Short- and long-term outcomes of small auto- and cryopreserved allograft skin grafting in those with >60%TBSA deep burn wounds

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ABSTRACT

Background: The shortage of autologous skin sources not only adds difficulty to the repair of extremely large-area deep burn wounds but affects the healing quality. The aim of the present study is to explore an ideal method for repairing large-areas burn wounds with low scar formation.

Methods: Between 2002 and 2014, we used grafting of small auto- and cryopreserved allo-skin to repair large-area residual burn wounds in wounds after 21 days 21 patients, and after early excision in 17 patients. The wound healing rate and quality were observed.

Results: The skin expansion rate was 1.9–1.16, and the mean area of wounds repaired after three weeks was 64.8 ± 7.3%TBSA, the wound healing rate was 91.8 ± 3.7%. The mean area of the early excision group was 65.9 ± 9.8 TBSA, where the healing rate was 94.5 ± 5.6%. After small auto- and cryopreserved allograft skin grafting, the epithelium of the auto-skin gradually replaced the allo-epidermis, and the allo-dermis persisted for a prolonged period. The dermal collagen fibers at the allo-skin grafting sites were well arranged. At 1–2-year follow-up, observation showed that the Vancouver Scar Scale total score was 4.304 ± 2.363, and we did not discern significant contracture and dysfunction in the large joints of the four extremities.

Conclusions: Small auto- and cryopreserved allograft skin grafting of small auto- and allo-skin not only raised the graft expansion rate but offers a stable wound healing rate. This new technique may provide an option for repair of large-area deep burn wounds.

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1. Introduction

Treatment of extremely large-area deep burn remains a clinical challenge. On the one hand, the survival rate is relatively low: 84–87.1% for 60–69%TBSA burns and 45–47.8% for more than 90%TBSA burns [1,2]. On the other hand, scar proliferation or even contracture and distortion seriously affect the appearance and function [3,4].

The shortage of autograft skin sources is the main important factor affecting the successful treatment and healing quality of critical burn patients [5]. Meek skin grafting,
microparticle skin grafting and cultured autologous epidermis grafting are among the conventional methods for quick repair of large-area burn wounds. However, the expansion rate of Meek skin grafting is limited (4–9 fold) [6]. Although the expansion rate of microparticle skin grafting is as high as 10–20 fold, graft survival rate is only 51.1% because it is largely affected by allo-skin viability, homogeneity and direction of microparticle skin distribution [7]. In vitro culture of autologous keratinocytes requires a long period for expansion and successful survival of the graft is unstable, fluctuating between 15% and 85% [8,9]. In addition, there are relatively large exposed wound areas between stamp-like skin or particles in Meek and microparticle skin grafting, and their coverage largely depends on epidermal cell migration, which is likely to cause cord-like scar formation. As cultured autologous keratinocytes grafting lacks the dermis, repeated erosion, scar proliferation and contracture after wound healing may seriously affect the patient’s appearance and function [9]. Some researchers attempted to first transplant the dermal substitute to deep burn wounds followed overlapping transplantation of stamp-like skin or cultured autologous epidermis. However, this procedure has to be completed in two steps, thus prolonging the time of wound repair. In addition, large-area burn patients usually have poor general conditions and low immunity, infection of the dermal substitute is likely to occur, which is also an important reason for the low success rate of grafting [10,11].

The present study reports our experience with small auto- and cryopreserved allograft skin grafting for the treatment of large-area and deep burn wounds. Our 12-year clinical observations demonstrated that the autograft expansion of this mixed grafting technique is as high as 9–16 fold, and the graft successful survival rate is more than 90%. In addition, persistence of the al-dermis as the dermal substitute may reduce scar formation. This technique provides an ideal approach to the repair of large-area deep burn wounds.

2. Methods

2.1. Patient

This study was carried out in the Burn Center of Changhai Hospital, a Burn Emergency Center in Shanghai. Thirty-eight patients with severe burns between 2002 and 2014 were reviewed retrospectively, including 21 with wounds older than 21 days from injury, and 17 patients with large-area wounds excised early.

Of the 21 patients with older wounds grafted at least 15 days from injury, 14 patients had wound eschar and the remaining 7 patients experienced skin grafting failure. The male/female ratio of the 21 patients was 15:6, with a mean age of 32.9 ± 5.1 years, a mean burn area of 84.9 ± 6.3%TBSA, and a mean residual wound area of 64.8 ± 7.3%TBSA at 20–35 days after burn. Of them, two patients had hypertension, one patient had diabetes, and one patient had obesity.

The male/female ratio of the 17 patients with early excision wounds was 13:4, with a mean age of 38.4 ± 8.5 years, a mean burn area of 89.4 ± 9.6%TBSA, and a mean wound area of 65.9 ± 9.8% TBSA in early excision at 3–15 days after burn. Of them, three patients were hypertensive, two patients were diabetic, and one patient was obese.

No patient was associated with other skin diseases, immunocompromised or immune defect and other underlying diseases as well as a medication history of hormones and immunosuppressants.

2.2. Wound preparation

For those with large-area wounds grafted at least 21 days from injury, multiple dressing changes were performed before surgery to remove necrotic tissues and control wound infections. Thorough wound debridement was performed during surgery. For wounds with eschar, this was removed together with the infected and necrotic subcutaneous tissue to the fascia; for wounds with granulation tissues, the superficial granulation tissues were excised deep to the fibrous plate. After complete hemostasis, the wounds were irrigated with normal saline (NS), hydrogen peroxide (HPO) and NS again for skin grafting preparation.

For those with full thickness burn wounds in the early stage of treatment, eschar excision was performed to the deep fascia and the wounds were irrigated with NS, HPO and NS again for skin grafting preparation.

2.3. Mixed grafting of auto-skin and cryopreserved allo-skin

The allograft skin was obtained from patients who died from traumatic injuries, cardiac arrest and other accidents after obtaining informed consent from the families or guardians and excluding malignant tumors, hepatitis B and C, syphilis, HIV and other infectious diseases. It was prepared into a 0.3–0.5 mm split-thickness skin, and cryopreserved in PBS containing 10% DMSO in liquid nitrogen for two months to one year. The histological structure of the cryopreserved allo-skin was observed by routine HE staining of the sections, and the viability was observed by CCK-8. The allograft skin was thawed in 42°C water bath, and prepared into 0.5 cm × 0.5 cm or 0.8 cm × 0.8 cm skin pieces using the Tanner-Vandeput Mesh Dermatome.

The patient was anesthetized, and the donor skin area was sterilized routinely to obtain a 0.3–0.5 mm split-thickness skin and prepare it into 0.5 cm × 0.5 cm in size for use. First, the small autograft skin was placed onto the wounds, with a 1–1.5 cm intervals, and then the small allograft skin was evenly grafted, allowing a 0.25–0.5 cm distance between the skin. The autoskin expansion rate was 1:9–1:16. The grafted skin was covered with fine-meshed gauze and dressed routinely.

2.4. Postoperative treatment

The dressing was opened at day 3 after operation, the inner layer dressing did not need to be changed and no antibiotics were used if there was no evidence of exudation in the inner layer of the gauze or sign of infection in the wound. In such cases, the wound was re-dressed with dry gauze, and the external dressing was changed every three days as long as it was kept dry. The inner-layer gauze was removed about 2
Fig. 1 – Autograft skin (0.5 cm × 0.5 cm) and allograft skin (0.8 cm × 0.8 cm) grafting. (A) During surgery. (B) Expansion of the box in Figure A, where the smaller skin (triangle) was the autograft skin and the larger one (star) was the allograft skin. (C) Dressing change 3 days after surgery.

Fig. 2 – Gross observation after operation. (A) On day 7 after-operation. (C) On day 14. (B) Expansion of the box in Figure C, the dermis of the small alloskin (star) was macroscopically visible.
Fig. 3 – Histologic HE staining showing the healing process. (A and B) Respective HE staining on day 10 and 21 after grafting, inside the two dotted lines were the small autograft skin (triangle) and small autograft skin pieces (star). (A) The auto-epidermis began proliferating, and gradually migrated to the allo-skin to cover the wound and partially replaced the allo-epidermis (solid-line arrow), and the remaining allo-epidermis was securely attached without vesicular formation or epidermal detachment seen (dotted-line arrow). (B) The auto-epidermis migrated to the allo-dermis and covers it completely 21 days after grafting, and the wound healed well.

weeks after operation if the grafted skin pieces grown to confluence.

In cases where local hematocele or secretion was observed, the inner-layer gauze was removed with caution and the wound was covered with sterile gauze impregnated with chlorhexidine or 5% mafenide solution, and dressed. No immunosuppressant or other anti-rejection medication was necessary during the course of treatment.

2.5. Calculation of the wound healing rate

Two weeks after small auto- and cryopreserved allograft skin grafting, the inner layer of fine-meshed gauze was removed to observe the wound healing rate. The wound healing rate = (graft area - no epithelization area)/graft area x 100%, where the area was calculated and expressed as the percentage of TBSA.

2.6. Observation of the wound healing process

Wound healing of the mixed grafting process was observed grossly. Upon informed consent from the patients, a small specimen was excised for observation of the histological structure. Part of the allo-skin carried tattoo in five patients who received mixed skin grafting, in whom the histological sections underwent routine HE staining before grafting and at 2 months or 2 years after grafting to observe the histological structure of the skin involving tattoo particles. Observation of the collagen matrix by Masson trichrome staining: The paraffin-embedded sections were de-waxed, hematoxylin stained, Masson trichrome stained, treated with phosphotungstic acid solution, re-stained with aniline blue liquid, dehydrated, hyalined and mounted.

Observation of collagen IV (Col IV), laminin (LN) and inflammatory reaction by immunohistochemical staining: The paraffin-embedded sections were de-waxed routinely, rinsed and sealed by addition of 3% BSA. After addition of antibody Col IV (1:100, Bioworld) and LN (1:200, Abcam), the sections were incubated at 4°C overnight, added with corresponding FITC fluorescence-incorporated IgG (1:300, Santa Cruz), and incubated at 37°C for 30 min. For observation of the inflammatory reaction, the sections were added with primary antibody CD11b (1:400, Abcam), CD4 (1:200, Abcam) and CD8 (1:200, Santa Cruz) and incubated at 4°C overnight, followed by addition of corresponding HRP-incorporated secondary antibody.

2.7. Scar assessment of functional and cosmetic outcomes

The patients were followed for 1–2 years to observe scar formation and function by an occupational therapist with the Vancouver Scar Scale (VSS). In VSS, pigmentation (0–2), pliability (0–5), scar height (0–3), and vascularity (0–5) are rated, the total score ranges from 0 to 15. The higher the score, the worse the scar [12,13].
2.8. Statistical analysis

All data were statistically treated using SPSS16.0 and expressed as mean ± standard deviation (SD).

3. Results

3.1. The histological structure and activity of the cryopreserved allo-skin

The morphological structure of the cryopreserved allo-skin was intact. There was no detachment between the epidermis and the dermis. No vacuolar formation was observed in epithelial cells. The skin activity was 50 ± 4%.

3.2. The outcome of the residual wound repair during the middle and late burn stages

Skin grafting for the later wounds was performed 22–42 days after burn. Of the 21 patients, 13 patients received 2 episodes of small auto- and cryopreserved allograft skin grafting, and eight patients received 3 episodes of small auto- and cryopreserved allograft skin grafting. The maximum graft area at a single operation was 36% TBSA, the maximum accumulative grafting area was 74% TBSA and the minimum...
was 55% TBSA, with a mean grafting area of 64.8 ± 7.3%TBSA. The wound healing rate was 91.8 ± 3.7%.

3.3. The outcome of wound repair during early escharectomy

In the 17 patients who received auto- and allo-skin mixed grafting after escharectomy at day 3–15 after burn, 9 patients received 2 episodes of small auto- and cryopreserved allograft skin grafting, and 8 patients received 3 episodes of small auto- and cryopreserved allograft skin grafting. The maximum graft area at a single operation was 41%TBSA, the maximum accumulative grafting area was 78% TBSA and the minimum was 52% TBSA with a mean grafting area of 65.9 ± 9.8%TBSA. The wound healing rate was 94.5 ± 5.6%.

3.4. The healing process of mixed grafting

Figs. 1–5 indicate the healing process of small auto- and cryopreserved allograft skin grafting in the right lower extremity. Fig. 1A and B indicate the situation during the procedure, where the auto-skin space was 1–1.3 cm and the expansion rate was 1:12. Skin attachment was good 3 days after operation, and vascularization was seen in part of the auto-skin (Fig. 1C). At day 7–10 after operation, the auto-epidermis began expanding in all directions, and the allo-skin remained intact without excoriation (Fig. 2A). At 2–3 week after operation, the auto-epidermis continued expanding, and finally fused together and replaced the allograft epidermis, while the allograft dermis was still macroscopically visible 2–4 weeks after grafting, the epithelium of the small exfoliated allograft skin was adhered with the inner-layer fine-meshed gauze or skin surface (Fig. 2B and C).

Fig. 3 is the histologic HE staining showing the process of how the autograft epidermis proliferated, migrated to the allo-skin, gradually covered the wound and finally replaced the allo-epidermis. Three weeks after grafting, continuous and wave-like staining of Col IV and LN was seen at the junction of the epidermis and dermis of autograft and allograft skin mixed grafting, while the staining between them was relatively weak and discontinuous (Fig. 4A–C. data of LN staining not shown). There was no evidence of inflammatory reaction at the site of allo-skin grafting (Fig. 4D–F). At 4–5 weeks after grafting, continuous and wave-like Col IV and LN staining was observed in all grafting areas, including the auto- and allograft skin grafting areas and areas between them.

The graft that contained the tattoo was the allograft skin, and at 2 months and 2 years after operation, the tattoo particles were seen inside the dermis, where the collagen fibers were arranged regularly without seeing formation of package structures, The dermal papilla-like structure formed at the junction between the epidermis and the dermis and was similar to the adjacent auto-dermal structure (Fig. 5).
3.5. Appearance and function after mixed grafting

One month after grafting, the healed grafted skin was gridlike and partially pigmented (Fig. 6A and B). After two months, scar formation was gradually seen between the grafted skin pieces with small amounts of vesiculation seen occasionally (Fig. 6C and D). The follow-up observation in 23 patients for 1–2 years after routine anti-scar treatment using elastic sleeves showed that mild scar proliferation was seen at the mixed grafting site, the appearance was smooth and flat, the skin was elastic (Fig. 6E and F). The VSS mean values of pigmentation, pliability, scar height, and vascularity were $1.174 \pm 0.576$, $1.565 \pm 1.273$, and $1.087 \pm 0.288$, $0.435 \pm 0.590$, respectively. The total score was $4.304 \pm 2.263$. No significant contracture and dysfunction were observed at the scarring sites of the knee, elbow and other large joints of the four extremities in 21 patients. Mild joint contracture was observed in the remaining two patients, whose articular function was reconstructed using simple plastic surgery.

4. Discussion

4.1. Mixed grafting expands the utilization ratio of autoskin and shortens the wound healing time significantly.

By using small auto- and cryopreserved allograft skin grafting, we expanded the autograft skin ratio to 1:9–1:16, which is similar to that of microskin grafting and higher than that of Meek grafting (1:4–1:9), thus greatly raising the utilization rate of autogenous skin. When the interval between the autoskin was 1 cm, the ratio of auto- and allo-skin was 1:3 and the expansion rate was 1:9 (Fig. 7B), thus making it possible to cover most wounds. As the space between the small skin is as short as 0.25 cm, the time for epidermis creeping and fusion was shortened. As a result, the wound could generally heal within 2–3 weeks. Without allo-skin, auto-skin grafting alone could only cover about 10% of the wounds (Fig 7A). In addition, the interval between these auto-skin was 1 cm, which is 4 fold as large as that in mixed skin grafting. Therefore, it would take
about 3–4 weeks for the epidermis to creep and fuse together, which is likely to cause local infection and necrosis of the small skin. Therefore, mixed grafting not only expands the rate of auto-skin but reduces the proportion of wound exposure and shortens the time of wound healing significantly.

In cases where the donor site is extremely limited, the expansion rate can reach 1:16 when the interval between the autoskin is expanded to 1.5 cm, and after the allo-skin is grafted between the auto-skin, the final interval between the small skin is 0.25–0.5 cm (Fig. 7D) As shown in Fig. 7C, the currently common clinical practice of simple auto-skin grafting without allo-skin usually uses the expansion rate of 1:4 and in such a case the interval between the skin is about 0.5 cm, indicating the expansion rate of mixed grafting is 4 fold as large as that of simple autoskin grafting under the same conditions, which is beneficial to making full use of the limited autoskin source to repair large-area wound.

It was found in our study that small auto- and cryopreserved allograft skin grafting offered a high graft survival rate and could be used not only for early escharectomy wound repair but for middle- and late-stage residual wound repair, thus maximally avoiding the unfavorable effects of surgical failure on severely burned patients. This may be attributed to small intervals between the grafts, small exposed wound areas and low risk of wound infection. In addition, in our practice of mixed grafting, we used fine-meshed gauze as the inner-layer dressing, which not only facilitates the small skin fixation but benefits the patency of wound drainage and prevention of local infection. The conventional grafting is unable to assure a stable survival of the graft skin, especially in middle-late stage residual wounds with bacterial colonization or infection [14,15]. Studies have shown that the large-sheet allograft skin has to be covered in micro-skin grafting, and a special biological membrane has to be used to cover the wound in Meek grafting, both of which may cause dissemination of the infection, especially in micro-skin grafting. In case wound infection occurs, the large-sheet allograft skin has to be removed, thus reducing the success rate of auto-skin grafting, or even causing sepsis and threatening life in severe cases [7].

**4.2. Long-standing existence of the dermal components after allo-skin grafting attenuates scar formation.**

No epidermal rejection was observed in the early stage of mixed grafting when vascularization of the allo-skin occurred. From 2 to 4 weeks after mixed grafting, the epidermal layer of the...
allo-skin was gradually covered and replaced by the auto-skin. However, the allo-dermis was still clearly visible on gross observation. With the lapse of time, the margin between the allo- and auto-dermis became vague and macroscopically invisible. As there is a lack of specific markers to identify the allo-dermis at present, it is difficult to trace the development and regression of the allo-dermis accurately. It was found in our study that after allo-skin grafting containing tattoo particles, they existed there for a long time. In addition, the dermal collagen fibers at the corresponding sites were well arranged and the histological structure remained intact. One year to 2 years follow-up observation in part of the patients showed that scar formation was mild and functional recovery was good, indicating that the existence of allo-dermis plays an important role in improving the quality of wound healing. Other studies have demonstrated that the auto-skin can produce a "skin island effect" to induce local immune tolerance, thus preventing acute rejection arising from allo-skin grafting [16,17]. Studies also found that collagen as the main component of the dermis could be long retained without being rejected by the body due to its weak antigenicity [18,19]. The long-existing allo-dermis can be used as a template to induce the formation of new dermis, which to some extent works as the dermal substitute, thus attenuating scar formation.

Our retrospective study corresponds to a low-level-of-evidence study. However, several difficulties impair the design of more strong-evidenced studies: small number of patient suffering extensive burns, great variability of protocols between burn centers, which make difficult prospective, controlled, multicentric studies [9]. In summary, small autograft and cryopreserved allograft skin mixed grafting significantly expands the autograft skin utilization ratio and offers a high graft survival rate. In addition, persistence of the allograft dermis as the dermal substitute may reduce scar formation. This technique provides an alternative option for quick repair of large-area deep burn wounds.

Conflict of interest statement

There are no competing interests or conflicts in the present paper.

Acknowledgments

This project was supported by the National Natural Science Foundation of China (81071555, 81120108015, 81372058, 81501665, 81571897), National Basic Research Program of China (973 Program, 2012CB518100), Shanghai Youth Sailing Program (14YF140550), Outstanding Young Scholars Program of Second Military Medical University. The authors have no conflicts of interest to disclose, nor financial interests to disclose.

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Please cite this article in press as: Shizhao J, et al. Short- and long-term outcomes of small auto- and cryopreserved allograft skin grafting in those with >60%TBSA deep burn wounds. Burns (2016), http://dx.doi.org/10.1016/j.burns.2016.07.017