TWO-STEP GRAFTING OF THE FULL THICKNESS SKIN DEFECTS IN PIGS USING THE COMPOSITE OF ATELOCOLLAGEN AND HYALURONIC ACID

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Abstract


The reconstruction of resistant and pliable skin in vivo is not possible without the substitution of its dermal component. To explore the feasibility of two-step grafting of full-thickness skin defects, an animal experiment was carried out. Twelve large white female pigs weighing 30 kg were used. The full-thickness wounds created were 5 x 5 cm in size.

The composite of bovine atelocollagen and hyaluronic acid (HyproDerm) was implanted as the dermal substitute in the first step. The overgrafting of this composite with a thin epidermal autograft followed as the second step ten days later.

Planimetric, histologic and clinical evaluations (using the Vancouver Scar Score) of healed wounds were carried out. Studied wounds were compared with those left for spontaneous healing and those grafted immediately using dermoepidermal graft without any dermal substitute.

1. Planimetric measurements have shown that the average area of the treated defects was 1 245.2 mm², the average area of untreated defects was 386.2 mm² and the area of immediately grafted defects was 677.8 mm², one month after the injury. These differences were statistically significant (p < 0.001).

2. Very good vascularisation and colonisation by fibroblasts with immediate production of collagen fibres, were observed microscopically.

3. The quality of reconstructed skin was superior to the untreated wound according to the modified Vancouver Scar Score (4. 91 points) treated wounds × 9. 25 points (non-treated wounds) × 5.50 (immediately grafted wounds) after one month.

The dermal substitute HyproDerm reduces the shrinkage of resurfaced wounds and improves the quality of reconstructed skin in pigs. Containing the harmless and approved materials, it is convenient for clinical use.

HyproDerm, dermal substitute, epidermal autografts, wound shrinkage, Vancouver Scar Score

The improved survival of severely burned patients - a result of better understanding and management of burn shock and early radical excision of necrotic skin (Pruitt and Mason 1997) - brought in the past decades the problem of reconstruction of the missing skin. New and/or improved methods of expansion of patient’s own skin epithelium were developed as life saving procedures. Cultured autografts are probably the most effective and popular among them (Gallico et al. 1984; Munster et al. 1990). However, the micrografting (Meek 1996), sandwich grafting (Alexander et al. 1981) and intermingled grafting (Yang et al. 1980) are also very important.

Nevertheless, for the reconstruction of durable and pliable skin, the renewal of the dermal component seems to be very important (Compton et al. 1989). This fact was recognized about 15 years ago and many attempts have been made to resolve this problem.
This paper describes one of the possible ways of dermal replacement: two-step grafting of full thickness skin defects in pigs using the composite of atelocollagen and hyaluronic acid (HyproDerm) as the first step and its subsequent overgrafting with very thin conventional epidermal autograft as the second step.

Materials and Methods

Twelve Large White female pigs weighing about 30 kg (± 2 kg) were used. General anaesthesia was provided using Stresnil, i.m. at a dose of 0.1 ml/kg and Hypnodil i.v. at a dose of 0.2 ml/kg during all procedures.

In each animal 6 full thickness skin defects were created paravertebrally. The size of each defect was 5 x 5 cm (Plate III, Fig. 1). The dermal substitute was immediately applied and fixed either by sutures or staples to the wounds 1-4.

The material consisted of the composite of atelocollagen and hyaluronic acid, as previously described (21). Our modification was called HyproDermTM. Its thickness was 3.5 mm (Plate III, Fig. 2). Thin polyurethan membrane was fixed onto the upper surface of the composite. Metallic staples and protective dressing containing gauze were used to fix the composite in the wound and adhesive tapes were applied at the end of the procedure (Plate IV, Fig. 3).

Two wounds were used as a control: wound No. 5 was left untreated (only covered) for healing per secundam. Wound No. 6 was closed immediately after the defect was created with a thin dermoepidermal graft.

The re-bandage of all wounds was performed on day 3 after the operation (Plate IV, Fig. 4).

Subsequently, 10 days after creation of the defects and dermal substitute application, the polyurethan membrane covering was removed and replaced by the same thin dermoepidermal autografts as wound No. 6 ten days previously. The wound beds showed good vascularisation at this time (Plate V, Fig. 5). Grafts were freshly harvested from the posterior legs. The dermoepidermal grafts were stapled and covered by the elastic bandage (Plate V, Fig. 6).

The samples for the histologic evaluation were taken before the application of the epidermal grafts (day 10), 7 days after over-grafting (day 17) and then 21 days after overgrafting, i.e. 31 days after creation of the defects.

At the same time (one month after the wounds were created) the size and shape of them were measured to assess the degree of wound contraction and the Vancouver Scar Score was used for quality assessment of healed defects.

Plate VI, Fig. 7 shows the appearance of a two-step grafted wound one week after overgrafting and Plate VI, Fig. 8 shows the wounds 3 weeks after overgrafting.

The evaluation of results obtained was performed:

a) planimetrically, using our original computer programme

The wounds were covered with sterile transparent membrane and their edges were painted with a surgical pen. Subsequently, the size and shape of wounds were transferred into the computer memory and the area was calculated in the specially developed computer programme.

The aim of this measurement was to follow up the degree of wound contraction in each of the wounds. The question was if the dermal substitute would be able to reduce it effectively.

b) histologically, using light microscopy (HE and AZAN staining)

The aim of this assessment was to follow up the structure of reconstructed skin, process of vascularisation and collagen ingrowth, and in the later phase the formation of new epidermis.

c) clinically, using the Vancouver Scar Score

The Vancouver Scar Score is often used for the description of the scar quality (Sullivan et al. 1990). We used it for the evaluation of all three types of healed wounds.

The scar assessments have been devised based on physical parameters of pigmentation, vascularity, pliability and scar height. All parameters were assessed independently and with increasing score being assigned to the greater pathologic condition.

Pigmentation:
0 = Normal: colour that closely resembles the colour over the rest of a person’s body
1 = Hypopigmentation
2 = Hyperpigmentation

Vascularity:
0 = normal: colour that closely resembles the colour over the rest of the body
1 = pink
2 = red
3= purple
Pliability:
0 = normal
1 = supple: flexible with minimal resistance
2 = yielding: giving way to pressure
3 = firm: inflexible, not easily moved, resistant to manual pressure
4 = bending: rope-like tissue that blanches with extension of scar
5 = contracture: permanent shortening of scar producing deformity of distortion

Height:
0 = normal
1 = < 2 mm
2 = 3–5 mm
3 = > 5 mm

Burn Scar assessment form

The results of planimetric measurements yielded the following values (Table 1):

<table>
<thead>
<tr>
<th>Pig No.</th>
<th>Wounds 1 – 4 (mm²)</th>
<th>Wound 5 (mm²)</th>
<th>Wound 6 (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 013.6</td>
<td>906.5</td>
<td>463.1</td>
</tr>
<tr>
<td>2</td>
<td>1 164.2</td>
<td>874.3</td>
<td>455.1</td>
</tr>
<tr>
<td>3</td>
<td>1 203.1</td>
<td>594.7</td>
<td>459.7</td>
</tr>
<tr>
<td>4</td>
<td>1 099.9</td>
<td>583.6</td>
<td>376.5</td>
</tr>
<tr>
<td>5</td>
<td>1 205.7</td>
<td>870.4</td>
<td>495.6</td>
</tr>
<tr>
<td>6</td>
<td>1 345.1</td>
<td>781.3</td>
<td>435.5</td>
</tr>
<tr>
<td>7</td>
<td>1 322.9</td>
<td>663.6</td>
<td>361.8</td>
</tr>
<tr>
<td>8</td>
<td>1 296.2</td>
<td>899.3</td>
<td>396.1</td>
</tr>
<tr>
<td>9</td>
<td>1 606.9</td>
<td>464.1</td>
<td>390.0</td>
</tr>
<tr>
<td>10</td>
<td>1 233.6</td>
<td>583.4</td>
<td>355.1</td>
</tr>
<tr>
<td>11</td>
<td>1 204.3</td>
<td>434.4</td>
<td>190.0</td>
</tr>
<tr>
<td>12</td>
<td>1 246.3</td>
<td>478.1</td>
<td>256.5</td>
</tr>
<tr>
<td>average value</td>
<td>1 245.2</td>
<td>677.8</td>
<td>386.2</td>
</tr>
</tbody>
</table>

The results were evaluated using analysis of variance.

Results

The results of planimetric measurements yielded the following values (Table 1):

Thirty one days after defect creation the average size of wounds 1 - 4 was 1 245 mm² (SD 146 mm²). The average size of wound 5 (healing per secundam) was 678 mm² (SD 180 mm²) and the average size of wound 6 was 386 mm² (SD 89 mm²).

The difference between wounds 1 - 4 and wounds 5 is highly statistically significant ($p < 0.001$). The mean difference between wounds 1 - 4 and 5 was 567 mm² (95% CI 396, 739).

The difference between wounds 1 - 4 and wounds 6 is also highly statistically significant ($p < 0.001$). The mean difference between wounds 1 - 4 and 6 was 859 mm² (95% CI 745,973).

Thirty one days after wounding, the contraction was most evident in the non-treated wounds (No. 5), followed by the immediately grafted wounds in No. 6. The least contraction was observed in wounds No. 1 - 4 i.e. those with the dermal substitute.
II. The histologic picture of the wounds was as follows (Plates VII and VIII, Figs 9 – 12):

The main features of the histologic follow up were the quick and intensive ingrowth of capillaries and collagen fibres into the substitute in the first days. Signs of inflammation were only moderately pronounced. The microscopic picture corresponded with the clinical finding of an almost ideal wound bed for grafting after 10 days of dermal substitute implantation.

In spite of this, the “take” of the grafts was relatively complicated in the histologic pictures. Necrotic areas appeared in the grafts and could be observed in the first days. The remaining living cells (fibroblasts and keratinocytes) were nevertheless able to form the new epidermis of quite a good quality (see Figs 9 - 12).

III. The Vancouver Scar Score

The mean difference between wounds 1 - 4 and wounds 5 was 4.3 (95% CI 3.7, 5.0). This difference was highly significant ($p < 0.001$).

The mean difference between wounds 1 - 4 and wounds 6 was 0.6 (95% CI 0.5, 1.6) This difference was not statistically significant ($p < 0.253$)

There was a conspicuous difference between wounds 1 - 4 and the wound 5 (untreated wound) in the degree of scarring. Less difference between the same wounds and wound 6 (immediately grafted) could be observed.

IV. Other observations made:

1. No difference could be found between the sutured (4.0 or 5.0 monofilament suture) and stapled grafts. Staples are quicker and easier to insert than sutures. No clinically significant irritation could be observed in either of the materials and both materials remained patent and safely fixed the dermal implant during the whole course of the experiment.

2. The 10-day interval between the implantation of dermal substitute and its overgrafting seems to be sufficient. At this time the tissue was freshly red and sometimes even slightly exceeding the level of surrounding skin (Fig. 5).

3. The first step of the procedure – dermal substitute implantation – is technically much easier than the second step – a successful “take” of the dermoepidermal graft on top of it.
Discussion

The replacement of dermis at present is performed using either the allogeneic acellular dermis (Pruniears et al. 1979; Heck et al. 1984; Cuono et al. 1986; Cuono et al. 1987) or using any form of collagen with (Bell et al. 1979; Boyce and Hansbrough 1988) or without autologous fibroblasts (Yannas and Burke 1980; Burke et al. 1981). Other synthetic biodegradable materials have also been used (Hansbrough et al. 1992; Van Dorp et al. 1998).

In Slovakia, a group of research workers explores a very similar material which differs from the presented one mainly in the cross-linking procedure (Koller et al. 1997; Vizárová et al. 1994; Vizárová et al. 1995).

Although the results obtained are convincing, the following topics should be taken into consideration:
1. Although pigs are probably the most suitable animals for the experimental reconstruction of skin, they are relatively complicated to treat and preventing them from damaging their wounds is problematic. The proper wound coverage must be reached and in spite of this it is hard to distinguish if the complicated dermoepidermal graft “take” is caused by its poor nourishment or by friction forces.
2. Assessing the wound contraction makes the use of any chambers, separating the wound from the surrounding skin, impossible. The growth of keratinocytes from the edges therefore takes part in the re-epithelisation of the wounds.
3. Due to the progressive adiposis and growth of the white domestic pigs it becomes impossible to ensure a reproducible long-term follow up.
4. The production of collagen and its reformation to fibrils and fibres is very impressive in the substitute. It is very probable that the results can be extrapolated for human wound healing. Also the vascularisation of the substitute is very quick and intensive.
5. As all the components of the dermal substitute used are known to be harmless for the wound, and the technology of their production corresponds to good laboratory practice, the obtained results therefore justify the clinical use of developed material.

In conclusion, the composite dermal substitute HyproDerm consisting of the mixture of atelocollagen and hyaluronic acid brought satisfactory results in our pig study. In comparison with the wound healing per secundam and even with the wound grafted immediately after wound creation, the wound shrinkage was much less pronounced 31 days after the operation. These results were statistically significant \( p < 0.001 \). Also the quality of reconstructed skin assessed by the Vancouver Scar Score was significantly better than that of the non-treated wound \( p < 0.001 \). Although this quality was even better than that of the wound grafted immediately, the difference is not statistically significant \( p = 0.253 \).
„Vancouverského skóre“ pro posuzování jizev (Vancouver Scar Score). Sledované rány byly porovnávány s defekty ponechané ke spontánnímu hojení a defekty ihned zatransplantovaného dermoepidermálního štěpem bez dermální náhrady.

1. Planimetrická měření ukázala, že měsíč po vytvoření ran byla průměrná plocha uzkoumaných defektů 1 245,2 mm², nelečených defektů 386,2 mm² a defektů ihned transplantiuvaných 677,8 mm². Tyto výsledky byly statisticky signifikantní (p < 0,001) .

2. Mikroskopicky byla pozorována velice dobrá vaskularizace a kolonizace fibroblasty s okamžitou produkci kolagenních vláken.

3. Kvalita rekonstruované kůže byla po měsíci lepší než u nelečených ran (podle modifikovaného „Vancouverského skóre“) 4,91 bodů (sledované defekty) × 9,25 bodů (kontrolní defekty bez léčby) × 5,50 bodů (okamžitě transplantované defekty).

Uprasá je redukce dermální náhrady HyproDerm kontrakci zhojených defektů a zlepšuje kvalitu obnovené kůže. Vzhledem k tomu, že obsahuje schválené neškodné materiály, je vhodné i pro klinické použití.

Acknowledgement
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Fig. 1. Large white female pig weighing 30 kg on the operating table. Six full-thickness skin defects (5 × 5 cm) just excised down to the muscle fascia paravertebrally.

Fig. 2. The composite dermal substitute HyproDermTM consisting of bovine atelocollagen and 6% hyaluronic acid. Polyurethan membrane fixed on the surface. Size 10 × 6 cm
Fig. 3. The composite dermal substitute HyproDermTM fixed with metallic staples onto the experimental wound. Dry gauze dressing on top of the substitute. Right wound still uncovered.

Fig. 4. Situation 3 days later. Substitute soaked with bloody wound secretion. Initial vascularisation can be seen.
Fig. 5. Ten days after HyproDerm™ implantation. The collagen fibres and vessels ingrowth cause the elevated wound bed to exceed the surrounding skin in the right wound. On the left, the control wound 5 (healing per secundam) below the level of surrounding skin.

Fig. 6. Overgrafting procedure. Thin dermoeipidermal graft adjusted onto the HyproDerm™ engrafted dermal substitutes in wounds 1 - 4.
Fig. 7. Completely adhering dermoepidermal graft one week after grafting. Some necrotic material on its surface. The red colour caused by the necrosis and loss of the superficial layers of epidermis due to the transitory ischaemia after grafting.

Fig. 8. The animal one month after experimental injury and 21 days after overgrafting. Note the difference between wounds 1-4 and wound 5 (upper right), which is not covered by crust.
Fig. 9. Typical histologic view of wounds 1-4 before overgrafting (day 10 post injury). The surface of vascularized dermal substitute covered by condensated layer of fibroblasts creating the outer barrier in wounds 1-4 without any difference. Below this layer reticular pattern of connective tissue is visible. Deeper continual layer of inflammatory cells can be seen. (HE).

Fig. 10. The typical histologic view of wounds 1-4 seven days after overgrafting. On the surface there is a layer of partially necrotic material, below this layer tangentially oriented collagen fibres. From the deeper layers the radially oriented collagen fibres are growing. In the dermoepidermal graft, necrotic areas can be seen and, in addition, undoubtedly live fibroblasts and keratinocytes (HE).
Fig. 11. The histologic picture of wounds 1-4 on day 31 after injury shows the structure of the graft closely similar to normal skin. The main differences: a) flat rete ridges compared to normal skin, b) thinner epidermis compared to normal skin, c) fine collagen bundles mostly tangentially oriented, d) low cellularity comparable to normal skin. (Left half of the picture – composite graft, right half of the picture – intact skin. HE).

Fig. 12. The same situation, another staining. Note the finer collagen bundles in the graft (right) than in normal skin (left). AZAN staining.