Histological and Biomechanical Evaluation of the Preserved Degenerative Dermis in Rat Autologous Skin Transplant Models after a Deep Second Degree Burn

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Summary

To describe the histological and biomechanical changes of the preserved degenerative dermis in rat autologous skin transplant models after a deep second-degree burn. 50 SD rats were divided into 5 groups randomly of 10 rats of each: 7-days group, 9-days group, 14-days group, 21-days group, and 60-days group. Deep second-degree burn wounds were prepared on the back of rats sized 3.5cm×3.5cm. Super tangential excision was performed on the burn wound to preserve the degenerative dermis. Then, autologous epidermis was grafted on the wound. After that, the histological changes of the preserved degenerative dermis tissues and the graft areas were observed by macroscopic, light microscope and electron microscope in the 7, 9, 14, 21, 60 days after the operation. Moreover, the tensile properties of healing deeply burned rat skin were also tested for each group at the same time points mentioned above**.** Results: (1) According to the macroscopic observation, 7 days after the operation, the grafted skin was fused with the area of burn wound; A few hairs were growing out on the skin at the 14th day; the injured skin recovered to normality by the 60th day. (2) Hyaline change occurred in the preserved degenerative dermis tissues based on the observation by light microscope. At the 7th day after operation, the dermis papillae and reticular layer could be discerned; by the 21st day, the thickness, structures and morphology of grafted skin were similar to the normal tissues. (3) 7 days after operation, ballooning changes were observed by the electron microscope in the mitochondria and endoplasmic reticulum of damaged cells and the number of the ribosomes was obviously reduced. The subcellular wound improved continuously and approached normality by the 21st day. (4) 9 days after the operation, the tensible strength and maximal strain of the grafting rat skin approached 70% and 90% of natural skin, respectively. (5) 60 days after the operation, the tensile performance of the healing rat skin recovered to the natural level.

Conclusion: The histological and biomechanical changes of the denatured dermis of a deep second degree burn wound may gradually recover to normality after being covered by autologous skin.

Introduction

Among all injuries, burn injuries have been recognized as a major cause of mortality in the relatively young population. With advances in the treatment of patients with severe burn injury, the mortality associated with burns has dropped dramatically (*John F et al., 2005; Lu K et al., 2004*). However, it is not reflected in the disabilities because of the formation of hypertrophic scar and contracture after healing following dermal destruction and loss (*Wu Z, 2004*). It has been

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indicated that loss of dermis is one of the principal factors that contributes to poor scar outcome after severe burn (*Peng YZ et al., 2005; Huang XY et al., 2001*). Therefore, strategies for dermal preservation are very important to improve the scar quality and new treatments of scar therapy have been as yet inadequate, which also become the hot points in this field.

In this paper, we report our recent experience with the autologous skin transplantation to rat models with a deep second degree burn. This new treatment has allowed us to preserve dermal tissues and achieve good results.

Materials and Methods

Animals

Adult male Sprague-Dawley (SD) rats (n=50, 200~300g, Experimental Animal Center of XiangYa Hospital, Central South University) were housed 6 per cage with free access to food and water, and were kept in a constant environment (22 ± 2°C, 50 ± 5% humidity, 12h light/dark cycle). All experimental animals were overseen and approved by the Animal Care and Use Committee of XiangYa Hospital, Central South University before and during experiments. Where appropriate, rats were anaesthetized with 10% chloral hydrate (0.5ml/100g) and were relieved pain with analgesics—Lappaconitine (10mg/kg) by i.p.injection.

Chemicals and reagents

All chemicals were of analytical reagent grade. Before the experiment, all of the vessels and tips for pipetting were dipped in strong HNO_3 for 24 h and then washed with ultrapure water. The water used was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Preparation of Rat Autologous Skin Transplant Model

For preparation of rat models with burn wounds, the hairs on the back of them were removed. An aluminium cylinder (3.76cm in diameter, 3.78cm in high) was heated to 75°C in the attemperator for 1h and placed onto the right back of rats for 10s to cause deep partial-thickness burn wound sized as 3.5×3.5cm. In order to confirm the degree of burn, the wound tissues were used to prepare pathological sections, which have been examined by special pathologists. There were no epidermis and papillar layer of dermis. Some fibroblasts in the dermis were necrotic. Collagen fibers, the density of which increased, obviously swelled and fused with each other. Some hair follicles atrophied and sphacelous. The normal tissues and muscles could be found under the degenerative dermis. The burn wounds of these rat models were shown to be deep second degree burns (Figure 1).

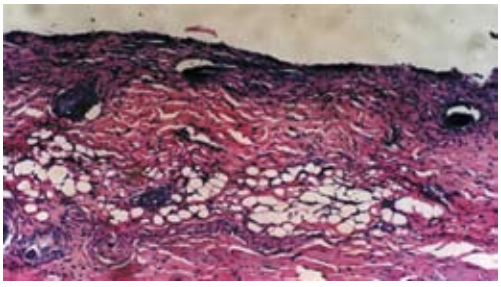

Figure 1. Observation on the tissues of deep second degree burn in rat models. There were no epidermis and papillar layer of dermis. Some fibroblasts in the dermis were necrotic.. Collagen fibers, the density of which increased, obviously swelled and fused with each other. Some of hair follicles atrophied and even sphacelous. The normal tissues and muscles could be found under the degenerative dermis.

During the debridement of the burn wounds in rats, the wound was washed and devitalized epidermis was removed. Super tangential excision was performed on 2~5 postburn days with the preservation of denatured dermis. After the rats were anaesthetized and fixed, the wound was covered with autogeneic epidermis obtained from their necks. The autogeneic epidermis was spread evenly and overlapped 2~3 cm at the junctions of separate pieces. Then it was covered with 3~4 layers gauze and firm bandage.

Finally, a self-stick bandage was applied and exposed to infrared ray for 3~5 days to dry.

Grouping

50 SD rats were divided into 5 groups randomly for 10 rats of each: 7-days group, 9-days group, 14-days group, 21-days group, 60-days group. The histological changes and biomechanical test were performed by the rats in different groups on 7th, 9th, 14th, 21st and 60th days after operation.

Histological changes

The histological changes of the preserved degenerative dermis tissues and the graft areas were observed by macroscopic, light microscope and electron microscope. The skin tissues were removed and fixed in 10% formalin, embedded in paraffin and examined in multiple consequent sections. The histopathologic study was carried out with HE (hematoxylin and eosin) staining and examined by light microscope. To observe the collagen fibers, Van Gieson(VG) staining was used. Additionally, other skin tissues were fixed by 3% glutaraldehyde and 1% osmic acid in order, and stained by uranyl acetate and citric acid. Then, the histopathologic changes in the structure of fibroblasts were observed by electron microscope.

Biomechanical test

The biomechanical testing of the graft epidermis occurred on the 7th, 9th, 14th, 21st and 60th after operation. A servo hydraulic testing machine (Eden Prairie, MN, Figure 2) was used to apply a tensile force to the graft epidermis. Stress was calculated by dividing the force by the tissue cross-sectional area, assuming a rectangular geometry. Strain was estimated as the tissue elongation divided by the initial tissue length. The ultimate strength (an indicator of strength) and elastic modulus (an indicator of stiffness) were obtained from the stress versus strain relationship.

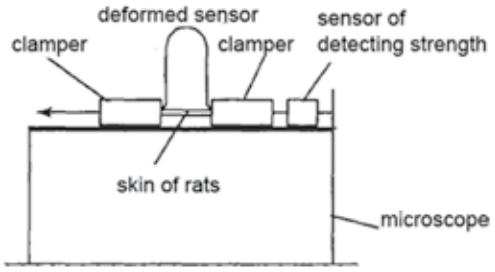

Figure 2. Schematic of the servo hydraulic testing machine used for the biomechanical testing.

Statistical Analysis

SPSS13.0 software for Windows (SPSS Inc, IL, USA) was used for analysis. Continuous variables were expressed as $\overline{X} \pm s$. Statistical analyses were performed by one-way ANOVA with the post-hoc Tukey's test applied for paired comparisons. A difference between means was considered significant if the p value was less than 0.05.

Results

Histological changes

Macroscopic, light microscopy and electron microscopy were used to observe the pathological changes of the graft epidermis at each time point in five different groups.

Macroscopic observation

The graft epidermis was alive and its blood supply had occurred on the 7th day. But it was not easy to isolate the graft epidermis from the wound after dermal sutures were removed until 9 days after transplantation. At the same time, the graft area without scar was redder and stiffer than peripheral skin. A few hairs had grown up slenderly on the graft area from 14 days of observation. By the 60th day, the graft epidermis had recovered to be normal.

Observation by light microscopy

HE staining: From the 9th day on, the graft epidermis became thicker and thicker, the four layers of which (containing horny layer, clear layer, granular layer and germinal layer) could be observed clearly. 14

days after transplantation, keratinized tissues in the epidermis appeared. The dermis also became thicker. The papillar layer and reticular layer could be observed. The hair follicles and sebaceous glands had atrophied. By the 21st day, the graft area had been recovered to normality (Figure 3).

VG staining: the density of collagen fibres had been increasing slowly from 7 days to 21 days after transplantation, with the fibre bundle also becoming thicker continuously. Finally, they fused with each other (Figure 3).

Observation by electron microscope

The cells near the basilar part of graft epidermis were polygonal or irregular shape. Their volumes became bigger with larger mitochondria and ribosomes 7 days after transplantation. The cell nucleus was round or an ellipse, the nucleoli of which could be observed clearly. On the 21st day, the mitochondria, endoplasmic reticulum and ribosome of cells were increased and recovered to be normal.

Biomechanical test

The maximum strain value ε and the tensible strength σ of the graft epidermis increased gradually after transplantation, the values of which at different time points are shown in Table 1. The recovery degree of these two indices at 7th, 9th, 14th, 21st, 60th were 85.71% and 62.40%, 89.29% and 64.73%, 92.86% and 68.99%, 96.43% and 78.68%, 98.21% and 97.67%, respectively (Table 2 and Figure 6).

Discussion

To our knowledge, surgical debridement and skin grafting is the commonest therapy for the deep partial thickness burn wound (*Yang XH et al., 2005*). Many burn surgeons also advocate early excision and grafting for this kind of burn wound

Figure 3. Histological changes of the graft area observed by light microscopy. A is the photograph of 7-days group, the papillar layer and reticular layer were found. The graft epidermis became thicker. The germinal cells were arranged in proper order like pillars. There have been some hair follicles and sebaceous glands. B is the photograph of 14-days group, the four layers (containing horny layer, clear layer, granular layer and germinal layer) and keratosic area of epidermis could be observed clearly. The thickness and shape of them recovered to be normal. The papillar layer and reticular layer became thicker continuously. The hair follicles and sebaceous glands began atrophy. C is the photograph of 21-days group, the graft area has recovered to normality and fibroblasts were well arranged.

Table 1. Maximal strain and tensible strength (MPa) of the graft epidermis in the rats of different groups. $X \pm s$.

Groups	Normal	7-days	9-days	14-days	21-days	60-days
Maximal strain	0.56±0.02	0.48 ± 0.07^{B}	0.50 ± 0.03^{B}	0.52 ± 0.03^{A}	0.54 ± 0.04^{C}	0.55 ± 0.05^{C}
Tensible strength	2.58±0.23	1.61 ± 0.15^{B}	1.67 ± 0.21^{B}	1.78 ± 0.13^{B}	2.03 ± 0.18^{A}	2.52 ± 0.33^{C}

"A" Refers to the comparison with normal skin, $p<0.05$; "B" Refers to the comparison with normal skin, $p<0.01$; "C" Refers to the comparison with normal skin, $p>0.05$.

Table 2. Comparison of the maximal strain and tensible strength of the graft epidermis in the rats of different groups with that of normal skin (%).

Groups	Maximal strain	Tensible strength
7-days to normal skin	85.71	62.40
9-days to normal skin	89.29	64.73
14-days to normal skin	92.86	68.99
21-days to normal skin	96.43	78.68
60-days to normal skin	98.21	97.67

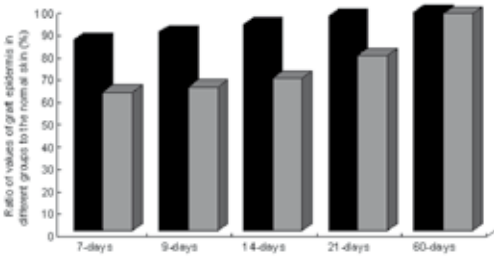

Figure 6. Comparison of the maximal strain and tensible strength of the graft epidermis in the rats of different groups with that of normal skin, which could indicate the recovery degree of the graft epidermis at the different time points after transplantation.

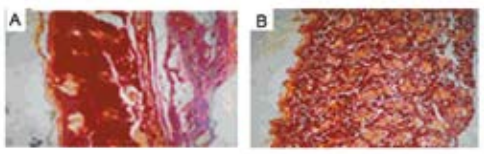

Figure 4. Observation of the collagen fibers with VG staining by light microscopy. A is the photograph of 7-days group; B is the photograph of 21-days group, the density of collagen fibre has increased slowly and fiber bundles became thicker. Finally, they fused with each other.

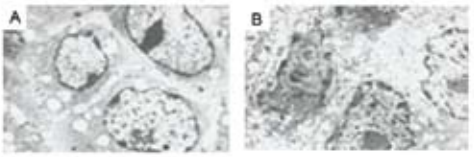

Figure 5. Histological changes of the graft area observed by light microscopy (2345x64). A is the photograph of 7-days group, most of the mitochondria and endoplasmic reticulum in cytoplasm showed ballooning changes. The number of ribosomes was obviously reduced comparing with normal cells. B is the photograph of 14-days group. There were plenty of mitochondria, endoplasmic reticulums and ribosomes in the cytoplasm. Cell nucleus and nucleolus could be observed clearly, which were similar with normal cells. Additionally, the number of esmosomes decreased but that of keratin filaments increased. The melanin granule particles were not found.

(*Liu Y et al., 2005; Xiangsheng F et al., 2006*). The technology of this treatment has been improved recently. However, it is so complicated and costs so much that its therapeutic value is limited. Therefore, a novel method, autologous skin transplantation, for the deep partial thickness burn wound has been introduced in this paper. In order to test our treatment, we created a kind of rat models with deep second degree burn wounds by thermal injury (75°C for 10s). This model provides a vehicle to observe the healing process of burn wounds at different time points after treatment. The macroscopic, histological and biomechanical analysis all support the hypothesis that the autologous skin transplantation heals a deep dermal burn injury in a scar-less fashion.

It has been demonstrated that the metabolism of cells in degenerative dermis of burn wounds is disordered (*Pfützner W et al.,2006; Cheng B et al.,2001*). As long as the excision is adequate, the skin graft will take and a scar will be formed. The amount of dermis remaining after excision will determine the quality of the scar and the degree of contraction

of the healing wound (*Tania C.S et al.,2006; Li GD, 2001*). If the burn wound is partial thickness, re-epithelialisation will occur from epidermal appendages in viable dermis. If viable dermis is removed during the excision, the scar may be less satisfactory for two reasons. Firstly, epidermal appendages are also removed and the burn can no longer heal by re-epithelialisation. Secondly, there will be less dermis to support the skin graft and this is associated with increased contracture. However, if the excision is inadequate the graft take will be poor. In this study, autogeneic epidermis obtained from neck of rats was used to cover the wounds after excision to preserve the degenerative dermis. Macroscopic, light microscopic and electron microscopic observation on the pathological changes of the graft epidermis at different time points indicat ed that hyaline of the degenerative dermis could become grey in colour (visible after eschar shaving to super layer) and the histological injury in the graft area could recover to be normal gradually within 60 days after transplantation.

There are two indices to reflect the biomechanical properties of graft materials, these are the maximum strain value ε and the tensible strength σ (*Wang W, 1999*). The tensible strength refers to the biggest tensible force, which the skin can support in each area (*Wang XQ, 2003*). The maximum strain value refers to that the skin is able to extend the maximum length under the biggest tensible force (*Lu S et al., 1999*). Only the force of unit area and the change of length under certain force can be considered as the endurance of the animal. So the tensible strength and the maximum strain value may reflect the resistant nature of the skin. The biomechanical properties of synthetic and biologic materials change with time. The decrease in maximum strain and the tensible strength of graft epidermis after transplantation found in our study is similar to that reported by others (*Peng Y, 2003; Chen J et al., 2002; Yu YR et al., 2006*). From 9 days after transplantation, the maximum strain value ε and the tensible strength σ have been demonstrated to recover continuously. By the l 60^{th} day, the two indexes have improved to be 98.21% and 97.67% compared with those of normal skin, which also indicate that the biomechanical properties are close to normality.

In conclusion, our initial experience shows that the autologous skin transplantation is very effective at preserving the denatured dermis of a deep second degree burn wound. The histological and biomechanical changes of the denatured dermis also may gradually recover to normality after being covered by the autologous skin.

Acknowledgements

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