Investigating human microskin grafting technique in a new experimental model

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1. Introduction

Major burns are life-threatening and several trials have been conducted to determine the best solution for covering the wound when the donor area is limited. The conventional method consists of repeated harvesting from the same limited donor site. Prolonged treatment is anticipated when using this method and a higher probability of complications is expected. Donor areas are limited in large burns, and searching for techniques to cover these areas using small skin grafts has lead to several experimental and human studies. Various techniques of intermingled skin grafts have been reported to cover large wound areas [1–5]. Keratinocyte culture was started over 20 years ago and their application in burn surgery.
has led to controversial results. Some centers reported successful results while others stopped using the method because of the low “take”, the 2-3 week waiting period, and the high cost. Nevertheless, it has a strong position in the armamentarium of the burn surgeon.

One of the oldest methods is cutting the skin and dividing it into small pieces (“stamps”) which are distributed over the recipient area. With time, this technique was improved and microskin grafts, which have an expansion ratio of more than 10:1, have been reported. Among the pioneers of microskin grafts and implantation of skin cell suspension was Gabarro [6]. He created small patches of skin graft by laying a sheet of skin over adhesive tape and subsequently cutting that into small pieces. In 1957, Najarian [7,8] decreased the patch size by using a food processor, thus producing a suspension of skin particles. The work of Zhang [9,10], published in 1986, renewed interest in this field.

In order to evaluate the healing process of skin grafting in humans, there is a need for a model that simulates human skin grafting. While reviewing the literature, we found that no work has been done using microskin grafts of human origin on animals. We present a novel experimental model to investigate the microskin graft technique. This model is based on grafting human skin onto athymic nude mice that cannot reject the graft. This model enables testing of new grafting protocols on an animal model, giving the opportunity to improve the technique. The present study has two goals:

a. To confirm the feasibility of this model for investigating human microskin grafts.

b. To determine whether this method of performing microskin grafts is applicable to clinical purposes when there is lack of donor sites in large burns.

2. Materials and methods

The study has been approved by the committee for the supervision of animal experiments, of the Technion, Israel Institute of Technology. Twenty female nude mice were randomly divided into a research group and a control group (14 mice in the research group, six in the control group). By removing a patch of full thickness skin, we created a 1.5 cm² wound on the backs of the mice. The human skin graft used in the study was harvested during an abdominoplasty procedure from a patient with no past medical history, after signing of the inform consent that has been approved by the Helsinki Committee of Rambam Medical Center. The thickness of the skin graft was 0.0015 in. Immediately after harvesting, the skin was minced and applied to the mice. The process from skin harvesting until mice wound dressing was less than two hours. For the research group, a 1 cm × 3 cm split thickness graft was minced using a food processor. This skin was placed in 20 cm³ Ringer lactate and was minced in short pulses of about five seconds each. This procedure took 10 min. In order to prevent overheating, ice water was used to cool the container (Fig. 1). The suspension thus created was uniformly spread on a synthetic fenestrated “Telfa” sheet (Tyco Inc., USA), sized 10 cm × 3 cm. The sheet was covered with minute skin pieces on areas 10 times larger than the original skin graft (expansion ratio of 1:10). Fourteen “stamps” were cut from the Telfa, each 1.5 cm², and applied onto the wounds (Fig. 2). Each was bandaged using adhesive tape and sterile metal clips to secure the graft. The mice in the control group were grafted with stamps of split thickness skin grafts. Both the control and the research groups were kept in identical conditions following the intervention. The animal care was in accordance to the Technion institution guidelines.

On the 1st and 11th days after the procedure, the size of each wound was measured using transparent paper. On the 11th day, we also measured the healed (epithelized) area and the open area. Upon completion of the experiment, biopsies were taken for histological examination. Measurement of the wounds was performed by one of the authors who was blind to the groups.

3. Variables tested

(A) Wound healing evaluation: the calculated percentage of the epithelized (closed) wound on the 11th day, compared to the wound area as measured on the 1st day. This was done by using transparent paper, and the areas were measured by a computerized system (Morphmat 10, ZEISS D 7082, Germany). Healing was calculated using
Degree of healing (%)
\[ \frac{\text{epithelialized area on the 11th day}}{\text{total wound area on the 11th day}} \times 100 \]

(B) Degree of wound contraction: the difference between the wound surface on the 1st and 11th days.

Degree of contraction (%)
\[ \frac{\text{wound area on the 1st day}}{\text{wound area in the 11th day}} \times \frac{\text{wound surface on the 1st day}}{\text{wound surface on the 11th day}} \times 100 \]

(C) Histological evaluation of the healing process. Upon completion of the experiment, biopsies were taken from 10 mice (7 in the research group, 3 in the control group). The biopsies were fixed in paraffin and stained in three different ways: Hematoxylin & Eosin, Trichrome, Sirius-Red. The following variables were measured:

a. Thickness of the epidermis, as an index of the quality of epithelization.

b. Thickness and quality of the dermis—the dermal layer in the wound is solely from the graft; thus, its quantity is an index of the success of the take.

c. Number of blood vessels—as an index of angiogenesis from the recipient bed.

d. Number of melanocytes—melanocytes are exclusively of human origin and are easily detected—the presence of melanocytes is a useful indicator of human tissue in the wound.

4. Statistical analysis

For both the research and control groups, the median, average and standard deviation values were calculated for: degree of contraction on the 11th day, percentage of epithelial coverage on the 11th day, thickness of the epidermis, thickness of the dermis, number of blood vessels and number of melanocytes. The results were processed using the SPSS statistical program. To find variance between the groups, the Mann–Whitney test was applied. In the analysis of one way variance (One way ANOVA), \( P < 0.05 \) was considered as statistically significant. Due to the different expansion ratios in the two groups, there was less grafted skin in the research group as compared to the control group; thus, the expected quality of healing in the micrografting will be less favorable. In the model used for the control group, in which the variance is known to be low, the sample size, therefore, was small.

5. Results

Our primary objective was to determine the feasibility of this animal model for grafting of human skin. We found good take of the minute skin graft created by grinding a sheet of skin. No graft rejection or surgical site infection was noted. Of the 20 mice that were examined, the wound dressing fell in three (of the research group), resulting in a complete loss of the skin graft—the wounds in these mice healed by contraction of the wound margins (Fig. 3). This was considered as a technical problem and not a failure of the graft taking. A contribution of the primary epithelial migration to the ultimate healing of the wound is not known and these mice were not included in the statistical analyses. The grafts took well in the other mice. Due to the nature of this model, as there were no significant differences between the groups, we can conclude that the new method of micrografting is comparable to the conventional method.

The healing rate (epithelial coverage) of the wounds on the 11th day was at average of 100% (±0%) in the control group and 97% (±5%) in the research group (Table 1 and Fig. 4). \( P > 0.05 \). The histological picture confirmed that the skin in the control

<table>
<thead>
<tr>
<th>Table 1 – Area measurements.</th>
<th>Research group, average</th>
<th>Control group, average</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td>Area, before grafting (µm)</td>
<td>151.96</td>
<td>124.96</td>
<td>( &gt;0.05 )</td>
</tr>
<tr>
<td>Area, after grafting (µm)</td>
<td>53.48</td>
<td>84.76</td>
<td>( &lt;0.005 )</td>
</tr>
<tr>
<td>Degree of wound contracture</td>
<td>63</td>
<td>30</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Degree of wound healing (%)</td>
<td>97</td>
<td>100</td>
<td>( &gt;0.05 )</td>
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</table>
group was of human origin and had normal papillary morphology. In the research group, the graft that seemingly covered the wound was actually a thin epithelial layer, without papillae (Fig. 5).

The average degree of wound contraction measured on the 11th day was 30% in the control group, compared to 63% in the research group \((P < 0.01; \text{Table 2})\).

The average thickness of the epidermis formed at the grafted site on the 11th day was 8.17 \((\pm 1.94)\) \(\mu\)m in the control group, compared with 4.45 \((\pm 4.17)\) \(\mu\)m in the research group, at the wound margins \((P > 0.05)\). The epidermis of the grafted area in the research group was found to be thicker at its margins, thinner towards the center of the wound, and detached from the deeper dermal layer. In the histological preparations, it was shown that native mouse epidermis is continuous with the epidermis formed over the grafted area (Fig. 5). Within the dermis there were groups of epidermal cells of human origin that remained vital but did not reach the surface (Fig. 6).

The average thickness of the grafted dermis as measured on the 11th day, was 37 \((\pm 11.14)\) \(\mu\)m in the control group and 92.46 \((\pm 21.26)\) \(\mu\)m in the research group \((P < 0.05; \text{Table 3})\). The dermis in the research group was found to be less organized and contained inflammatory tissue that was made of blood vessels, fibroblasts and inflammatory cells (Figs. 7–9).

To count the blood vessels, we used a 40× magnification microscope. We counted the blood vessels in five different histological sections and used the mean of those five measurements as the final number of blood vessels in each section. The number of blood vessels was an average of 32.67 \((\pm 10.15)\) in the control group and 37.34 \((\pm 20.10)\) in the research group \((P > 0.05)\).

The average number of melanocytes on the 11th day was 41.93 \((\pm 5.75)\) in the control group and 8.43 \((\pm 6.47)\) in the experimental group \((P < 0.05)\).

6. Discussion

Many researchers have tried to find the best way of expanding the available skin for grafting in extended burn patients. Gabarro [6] obtained small grafts after multiple cuts performed longitudinally and transversally. Nystrom [11] described good clinical outcomes after using his device to cut the skin into 1 mm² pieces. Lai [12] reported a single way of cutting the skin into small pieces. The skin sheet was laid on the dermacarrier, which was passed at various angles through the mesher, thereby producing skin pieces of 0.4 mm². These small pieces were randomly placed on the wound and then covered by pig xenograft. The skin graft take was good with satisfactory wound healing and cosmetic result. Small pieces of graft (1 mm × 1 mm) were obtained also by Inoue [13] using a mesher. The grafts were minced into small parts by cutting only twice on a 3:1 plate. Blair [14] performed an experimental study on pigs. Small pieces of 200 \(\mu\)m × 200 \(\mu\)m “diced” skin grafts were obtained with a histological tissue slicer. An algebraic method of calculating the required donor area surface according to the side-length of micropatch was reported by Lin [15]. At present, there is no perfect device for obtaining small skin pieces. Finding such a device would contribute to obtaining a better experimental protocol.

Various animal models have been used to study the results of the microskin graft technique. The work of Zhang [9,10] was done on rabbits. He took a small skin graft cut into 1 mm² pieces and scattered back onto the wound. A rabbit skin

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**Table 2 – The degree of wound contraction \((p < 0.01)\).**

<table>
<thead>
<tr>
<th></th>
<th>Experimental group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before grafting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After grafting</td>
<td>160</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>100</td>
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<td></td>
<td>40</td>
<td>20</td>
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<td></td>
<td>20</td>
<td>0</td>
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</tbody>
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**Fig. 5 – After implantation; the continuity of the epidermis from the wound borders can be seen. (a) Mice epidermis; (b) border with more flat epidermis without papillae and melanocytes; (c) toward center implantation, the epidermis becomes thinner until it disappears (Trichrome coloration; 40× magnification).**

**Fig. 6 – Melanocytes group (black arrows) located under the dense collagen fibers.**
allograft was placed on top as temporary wound coverage. The outcome in this trial was good, both regarding the healing time and the histological analysis. Rabbits were also used by Lin [16], who demonstrated that expanded allogenic and microskin autografts can proliferate together to resurface the wound. In a different study, the same author [17] observed that micrografts proliferated with an increased number of mitoses when allogenic grafts were treated with ultraviolet irradiation, although the regeneration was slower than in the control group. Fang [18] used Lewis rats to investigate microskin grafts. Gilhar [19] was the first to use mice to test human hair growth.

Our study was performed on nude mice which allowed us to investigate human skin. The large number of newly formed blood vessels in the grafted area served as one of the parameters for the quality of the take of the grafts in our study. In both the research and the control group, similar numbers of blood vessels were observed ($P > 0.05$), compared to the relatively lower number of blood vessels outside the grafted area. The presence of melanocytes was used to identify the human source of the examined tissue. Since nude mice lack melanocytes, these cells act as a natural marker for human skin. There was a considerable difference in the number of melanocytes between the research and control groups due to the different expansion ratios.

### Table 3 – The histological findings.

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Research, average</th>
<th>Group, standard deviation</th>
<th>Control, average</th>
<th>Group, standard deviation</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of the dermis at the implantation area ($\mu$m)</td>
<td>92.46</td>
<td>21.26</td>
<td>37.00</td>
<td>11.14</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>Thickness of the epidermis at the margins of the implantation area ($\mu$m)</td>
<td>4.45</td>
<td>4.17</td>
<td>8.17</td>
<td>1.94</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>Thickness of the epidermis at the center of the implantation area ($\mu$m)</td>
<td>0.00</td>
<td>0.00</td>
<td>8.17</td>
<td>1.94</td>
<td>$0.05&gt;$</td>
</tr>
<tr>
<td>Number of blood vessels at the implantation area</td>
<td>37.34</td>
<td>20.10</td>
<td>32.67</td>
<td>10.15</td>
<td>$0.05&lt;$</td>
</tr>
<tr>
<td>Number of blood vessels outside the implantation area</td>
<td>3.63</td>
<td>1.49</td>
<td>4.60</td>
<td>1.41</td>
<td>$0.05&lt;$</td>
</tr>
<tr>
<td>Number of melanocytes at the implantation area</td>
<td>8.43</td>
<td>6.47</td>
<td>41.93</td>
<td>5.75</td>
<td>$0.05&gt;$</td>
</tr>
</tbody>
</table>

The wound contraction phenomenon plays an important role in the formation of undesirable hypertrophic and keloid burns. The dermis in the grafted area has great importance. Skin that lacks dermis, as in using keratinocyte cultures for grafting, is vulnerable to trauma and offers fragile coverage. In our model, we found a good “take” of the dermis although there was no polarity of the micrografts in terms of locating the dermis on the recipient site. There was no doubt that the dermis found in the research group was of human origin because of its similarities to that of the control group, as shown by the histological analysis.

### Fig. 7 – Research group. Intake of the dermis and collagen concentration in different areas can be seen. In the deep part, the muscle layers are colored in green (Sirius-red coloration; 40× magnification). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

### Fig. 8 – All skin layers in the research group. The dermis structure can be seen in the left upper corner; *Intake of the dermis;* (a) mice epidermis; (b) skin graft area.

### Fig. 9 – The skin grafted area in the control group. Left upper corner shows the dermis structure (100× magnification) *Collagen structure;* (a) mice epidermis; (b) skin graft epidermis.
scars and is an undesirable sequel in skin grafting. In our study, we found significant wound contraction in the research group compared to the control group (63% versus 30%). When the dermis is well organized, the contraction diminishes. In the present model, due to the random cellular alignment, an unorganized dermis formed, containing excessive granulation tissue. Fang [18] also observed that wound contracture after microskin autografts was greater than in the sheet isograft group. Most of the contracture for the micrograft group (43% of the original size) appeared at 7 weeks post-surgery while it occurred at 4 weeks post-grafting in the autograft group sheet (72% of the original size). Significant contracture in the microskin graft technique was reported also by Cox [20].

There are several reports in the literature of using microskin grafts on humans. Blair [14] used this technique successfully on five patients with chronic leg ulcers. Lin [21] used a modified Tauner-Vandeput mesh dermatom to obtain scalp microdermis grafts which were used in humans. No skin appendages were found. According to this author, scalp microdermis grafts are better than regular microskin grafts. He noticed unstable skin condition in some patients which persisted for about three months. No “rete ridges” or other skin appendages were identified in our research group. For this reason, this technique is potentially more life-saving and perhaps should not be used when considering grafting over joints, face, back or other areas where strong support is needed. In a different study [22], Lin successfully used microskin grafts with pig skin xenograft overlays to cover different full thickness wounds. This microskin graft technique is more widely used in China than in any other country and is considered as one of the factors that have lowered the mortality rate in very extensive burns [23].

In our present study, we have introduced a new model for investigating human microskin grafts. In this study with an animal model, a food processor was used to mince the skin and the skin suspension was spread on “Telfa”. Although there was a good take of the grafts, the quality of the micrografts was significantly worse than of sheet grafts. In future research, by creating a different size of skin graft with different methods of grinding, or by improving the grafting technique, we hope to improve the graft “take” and to significantly reduce the wound contracture.

**Conflict of interest**

None.

**References**


