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Mechanical properties of skin graft wounds

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SUMMARY. **In female Wistar rats the mechanical strength development of the wound between a skin graft and the neighbouring intact skin (graft wound) was compared with that of ordinary incisional wounds after 4, 7, 14 and 21 days of healing. In one group of rats a 35 × 20 mm skin graft including the subcutaneous muscle was raised and replaced *in situ* on the left side of the back and a 35 mm incisional wound was made on the right side. In another group a 35 mm incisional wound was made on the right side of the back only. After 4 days the maximum load, maximum stiffness and relative failure energy of the graft wounds were 49, 43 and 40% less respectively than those of the incisional wounds from the same animals and after 7 days the maximum load and maximum stiffness of the graft wounds were reduced by 26 and 29%. However, after 14 and 21 days no differences in mechanical properties were found between these two types of wounds. Compared with the incisional wound from rats without graft the maximum load, maximum stiffness and relative failure energy of the graft wound were reduced by 57, 58 and 44% after 4 days, 59, 62 and 54% after 7 days, 37, 38 and 29% after 14 days and for maximum load and maximum stiffness a reduction of 33 and 31% was found after 21 days of healing.**

The healing of a full thickness skin graft is dependent on the adherence of the graft both to the neighbouring intact skin and to the wound bed. The healing process has been evaluated by blood flow measurements, histological examination and biochemical examination of mucopolysaccharide synthesis and collagen synthesis, deposition and degradation.[1–5] The mechanical properties of a healing wound are mainly a result of the net deposition and stabilisation of collagen in the wound.[6,7] Mechanical strength of skin grafts has only been measured by Tavis *et al.*,[4] who measured the adherence of the graft to the wound bed by tensiometry after 72 h of healing. However, the deposition of collagen fibrils does not take place until after 2–3 days of healing when evaluated by electron microscopy,[8] although a weak increase in collagen α1(I) mRNA has been found in fibroblasts of wounds after one day of healing.[9] The aim of the present study was to examine the mechanical strength development in the incision between the graft and the skin after 4, 7, 14 and 21 days of healing and compare this strength with that of ordinary incisional wounds.

Materials and methods

Female Wistar rats (Møllegård, Ll. Skensved, Denmark), 210–220 g (90 days old) were used. All rats were caged separately with free access to tap water and pellet food (Altromin Diet, Chr. Pedersen, Ringsted, Denmark), with 12 h light and 12 h darkness.

The operations were performed under general anaesthesia (sodium pentobarbital 50 mg/ml, 0.1 ml per 100 g body weight intraperitoneally), using sterile technique. Mechanical testing was performed after 4, 7, 14 and 21 days of healing. 12 rats were included in each of the groups tested after 4 and 21 days and 8 rats were included in each of the groups tested after 8 and 14 days. After each healing period, two groups of rats were tested. One group had both an autograft and a linear incisional wound and one group had a linear incisional wound only.

Graft wounds (A) and incisional wounds (B), Fig. 1. The rats were shaved and on the left side of the back 10 mm from the midline, using the thoracolumbar transition as midpoint, a 35 × 20 mm piece of full thickness skin including the s.c. muscle was removed and then immediately replaced *in situ* in the wound bed. At each end and at the middle of each incisional line a Prolene 6-0 (Ethicon, Norderstedt, Germany) suture was placed intracutaneously, and the wound edges were further approximated with a transparent dressing (Tegaderm, 3M, St. Paul, Minnesota, USA) which covered the whole graft (A).

On the right side, a 35 mm linear incisional wound through epidermis, dermis and the s.c. muscle was made 30 mm from the midline, again using the thoracolumbar transition as midpoint. A Prolene 6-0 suture was placed at each end and at the middle of the wound and approximated with Tegaderm (B).

A piece of gauze was placed on the back and a piece of cotton, soaked in water and subsequently squeezed, was placed over the graft to ensure contact between the graft and the underlying fascia. Another piece of gauze was placed on the back, and finally the rats were wrapped with Tensoplast (Smith and Nephew, Hull, England). The bandage was kept on for the entire healing period.

Incisional wound (C) Fig. 1. An incisional wound was made on the right side as with the incisional wound type B. The wound was closed and bandaged like the incisions in the grafted rats.

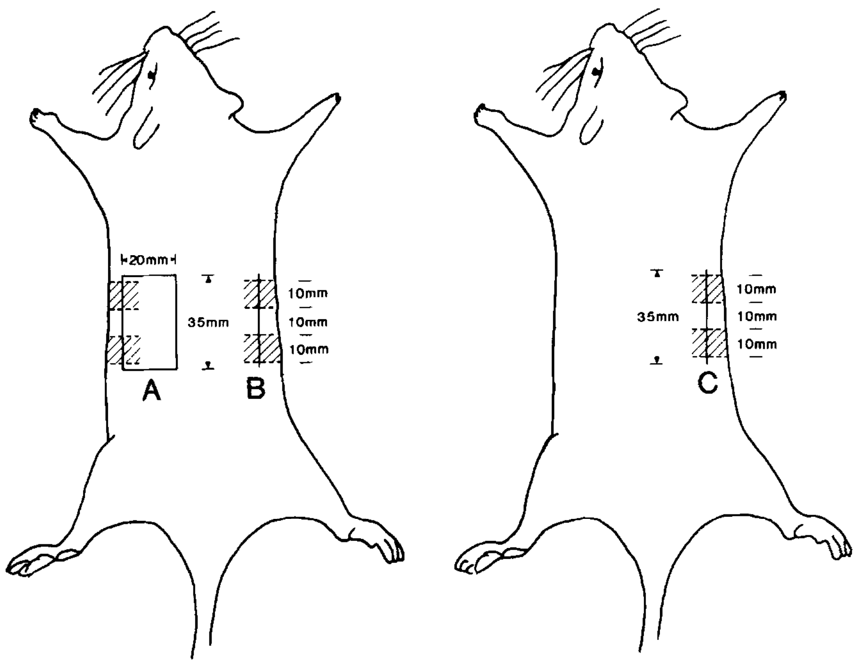

Fig. 1

Figure 1—The figure shows schematically how the operations were made. The rat to the left had both a graft (A) and an incisional wound (B). The rat to the right had an incisional wound (C) only. The dashed areas represent the wound strips punched out for mechanical testing.

Mechanical testing

After the healing periods, the rats were killed with an overdose of sodium pentobarbital. Before they expired, the Tegaderm was gently removed and the dimensions of the grafts and incisional wounds were measured. The back skin was removed and the underlying loose soft connective tissue was cut off.

From the lateral wound of the graft and from the incisional wounds two strips including the wound, 10 mm in width, were cut out perpendicular to the wound at a distance of 5 mm from the suture in the middle of the wound using specially mounted razor-blades (Fig. 1). From these strips the s.c. muscle was removed except for 2 mm next to the wound, to avoid damage to the wound. The strips were then wrapped airtight and kept at 4°C until mechanical testing, which was performed within 1 h. Before testing, the strips were soaked in Ringer's solution (pH 7.4, 21°C) and the dimensions measured optically at 10 times magnification.

The mechanical testing was performed in a materials testing machine (Lorentzen & Wettres TCT 5, Stockholm, Sweden) in which the strips were mounted with a jaw space of 10 mm. After mounting, the strips were immersed in Ringer's solution (pH 7.4, 21°C) and the wounds stretched at a constant speed of 10 mm per min until failure. Load and deformation were registered continuously on an X-Y recorder and the curves were fed into a computer by a digitiser for further calculations. The load data of the wounds were corrected by the width of the specimens, as the thickness and hence the cross-sectional area of graft and intact skin differed. Strain values were calculated by dividing deformation values with the original length of the specimens. The original length was defined as the sum of the jaw-space and the deformation that occurred at load values of 0.005 newton per mm width of the wound strips. Relative failure energy, representing the energy absorbed by the specimen during stretching until failure, equalled the area under the load-strain curve. Maximum stiffness was the maximum slope of the load-strain curve and was measured over a distance of 1% strain. The mean values with SEM for each group were calculated from the means obtained from each rat. The procedure of mechanical testing and subsequent obtaining of mechanical data has been described in detail previously.[10,11]

Weight. The rats were weighed at the time of operation and at the time of testing.

Statistical analysis. For differences between the groups the Kruskal–Wallis test was used, followed by the Mann–Whitney *U*-test. The Wilcoxon test for matched pairs was used for the comparison of graft wounds and incisional wounds from the same animal. $p < 0.05$ was considered statistically significant.

Results

Five rats were excluded from the study. Three rats damaged the grafts, one rat died on the second and another on the third day postoperatively for unknown reasons.

Dimensions (Table 1). After 4 days of healing, the dimensions of the grafts were identical to the values at operation. The length of the grafts did not change during the healing periods, whereas a decrease in width was found (10% after 7 days, 20% after 21 days). No changes in length of incisional wounds were measured during the healing periods.

Weight changes (Fig. 2). Both groups showed a weight loss of 6% for the first 4 days postoperatively. On day 7 postoperatively, the animals with grafts still showed a weight loss of 6%, whereas the weight loss in the animals with an incisional wound only had been reduced to 3%. Compared with the weight at operation, none of the groups had gained weight until day 21 and at this time the rats with an incisional wound only had an increased weight gain compared with rats with both a graft and an incisional wound.

Table 1 Dimensions of the full thickness grafts after 4, 7, 14 and 21 days of healing. At the time of operation, the length of the grafts was 35 mm and the width 20 mm

Healing period (days)	*n*	*Length (mm)*	*Width (mm)*
4	11	35±0.0	20±0.0
7	7	34±0.4	18±0.5*
14	8	34±0.4	19±0.7
21	11	34±0.7	16±0.3*

Mean ± (SEM).
*: $p < 0.05$ compared with the value at the time of operation.

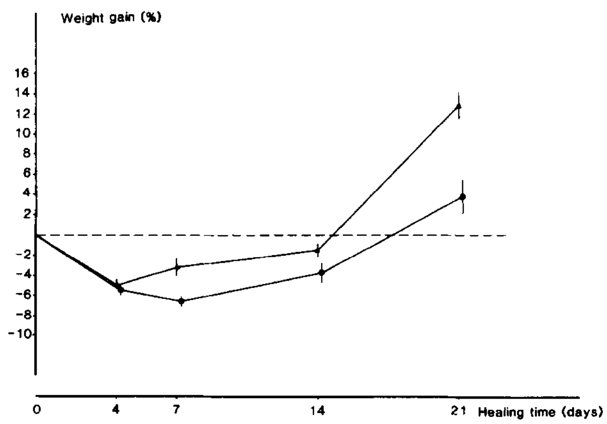

Fig. 2

Figure 2—Weight changes in percent of weight at the time of operation. ●: Rats with graft and incisional wound. ▲: Rats with incisional wound only. Mean values with SEM are depicted.

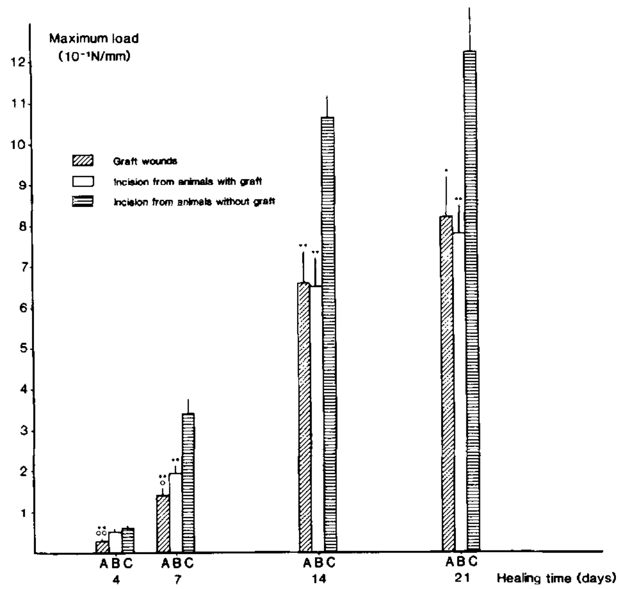

Fig. 3

Figure 3—The figure shows the maximum load per mm width of the wound strip as a function of healing time. Mean values with SEM are depicted. N = newton o: $p < 0.05$, oo: $p < 0.01$ compared with incisional wound from the same rat (type B).
*: $p < 0.05$, **: $p < 0.01$ compared with rats having incisional wounds only (type C).

Mechanical properties (Table 2 and Fig. 3). All the tested strips ruptured at the incision.

4 days of healing: The maximum load, maximum stiffness and relative failure energy of the graft wounds (type A) were reduced by 49, 43 and 40% compared with the incisional wound from the same animals (type B) and 57, 58 and 44% compared with animals with incisional wounds only (type C). No differences were found between the two types of incisional wounds (type B and C).

Table 2 Biomechanical properties of full thickness skin graft wounds and skin incisional wounds in rats. Values corrected for the width of the skin strips

Healing period (days)	*Wound*	*n*	*Maximum load (10^{-1} N/mm)*	*Maximum stiffness (10^{-1} N/mm)*	*Relative failure energy (10^{-3} N/mm)*	*Strain (%)*
	type A: Graft wound	11	0.26±0.05°°**	3.0±0.6°**	2.4±0.5°*	17±1.4
4	type B: Incision (+graft)	11	0.51±0.06	5.3±0.8	4.0±0.4	20±1.2
	type C: Incision (−graft)	12	0.60±0.06	7.2±0.8	4.3±0.3	18±0.8
	type A: Graft wound	7	1.4±0.15°**	15±1.5°**	11±1.7**	21±1.5
7	type B: Incision (+graft)	7	1.9±0.17**	21±2.6*	16±1.9	25±2.3
	type C: Incision (−graft)	8	3.4±0.35	42±5.2	24±3.5	22±2.0
	type A: Graft wound	8	6.7±0.76**	59±7.2***	60±1.1**	27±1.6
14	type B: Incision (+graft)	8	6.5±0.65**	65±10.2*	47±2.6**	25±1.2
	type C: Incision (−graft)	8	10.7±0.48	95±8.6	84±5.9	28±1.6
	type A: Graft wound	11	8.2±0.94*	55±5.4*	89±11	34±1.5
21	type B: Incision (+graft)	11	7.8±0.67**	62±5.7*	75±8*	32±1.4
	type C: Incision (−graft)	10	12.2±1.08	82±6.1	117±13	31±0.8

Mean ± SEM; N = newton.
°: $P < 0.05$, °°: $P < 0.01$ compared with incisional wounds from the same rat (type B).
*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$ compared with rats having incisional wounds only (type C).

7 days of healing: The development of maximum load and maximum stiffness of the graft wounds (type A) was reduced by 26 and 30% compared with the incisional wounds from the same animals (type B) whereas no differences were seen in relative failure energy. The maximum load, maximum stiffness and relative failure energy of the graft wounds (type A) were reduced by 58, 65 and 52% compared with the type C incisions. When comparing type B and type C incisional wounds, a reduction in maximum load and maximum stiffness of 43 and 50% was found in the type B wound whereas the relative energy did not differ significantly.

14 days of healing: No differences were found in any of the mechanical parameters between the graft wounds (type A) and the incisional wounds from the same animals (type B). Compared with the type C incisions the graft wounds (type A) showed a reduction in the development of maximum load, maximum stiffness and relative failure energy of 37, 38 and 29%. In the type B incisions the maximum load, maximum stiffness and relative failure energy were reduced by 39, 32 and 44% compared with type C incisions.

21 days of healing: None of the mechanical parameters in the graft wounds (type A) and the incisions from the same animals (type B) differed from each other. The graft wounds (type A) showed a reduced development of maximum load and maximum stiffness by 33 and 33% compared with type C incisions. The maximum load, maximum stiffness and relative failure energy of type B incisional wounds were reduced by 36, 24 and 36% compared with type C incisions.

No differences in strain at maximum load were found among the groups in any of the healing periods.

Discussion

In the wound between the skin grafts and the neighbouring intact skin the development of maximum load, maximum stiffness and of energy absorption during stretching until failure (relative failure energy) was decreased after 4, 7 and 14 days of healing compared with the values of incisional wounds from animals without a graft. After 21 days of healing, the maximum load and maximum stiffness were lower in the graft wounds, whereas the failure energy did not differ significantly. However, the relative elongation of the wound strips (strain at maximum load) did not differ between the graft wounds and the incisional wounds from ungrafted rats. This demonstrates that when stretching a skin graft by applying only small forces on the wound, it can be stretched as much as an ordinary incisional wound without rupturing.

The mechanical strength of soft connective tissues is mainly mediated by the collagen,[12] and in a healing wound substantial amounts of collagen are usually not present until after 2–3 days of healing. The pattern of collagen deposition in the graft wounds in contrast to the graft itself has not been described so far. Klein and Rudolph[2] evaluated the collagen deposition in skin grafts during healing by labelling rats with ^{14}C-proline for 4–6 weeks before grafting. Then they measured the change in amount of unlabelled collagen in the graft, and considered that as newly formed collagen. By this means, they found that the gain of new collagen in the graft itself was only moderate in thick full-thickness skin grafts raised on the back (unlabelled collagen comprised less than 10% of the original collagen after 21 days of healing) compared with thin full-thickness skin grafts raised on the abdomen (unlabelled collagen comprised more than 50% of the original collagen after 21 days of healing). Although their paper does not describe the event taking place in the incision between graft and neighbouring skin, the data indicate that a reduced collagen formation capacity is also to be expected in the incision between skin and graft.

The difference in strength between graft wounds and incisional wounds from ungrafted rats was not affected by retraction in the wounds, as the length of the grafts was only reduced by 1–3% (not significantly) and no reduction was found in the length of the incisional wounds.

After 14 and 21 days, the strength of the graft wound does not differ from the strength of the incisional wound on the opposite side of the back, and both wounds possess less mechanical strength than the incisional wounds from animals without graft. This indicates that at these healing intervals, systemic factors which inhibit wound healing are also induced in the grafted rats.

One such factor may be the nutritional status of the animal. In our study the weight loss in rats with grafts was larger than in rats without grafts and further, the regaining of weight was slower. Delany *et al.*[13] recently showed that postoperative protein deprivation did not influence the tensile strength of rat skin incisional wounds after 6 days of healing. In addition, Peacock[14] found that protein deprivation did not influence the tensile strength of skin wounds after 6 days of healing. However, at later healing times up to 21 days of healing he found that protein deprived rats showed less tensile strength of the skin incisional wounds than those normally fed. Fogdestam[15] showed that delayed closure of a skin wound could inhibit the strength development in a simultaneously induced but primarily sutured incision on the opposite side of the back skin in rats after 10 days of healing. The later the delayed primary suture was performed, the more pronounced was the strength inhibition of the primary closed incision. Further, the postoperative weight loss of the rats increased linearly with decreasing strength of the primarily closed wounds.

In conclusion, our study shows that the healing capacity in terms of mechanical strength in wounds from composite skin grafts is reduced compared with incisional wounds from ungrafted rats during the first 21 days of healing. The presence of a graft induces inhibition of the strength development in a simultaneously present incisional wound. The inhibited wound healing of the grafted animals was achieved without exogenously applying a catabolic state in addition to that already present as a result of the operation. This provides us with a model to investigate the influence of growth factors and growth hormone on compromised wound healing.

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