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Assessment of Two Commonly used Dermal Regeneration Templates in a Swine Model without Skin Grafting †

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Abstract: In the medical care of partial and full-thickness wounds, autologous skin grafting is still the gold standard of dermal replacement. In contrast to spontaneous reepithelializing of superficial wounds, deep dermal wounds often lead to disturbing scarring, with cosmetically or functionally unsatisfactory results. However, modern wound dressings offer promising approaches to surface reconstruction. Against the background of our future aim to develop an innovative skin substitute, we investigated the behavior of two established dermal substitutes, a crosslinked and a non-crosslinked collagen biomatrix. The products were applied topically on a total of 18 full-thickness skin defects paravertebrally on the back of female Göttingen Minipigs—six control wounds remained untreated. The evaluation was carried out planimetrically (wound closure time) and histologically (neoepidermal cell number and epidermis thickness). Both treatment groups demonstrated significantly faster reepithelialization than the controls. The histologic examination verified the highest epidermal thickness in the crosslinked biomatrix-treated wounds, whereas the non-crosslinked biomatrix-treated wounds showed a higher cell density. Our data presented a positive influence on epidermal regeneration with the chosen dermis substitutes even without additional skin transplantation and, thus, without additional donor site morbidity. Therefore, it can be stated that the single biomatrix application might be used in a clinical routine with small wounds, which needs to be investigated further in a clinical setting to determine the size and depths of a suitable wound bed. Nevertheless, currently available products cannot solely achieve wound healing that is equal to or superior to autologous tissue. Thus, the overarching aim still is the development of an innovative skin substitute to manage surface reconstruction without additional skin grafting.

Keywords: animal model; dermal regeneration; Integra; Matriderm; skin substitute; wound healing

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1. Introduction

In the human body, the skin is the largest organ and serves as a barrier to the external world. The epidermis is a stratified epithelium with proliferating basal and differentiated suprabasal keratinocytes. The dermis consists of an extracellular matrix with interwoven collagen fibrils, some elastin and glycosaminoglycan, to give physical strength and flexibility to the skin, and fibroblasts. These are able to produce remodeling enzymes, which are important for wound healing (e.g., collagenases and proteases). Skin injuries through trauma, disease, burns, surgery, or even chronic wounds can have drastic effects. In contrast

to spontaneous reepithelializing of superficial wounds, deep dermal wounds often lead to delayed healing, disturbing scarring and cosmetically or functionally unsatisfactory results.

The ultimate goal in plastic, reconstructive and burn surgery is to produce skin analogues similar to natural skin. To achieve better outcomes with a lower risk of mortality and better functional results, early permanent wound closure is recommended. Autologous skin grafting is still the gold standard of dermal replacement in partial and full-thickness wounds. The demand for modern skin substitutes is high [1–3]. Various artificial skin replacement products have been developed recently, and their use is increasingly widespread in clinical practice [3–5]. The scaffolds must meet certain requirements, besides clinical effectiveness, easy handling and safe application to the patient. Other necessary scaffold properties are:

- Biocompatibility and biodegradability;
- Ability to guide regenerative skin elements;
- Similarity to the physical strength and flexibility of normal skin;
- The 3D-matrix of naturally existing substances of the human body.

At present, acellular skin substitutes produced through lyophilization and phase separation techniques are the most convincing imitation of the extracellular matrix of the skin [6]. Many researchers are pursuing new approaches to tissue engineering. Some currently available products offer promising properties such as protecting the wound from fluid loss and microbial invasion. They support dermal cell migration and neoangiogenesis and reduce the development of scar tissue [7]. Against the background of our future aim to develop an innovative skin substitute, we investigated the behavior of two established dermal substitutes, a crosslinked and a non-crosslinked collagen biomatrix in this study. The products were investigated in full-thickness skin defects as a promoter for epithelialization without additional skin grafts as an alternative, novel approach to the originally intended use in combination with skin grafting and directly compared with a particular focus on neodermal formation.

2. Materials and Methods

In this trial, full-thickness skin defects were generated paravertebrally in female Göttingen minipigs due to the physiologic concordance to human skin [8,9]. The animals were provided from Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark, aged 39 weeks (±12 days) and weighed 22.6 kg (±1.4 kg). Animals were treated according to the German Law on the Protection of Animals, and the study was performed with permission from the local Animal Welfare Committee (approval code AT 1/12).

On the first day of the study, the Göttingen minipigs were anesthetized and their backs were shaved, surgically disinfected in a standardized manner and the outline of the wounds was tattooed. Eighteen full-thickness skin defects (2.0 cm diameter, 0.6 cm depth) were created, and a single dressing was applied with either a crosslinked biomatrix (n = 6) or a non-crosslinked biomatrix (n = 6). Control wounds remained untreated (n = 6). The crosslinked biomatrix (Bilayered Integra® by Integra Life Sciences Corporation, Plainsboro, NJ, USA) is an acellular crosslinked collagen type I and chondroitin-6-sulphate based bilayered biomatrix with a polysiloxane polymer layer. It is produced from bovine tendon and shark glycosaminoglycan. The silicone was left on the wound for seven days. The non-crosslinked biomatrix (Matriderm® 1.00 mm by Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany) is based on a compound from lyophilized collagen type I, III, V and α-elastin in a decellularized non-crosslinked biomatrix of bovine origin. The wound dressings were applied to the wounds in a randomized manner with a standardized separation distance of 6.0 cm sealed with adhesive foil (Smith & Nephew Orthopaedics GmbH, Tuttlingen, Germany) to protect the implants, avoid cross-contamination and prevent bacterial contamination. Additionally, the minipigs wore customized minipig jackets (Ellegaard Minipig Jacket Large Full Body, Lomir Biomedical Inc., Notre Dame de L'Ille Perrot, QC, Canada), as previously described [10–13].

Bandage renewal and photographic documentation were performed every second day using a digital camera with a tripod to determine the exact distance. The evaluation was carried out planimetrically by importing the photos into Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA) and calculating the percentage of epithelialization during the study period of 21 days [14]. After 20 days, original circular wound areas were excised, and samples were processed for histopathological evaluation. Histological slices were stained with hematoxylin and eosin to analyze the resulting neodermal thickness and cell density. The thickness of the neoepidermis was determined repetitively from the basal layer to the stratum corneum of the epidermis, and the number of keratinocytes was quantified within a rectangular area of 100 × 50 µm (5 mm^2), both in three different sections with an interval of 100 µm. Pictures were taken with a digital microscope camera (AxioCam ERc 5s, Carl Zeiss Microscopy GmbH, Jena, Germany) connected to a Zeiss microscope (Axio Observer.Z1, Carl Zeiss Microscopy GmbH, Jena, Germany) with the ZEN blue edition (2011) microscopy software (Carl Zeiss Microscopy GmbH, Jena, Germany). The study was carried out after approval of the local Animal Welfare Committee in accordance with the German Animal Protection Act. Statistical evaluation was performed using SPSS software version 20.0. The results were analyzed with the Wilcoxon rank-sum test with a defined p-value ≤ 0.05.

3. Results

All templates were examined macroscopically and histologically, and upon completion of the study, epidermal coverage was complete without any rejection reaction in any animal (Figure 1).

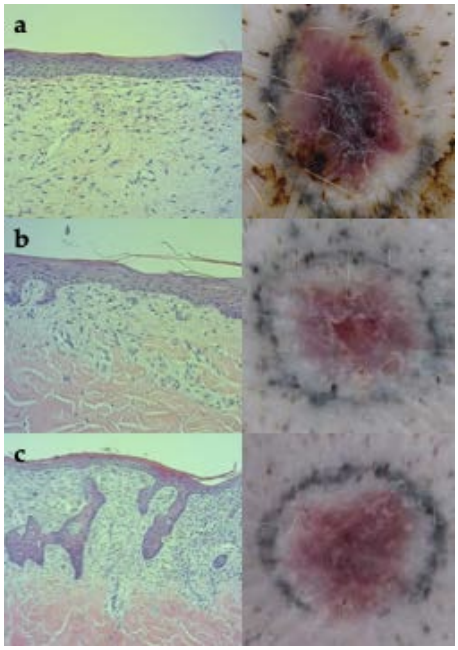


Figure 1. Macroscopic and microscopic images from tissue stained with hematoxylin and eosin of the excised former sore center in a representative (**a**) untreated wound and a wound treated with (**b**) a crosslinked and (**c**) a non-crosslinked biomatrix at 20 days after a single dressing application. All biomaterials presented a continuous epidermal layer and complete wound closure.

3.1. Planimetric Evaluation

Wound closure time decreased significantly in the treatment groups. For the control wounds, a complete epithelialization was observed after 13.50 ± 1.19 days. Planimetric analysis of wounds treated with the crosslinked biomatrix showed complete wound closure after an average of 10.00 ± 1.15 days. The non-crosslinked biomatrix-treated wounds were closed after 10.67 ± 0.94 days. Thus, the two treatment groups showed significantly

faster wound closure than the controls. The mean time for complete wound closure was accelerated by 3.50 days ($p < 0.0001$) and 2.83 days ($p = 0.0002$), respectively (Figure 2).

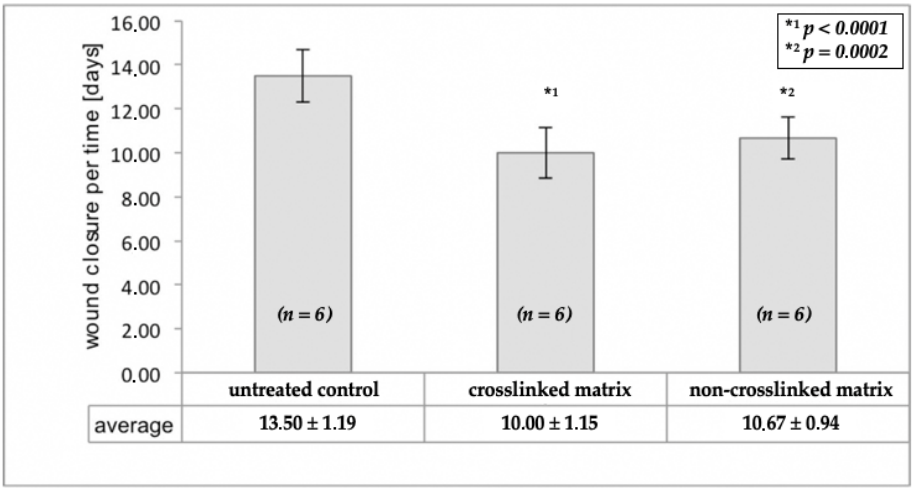
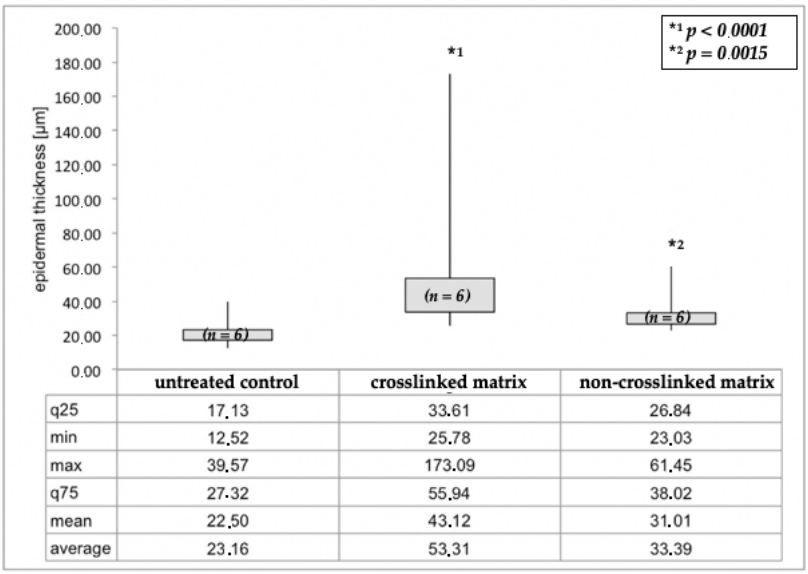


Figure 2. Time in days to complete epithelialization of control wounds and treated wounds with the crosslinked and non-crosslinked biomatrices. The wound closure was significantly increased in the treatment groups. * Statistically significant results.

3.2. Histological Analysis

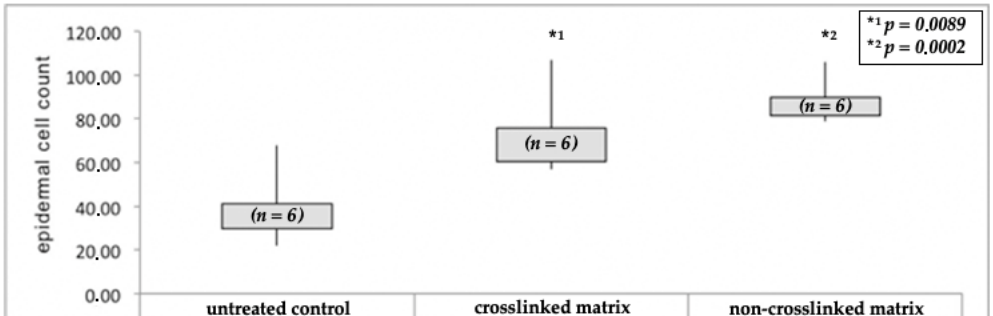
Further, the evaluation was carried out in the histological slices. In untreated control wounds, the mean neoepidermal thickness was 22.50 µm with a range of 12.52–39.5 µm. Epidermal thickness had increased from treatment with the non-crosslinked biomatrix at a mean thickness of 31.01 µm (range 23.03–61.45 µm; $p = 0.0015$) and from the crosslinked biomatrix at a mean of 43.12 µm (range of 25.78–173.09 µm; $p < 0.0001$), as shown in Figure 3. Thus, the histological examination revealed higher epidermal thickness in the crosslinked biomatrix-derived neoepidermis. Rete ridge-like formations were visible in both treatment groups (Figure 1).



	untreated control	crosslinked matrix	non-crosslinked matrix
q25	17.13	33.61	26.84
min	12.52	25.78	23.03
max	39.57	173.09	61.45
q75	27.32	55.94	38.02
mean	22.50	43.12	31.01
average	23.16	53.31	33.39

Figure 3. Epidermal thickness of untreated wounds versus crosslinked and non-crosslinked biomatrices-derived neoepidermis in three different sections per sample with an interval of 100 µm in micrometers. Histologic examination verified highest epidermal thickness in the crosslinked biomatrix-treated wounds. * Statistically significant results.

In comparison, the mean epidermal cell count for untreated controls amounted to 41,00 cells/5 mm^2 (range of 22.00–68.00 cells/5 mm^2). Higher values of cell density were seen in the crosslinked biomatrix-treated groups with 69.50 cells/5 mm^2 (range of 57.00–107.00 cells/5 mm^2, p = 0.0089), whereas in the non-crosslinked biomatrix-treated wounds the highest values for mean neoepidermal cell count with an average of 84.50 cells/5 mm^2 (range of 79.00–106.00 cells/5 mm^2, p = 0.0002) could be observed. The templates were fully integrated into the host tissue with the colonization of epidermal minipig cells within all sections at statistically significant levels (Figure 4).



	untreated control	crosslinked matrix	non-crosslinked matrix
q25	29.75	60.50	81.50
min	22.00	57.00	79.00
max	68.00	107.00	106.00
q75	47.75	88.25	99.50
mean	41.00	69.50	84.50
average	41.33	75.83	89.83

Figure 4. Neoepidermal cell density in untreated, the crosslinked and non-crosslinked biomatrices-derived neoepidermis within a section of 100 µm width taken repetitively in three different sections. Histologic examination demonstrated the densest colonization of neoepidermal cells in the non-crosslinked biomatrix-treated wounds. * Statistically significant results.

To sum up, treatment groups showed overall improved wound healing with accelerated wound closure, thicker neoepidermal tissue and increased epidermal cell density in comparison to untreated wounds.

4. Discussion

Full-thickness skin injuries, such as deep burn wounds, generally require surgical intervention in addition to sufficient debridement. In medical care, autologous skin grafting is still the gold standard of dermal replacement [15–17]. However, when harvesting a skin graft, a new wound surface will arise and might result in hypertrophic scarring, severe pain, unsatisfactory cosmetic and functional complications or even additional chronic wounds in patients with microcirculation disorders [18]. In burn patients, the harvesting of a graft leads to a larger wound area with an increased risk of fluid loss, infection and temperature loss. Moreover, in extensive burns, the donor region is limited, so the overall aim in plastic, reconstructive and burn surgery remains the use of skin analogues similar to natural skin. There are various manufactured skin substitutes, but at present, there is still no commercially available skin substitute that can restore all necessary characteristics of the native skin [19]. Beginning in the early 1980s, tissue engineering developed some skin replacement products with promising results with deep dermal burn wounds and for use in reconstructive surgery [20–23], including the analyzed crosslinked and non-crosslinked biomatrices. Dermal substitutes such as these are mainly acellular 3-dimensional scaffolds and of allogenic, xenogenic or synthetic origin, which nourish the epidermal layer or an epidermal graft (i.e., skin grafts). Both analyzed products contain collagen, the key component of the human skin. Cell migration takes place along the collagen fibers in an organized manner.

The non-crosslinked biomatrix is used for dermal regeneration with promising results comparable to pure full-thickness grafts but with marginally slower regeneration compared with split-thickness skin grafting only [24]. Even in wet conditions it retains its stability and elasticity [25] and improves the biomechanical properties of the regenerating tissue due to the elastin component [26]. It reduces wound contraction and scar formation, so the reconstructed skin is stress-resistant sooner and patients' rehabilitation is faster, shortening their hospital stay and sophisticating long-term results [27,28].

The crosslinked biomatrix also serves as a dermal regeneration template. The dermal matrix with its open pore structure promotes the ingrowth of fibroblasts and endothelial cells [29]. Its silicone layer functions as a temporary barrier preventing fluid loss and protects against mechanical influences and bacterial contamination [20,25,30–33]. Usually, the silastic sheet may be removed 15 to 28 days after placement [29]. In our study, it was left on the wound for seven days because we already observed wound healing and did not want to integrate the silicone into the wound and compromise epithelialization. Klein et al. already showed that deep facial burns tolerate the removal of the silicone layer after seven to ten days without difficulties [34]. By applying the crosslinked biomatrix complex, skin defects, even with exposed anatomical structures, can be managed and this process might even reduce the necessity for reconstructive techniques, such as local or free flap transplantation [35]. Nevertheless, artificial skin replacement products have disadvantages, including their relative expense, the risk of infection complications and, if the treatment is insufficient, patients will suffer fragile wounds and consequent surgical revisions [36].

The crosslinked and non-crosslinked biomatrices are usually applied together with split-thickness skin grafting, as Böttcher–Haberzeth et al. did in rats [5]. They demonstrated that the crosslinked and non-crosslinked biomatrices offer promising approaches for one-step closure with a neonatal rat epidermis. The crosslinked biomatrix-derived neodermis was thicker than in the non-crosslinked biomatrix-treated wounds, and cell density was higher in the non-crosslinked biomatrix-derived neodermis, same as in our study concerning the neoepidermis. The analyzation of the data with a particular focus on neoepidermal formation, even though the products are intended for use in dermal replacement, represents a further discrepancy in the recommended handling. Nevertheless, in the present study, the two biomaterials were investigated as a dermal replacement and a single promotor for epithelialization at the same time without additional skin grafting, and, therefore, no donor site morbidity. Both treatment groups showed significantly faster reepthelialization than the controls, which suggests the presumption that the dermal substitutes offer a better surface for keratinocytes to move from the wound edges. Furthermore, the histologic examination demonstrated rete ridge-like formations especially in the non-crosslinked biomatrix-treated wounds. These are advantageous because the surface area of the epidermis is increased at the dermoepidermal junction, and the cells of the epidermis receive their nutrients through improved contact with the blood vessels in the dermis. In addition, rete ridges provide a niche for stem cells, protected from environmental stress with epidermal shearing, such as shear forces. With this, support of skin regeneration could be observed through the application of the dermis replacement products and can even reduce the need for local or free flap-plastic defect coverage [35].

However, only minor wounds reepithelialize without the need for transplantation [37] as also shown by Fulchignoni et al. They recently published a clinical study, comparing the use of the non-crosslinked biomatrix with or without grafting in the treatment of fingertip tissue loss [38]. So, in the current study, it would have been an interesting point to analyze wounds of increasing sizes. Moreover, additional data on the functionality of the skin and vascularization would have been further interesting aspects.

This led us to look again at this issue. The experimental setup chosen in our study turned out to be a reliable option as a wound-healing model [9]. In general, porcine skin shows recognizable similarities to human skin in terms of its anatomical composition and physiological behavior, as described extensively in the literature [39–41].

5. Conclusions

In conclusion, this trial showed comparable biological behavior with successful wound closure using crosslinked and non-crosslinked biomatrices in full-thickness wounds. Our data presented a positive influence on the epidermal regeneration and support of epithelialization with the chosen dermis substitutes even without additional skin transplantation and, thus, without additional donor site morbidity. Therefore, it can be stated that the single biomatrix application might be used in small wounds, which needs to be investigated further in a clinical setting to determine the size and depths of a suitable wound bed. Nevertheless, currently available products cannot solely achieve wound healing that is equal to or superior to autologous tissue. Thus, the overarching aim still is the development of an innovative skin substitute to manage surface reconstruction without additional skin grafting.

Author Contributions: Conceptualization and methodology, W.E., M.H. and A.R.-S.; software, J.-O.B. and M.H.; formal analysis, J.-O.B. and M.D.; validation, investigation, and data curation, W.E., J.-O.B. and M.H.; resources, A.R.-S. and A.D.; writing—original draft preparation, W.E. and M.D.; writing—review and editing, W.E.; visualization, M.H. and M.D.; supervision and project administration, A.R.-S. and A.D.; funding acquisition, W.E.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki. Animals were treated according to the German Law on the Protection of Animals, and the study was performed with permission from the local animal welfare committee (approval code AT 1/12) of the University of Tübingen in Germany.

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Conflicts of Interest: The authors disclose no conflict of interest.

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