressing. The sutured STSG survival rates were as follows: 87% when attached to bone with periosteum, 94% when attached to bare bone, and 74% when attached to bare bone using fibrin glue.

Conclusions: The survival of STSGs attached to bare bone was comparable to that of STSGs attached to bone with periosteum when grafts were protected with the skin-subcutaneous flap. The STSGs that were fixed with 0.1 cc of fibrin glue demonstrated poorer survival rates than those attached with sutures and were associated with more seromas.


POORLY VASCULARIZED TISSUES, including exposed cortical bone, cartilage without perichondrium, and fascia, are considered by most authors to be poor recipients of skin grafts.1-4 A study by Stallings et al5 of skin grafting over bare bone in rabbits showed only a 22% graft take compared with 77% for skin grafting over periosteum. Nevertheless, skin grafts have been applied to head and neck soft tissue defects with bare bone in a variety of situations: coverage of canal wall or mastoid bone in otologic surgery,6,7 lining of the orbital wall after exenteration,8,9 repair of total scalp defects with exposed calvarium,10,11 and reconstruction of composite resection defects with exposed mandible.12 However, some authors advocate delayed skin grafting in such situations to allow the development of a vascularized recipient site with granulation tissue.7,11,12

Clinical evidence suggests that skin grafting on bare bone is feasible, although many surgeons remain skeptical. Such a technique is important in head and neck reconstruction (1) to facilitate more rapid wound healing and to decrease the incidence of infection in the case of an open defect; (2) to provide coverage of large defects that cannot be closed primarily, especially while awaiting oncologic margins; (3) to allow surveillance in defects with a high propensity for recurrence; and (4) to eliminate the need for additional procedures and/or dressing changes while awaiting the development of a granulation bed.

In the present study, we applied a rat model to investigate split-thickness skin graft (STSG) attachment to bone lacking periosteum and the effects of fibrin glue on STSG survival.

METHODS

The study design and procedures were approved by and performed in accordance with guidelines of the Animal Care and Use Committee of Stanford University, Stanford, Calif.
In experiment 1, a total of 40 male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Calif) weighing 250 to 300 g were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (3.0 mg/kg), administered intraperitoneally. The injections were repeated as needed. The dorsal hair and the scalp hair were shaved with an electric trimmer, followed by the application of depilatory cream to remove any remaining hair. The skin was cleaned with a povidone-iodine solution. Next, mineral oil was applied and the skin graft was harvested from the dorsum using a miniature skin graft knife with the guide set at the second setting, yielding a graft with an approximate thickness of 0.46 to 0.51 mm (Figure 1). The donor sites were dressed with gauze permeated with 3% bismuth tribromophenate in a petrolatum blend (Xeroform; Sherwood Medical, St Louis, Mo), and the STSG was placed into sterile saline.

The scalp skin was then removed with scissors, creating a 1.0-cm defect lined by periosteum and flanked by the temporalis muscles. The STSGs were trimmed to fit the round 1.0-cm scalp defects and sutured in place with a 5-0 nylon suture in 10 of the animals (Figure 2A). In 22 animals, the periosteal layer was removed with a periosteal elevator before placement of the STSG. In 10 of the animals with defects lacking perios- teum, the STSG was fixed to the defect with 0.1 cc of fibrin glue (Tisseel; Immuno AG, Vienna, Austria), while the STSG was sutured in place in the remaining 12 animals. Bolsters consisting of permeated gauze dressing and cotton were tied over the graft sites (Figure 2B). The animals were then examined at day 14 and graded on the percentage of graft survival (0%-100%) by 2 of us (L.E.T. and W.E.F.) and an independent observer, who was blinded to the treatment group (Figure 2C). The averages were then calculated for each treatment group. Eight animals died shortly after the anesthesia was administered, presumably from anesthetic complications.

In experiment 2, the study design was modified according to methods described by Wang et al14 to prevent the animals from disrupting the STSG sites. A posteriorly based U-shaped scalp flap was created by elevating the skin and subcutaneous tissue off the underlying periosteum and muscle, which (1) acted as a biologic dressing for the recipient graft sites, (2) prevented the animals from tampering with the STSGs, and (3) eliminated the need for bolster dressings. In 6 animals, the harvested STSG was placed over periosteum and secured with nylon suture (Figure 3A). Next, a piece of biosynthetic wound dressing (Biobrane; Bertek Pharmaceuticals Inc, Sugar Land, Tex) was placed over the graft to separate the environment of the STSG from that of the skin flap, thus eliminating any potential contribution to graft survival from the skin flap. Finally, the skin flap was then placed back in neutral position and sutured with 5-0 polybutester (Novafil; Davis & Geck, Danbury, Conn). In 12 more animals, the periosteum was removed with a periosteal elevator before placement of the STSG. In 5 of the 12 animals, fibrin glue was used to fix the graft to the bare bone. All animals were killed on day 14. The skin flap and the biosynthetic wound dressing were elevated, and STSG survival was assessed (Figure 3B). Two animals in experiment 2 had postoperative wound infection and were killed on day 1. The skin and underlying bone were excised and fixed in 10% neutral buffered formalin. After decalcification, the tissues were serially sectioned, routinely processed, and embedded in paraffin wax. Five-micron sections were prepared and stained with hematoxylin-eosin, Masson trichrome, and elastic von Gieson. The slides were reviewed in a blinded...
fashion by 2 of us (G.J.B. and W.E.F.) to assess the quality of the graft, thickness of the periosteal layer, presence of granulation tissue, inflammation, and vascularity.

RESULTS

In experiment 1 (Table 1), in the STSG-periosteum group (n = 10), the average STSG survival rate was 38% as graded by the 2 surgical investigators and 32% as graded by the independent investigator. In the STSG–bare bone group (n = 12), all 3 investigators found the graft survival rate to be 6%. In the animals in which the STSG was fixed to bare bone with fibrin glue, the average survival rate of the STSG was 10% as graded by the surgical investigators and 9% as graded by the independent investigator. The glue caused a local inflammatory reaction with frequent seroma formation under the skin graft. Bolsters did not stay in place longer than 2 days after graft placement owing to scratching by the animals.

In experiment 2 (Table 2), the STSGs attached to bare bone resulted in the highest average graft survival rate (94%) compared with those attached to bone with periosteum (89%) and those fixed to bone with fibrin glue (73%).

A total of 19 animals were submitted for histopathologic examination. Accurate classification of the specimens into 2 groups was possible in all cases: group A comprised STSGs attached to periosteal-lined bone (n = 5) and group B comprised STSGs attached to bare bone (n = 14). In group A, the periosteal layer was found between the cortical bone and spindled fibrous tissue in the deep dermis and/or subcutis (Figure 4A and B). The periosteal layer ranged from 0.05 to 0.11 mm in thickness and was composed of scattered stellate and spindled fibroblastic cells embedded in a loose, edematous stroma. Rare histiocytic cells, mast cells, and lymphocytes, as well as small arterioles and arteries, were also found in the periosteal layer. In group B, the deep dermis and/or subcutis contained a thick layer of spindled myofibroblastic cells and chronic inflammatory cells embedded in mature collagenous tissue stroma that abutted the cortical layer of bone (Figure 4, C and D). The vasculature consisted of a mosaic proliferation of capillaries and arterioles. Small arteries were found in the deep dermis, near the interface with the myofibroblastic layer. In some cases, microscopic foci of new lamellar bone were noted at the interface of bone and fibrous tissue.

Table 1. Experiment 1

<table>
<thead>
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<th>Variable</th>
<th>% Graft Survival as Graded by Surgical Investigators</th>
<th>% Graft Survival as Graded by Independent Investigator</th>
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<tbody>
<tr>
<td>STSG-periosteum (n = 10)</td>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td>STSG-bone (n = 12)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>STSG-bone, fibrin glue (n = 8)</td>
<td>10</td>
<td>9</td>
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Abbreviation: STSG, split-thickness skin graft.

Table 2. Experiment 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>% Graft Survival as Graded by Surgical Investigators</th>
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<tbody>
<tr>
<td>STSG-periosteum (n = 6)</td>
<td>89</td>
</tr>
<tr>
<td>STSG-bone (n = 7)</td>
<td>94</td>
</tr>
<tr>
<td>STSG-bone, fibrin glue (n = 5)</td>
<td>73</td>
</tr>
</tbody>
</table>

Abbreviation: STSG, split-thickness skin graft.

COMMENT

Skin grafts are considered free tissue transfers and thus are entirely dependent on the recipient wound bed for survival. Thiersch13 first described the stages of wound healing after skin transplantation in 1874, and Converse and Ballantyne16 described the plasmatic circulation of skin grafts in 1937. Initially during the stage of imbibition in the first 24 to 48 hours, the skin graft receives nutrients and oxygen via diffusion and absorbs a serulike transudate from recipient site vessels. Beginning on day 2 after transplantation, inosculation occurs when anastomoses form between the graft and host vasculature. Finally, there is an ingrowth of host vessels into

Figure 3. Experiment 2. Split-thickness skin graft attached to bone with overlying skin flap (A) and attached to bare bone 2 weeks after surgery, showing 100% take (B).

Figure 4. Split-thickness skin graft attached to periosteal-lined bone (A) and to bare bone (B).
the graft during the process of neovascularization, with a well-established blood supply by days 5 through 7.

Historically, skin graft survival on bone lacking periosteum has been considered poor. One of the few studies to show that STSGs can attach to bare bone was performed by Stallings et al., who found a 22% average graft attachment to bare bone compared with a 77% attachment on bone with an intact periosteum.

In experiment 1 of our study, an animal model was developed in which we found a higher percentage of graft attachment to bone with periosteum (38%) compared with attachment to bare bone (6%). The hemostatic and adhesive properties of fibrin glue have been well recognized, and the use of fibrin glue has been shown to increase skin graft survival in infected wound beds. In cases in which fibrin glue was applied to bone without periosteum before placement of the STSG, there was no significant increase in the percentage of graft survival (10%). The bolsters were found to have fallen off the animals by day 2 after grafting. In the second part of this study, a posteriorly based skin and subcutaneous tissue flap modeled after Wang et al. was used as a biologic dressing for the skin graft recipient site. This flap prevented the animals from scratching at the grafts and eliminated the need for bolster dressings. Also, a biosynthetic wound dressing was placed between the skin graft and the flap to create an occlusive environment and to prevent the possibility of nutrients from the flap contributing to graft survival. However, we do recognize that placement of the STSG under a tissue flap does not mimic the typical clinical skin graft recipient bed because it provides a moist and warm environment as well as additional protection against sheer forces that may prevent graft adherence.

In experiment 2, the percentages of STSG attachment to bone with and without periosteum were comparable, with 89% for the former and 94% for the latter. On histopathologic examination, host vessel ingrowth was demonstrated for STSGs applied to periosteal-lined bone as well as to bare bone. Again, the application of fibrin glue did not improve graft survival and was found to result in seroma formation.

In conclusion, the Sprague-Dawley rat was found to be a reliable animal model for the study of STSG attachment to bone without periosteum. Split-thickness skin graft survival on bone with and without periosteum was found to be similar. Finally, the application of fibrin glue resulted in poorer graft survival on bare bone.

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REFERENCES


Announcement

New Online Submission and Peer Review System. The Archives of Otolaryngology—Head & Neck Surgery editorial office is now using an online manuscript submission and peer review system developed by ejournalPress that serves the needs of authors, reviewers, and editors. See http://www.archoto.com for more detailed information.