Comparison of Glyaderm with Different Dermal Substitute Matrices in a Porcine Wound Model

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ABSTRACT

Closure of extensive burn wounds with widely expanded autologous Split-Thickness Skin Grafts (STSG) is associated with undesirable scar formation and contraction, due to the lack of dermis. Various materials for dermal replacement have been developed, either from xenogeneic or allogeneic origin and are placed in the wound underneath a thin STSG in order to improve scar quality. A porcine wound model was used to compare available acellular dermal substitutes with a prototype prepared from glycerol preserved human skin: Glyaderm.

Keywords: Dermal substitute, Burns, Glyaderm, Graft take, Surgery, Scar quality

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INTRODUCTION

Extensive full thickness burn wounds can be closed with widely expanded autologous Split-Thickness Skin Grafts (STSG) after excision of the necrotic tissue. The results obtained with this standard surgical technique are less favorable with respect to contraction and the quality of the resulting scar tissue, mainly due to the lack of the dermal layer. Several materials for dermal replacement have been developed. These substitution materials can be placed underneath the STSG and serve as a scaffold into which cells can migrate and repair the wound, ultimately resulting in less scar tissue formation and contractures.

Nowadays, different dermal substitutes such as Alloderm, Matriderm and Integra are available on the market, but the benefit and cost-effectiveness of these materials is still under discussion. Alloderm (Life cell Corp., Branchburg, NJ) is an cellular dermal substitute processed from cryopreserved human cadaver skin. When used with very thin STSG the take rate of Alloderm was improved and in the long term less scarring and contracture were reported consists of cross-linked bovine collagen and chondroitin-6-sulfate covered with a silicon layer to temporarily close the wound. Integra is applied during the first operation after meticulous debridement and preparation of the wound bed. After a period of 2-4 weeks, the silicon layer is removed during a second operation and a STSG is placed to close the wound. The silicon layer serves as a barrier against bacteria, it controls water evaporation and provides mechanical support. Several studies using Integra have reported less hypertrophic scar formation but also increased risk of infection mainly underneath the silicon layer. Another dermal substitute, which is now commercially available as MatriDerm (MedSkin Solutions Dr. Suwelack, Billerbeck, Germany) consists of a lattice of bovine collagen coated with elastin hydrolysate. Promising results were obtained but on the long term (1 year after application), no significant difference was observed when compared to wounds treated with only split skin grafts except for a less visible mesh pattern in the scar. The possibility of MatriDerm to be used in a single-step procedure can be a practical advantage but comparative clinical data are limited. In a small clinical trial improved elasticity of the scar was observed in the group treated with Matriderm and sheet auto grafts compared to wounds treated with sheet auto grafts only. There were no significant differences within the groups if wounds were closed with meshed auto graft.

MATERIALS and METHODS

Human participants were not involved in this study. The experiments with animals were approved by the animal welfare committee of the Vie Universities Medical Centre, Amsterdam. Human skin was obtained from donors with consent for research according to the Dutch Law on Organ donation.

Dermal substitute materials

Glyaderm was prepared from human donated skin by the Euro Skin Bank as described earlier using low concentrations of NaOH (0,06 M). The skin was obtained from donors with consent for research related to the field of transplantation. DED was prepared according to the method in literature using incubation in Phosphate Buffered Saline (PBS). Cellular dermal tissue was prepared by repeated washing of glycerol Preserved Donor Skin (Euro Skin Bank, Beverwijk, the Netherlands) in sterile PBS (PBS-dermis) supplemented with 50 IU/ml-1 penicillin G; 50 g/ml -1 streptomycin (Gibco, Paisley, U.K.), for 3 weeks at 37°C and further stored at 4°C (no longer than 6 months). Alloderm was ordered from Life cell (Lifecell Corp., Branchburg, NJ) and Integra from Life sciences Corp, Inc, Plainsboro, NJ)

Animals

Twelve female Yorkshire pigs (weight 30-35 kg) were used. We used the same animal model as described earlier. A grid was tattooed one week before the first surgical procedure, by cutting the skin with a scalpel till sub-epidermal depth and applying tattoo ink. In this way, we can measure wound contraction and correct for the growth of the animals.

EXPERIMENTAL PROCEDURES

One stage procedure

We prepared four full thickness excision wounds of 4 × 4 cm on each flank of the animal, under general anesthesia. The dermal matrix materials that were tested (Glyaderm, Alloderm and DED) were meshed 1:1,5 and sutured into the wounds. We used an Aesculapius dermatome to harvest autologous split skin (0.2-0.3 mm thickness) from the back of the animal. The autologous skin was meshed 1:3 and was sutured on top of the dermal matrix.[1] finally; skin grafts were covered with SurfaSoft. Further wound dressing was performed as described earlier and consisted of cotton gauzes soaked in 0.9% NaCl which were fixed using adhesive bandages (Curafix). The dressings were protected from mechanical disturbance using an elastic bandage (Tubigrip).

Two-stage procedure

The animal model was slightly adjusted, again 4 full thickness excision wounds of 4 × 4 cm were prepared under anesthesia on each side of the animal, but then the control wounds were directly covered with autologous split skin meshed 1:3. Glyaderm and Integra were meshed 1:1,5 and sutured into the wounds with Surf soft on top of the Glyaderm. Iso-Betadine gel was added on the Surf soft and on the Integra. Wounds were further dressed with cotton gauze soaked in 0.9% NaCl which were fixed using Cur fix and covered with Tub grip. Dressings were changed at day 4 post surgery. Seven days later, the second operation was performed.[2] We removed the dressings and the Surf soft from the Glyaderm. Also, the silicon layer was peeled off from the Integra. Autologous split skin grafts were harvested from the back of the pig, meshed 1:3 and sutured on top of the dermal substitutes using Surf soft for final coverage. Wounds were dressed as described above.

Evaluation of wound healing

Wound dressings were changed on day 4 and 7 post surgery, checked for any signs of infection. Thereafter, wound dressings were weekly changed until the wounds were completely closed. We carefully removed the Surf soft at day 7 after transplantation of the autologous skin and the take rate of the skin was scored. We took biopsies (4 mm) at day 7 and 14 after wounding for histology. Wounds colonized with bacteria were excluded from analysis. The pigs were sacrificed at 8 weeks after operation. After macroscopic inspection of the scars and measurement of wound contraction using planimetry, we excised large biopsies covering the full width of the wound for histology. We took digital photographs to evaluate the macroscopic aspects of wound healing evaluation at day 7, 14 and day 56 after operation.

Planimetry

This was performed as described in earlier studies. Briefly, we measured wound contraction by tracing the edges of the wound and the tattoo grid on transparent film. Then Visitrak was used to measure contraction, expressed as a percentage of the original wound area, corrected for the growth of the animals.

After 10 min fixation in acetone, slides were incubated with the sections with the SMA antibody diluted in PBS for 45 min at room temperature. Thereafter the slides were washed three times with PBS followed by incubation with a secondary antibody conjugated with horseradish-peroxidase (rabbit anti mouse, Dako, Glostrub, Denmark) for 1 hour at room temperature. After a washing step using PBS, the slides were incubated with diaminobenzidine (Dako) to stain the positive cells (dark brown color).

Two independent observers analyzed the stained sections and scored the influx of cells. The haematoxilin sections were used to analyze the influx of inflammatory cells in the wound. In the large biopsies taken at the end of the experiment at week 8 after operation, an ocular grid was used in the microscope to quantify the areas in the dermal matrices with inflammatory cells. My fibroblasts presence was quantified on digital images of the stained sections and analyzed using Lucia G software. Since pericytes in the blood vessels are also positive for smooth muscle actin, these structures were first excluded from the regions of interest before quantification.[3] The effects of the treatments on wound contraction, the inflammatory response and number of my fibroblasts were tested for significant differences with the Mann-Whitney test using Instat software. We considered a $P < 0.05$ to be statistically significant.

DISCUSSION

The aim of this study was initially to compare the Glyaderm prototype with other human dermal matrix types; Alloderm and De-Epidermises Cellular Dermis (DED). The NaOH method to de-cellularize human donor skin (Glyaderm) is more stringent. Less cells and remnants of appendages could be detected in sections of the material. This could explain the milder inflammatory response in the wounds treated with this prototype compared to wound treated with Alloderm or DED. As a consequence, wounds treated with the NaOH prototype showed better results with respect to color and smoothness of the scar although there were no significant differences in wound contraction. Nevertheless, this could only be observed in wounds with a good survival (take rate) of the autologous split skin graft. Survival of STSG decreased when placed on a dermal matrix compared to the control wounds covered with only autologous STSG. The take rate on Glyaderm was lower than on Alloderm but higher compared to DED. The NaOH may have caused some damage to the basal membrane molecules which are important for outgrowth of the epithelial cells as has been shown in vitro and vivo. The method for processing DED preserves intact basal membrane molecules but the inflammatory response in the matrix may hamper the ingrowth of blood vessels from the wound bed. Although the inflammatory response was lower with Glyaderm, no significant differences were observed with respect to wound contraction. The number of my fibroblasts, which are known to be related to contraction and scarring, did not differ. For the initial survival during the first days,

the autologous skin graft depends on diffusion of nutrients from the wound fluid before newly formed blood vessels are connected. It takes at least 4 days for the endothelial cells to invade the relatively tightly woven collagen and elastin fibers from these human skin derived matrices.

Thereafter, we tested the Glyaderm prototype in a two-stage procedure with a one week interval between the first operation and the second operation applying the autologous split skin graft. In our porcine model, this time period was sufficient to reach a take rate comparable to control wounds without Glyaderm as well as wounds treated with Integra. The good survival of the STSG indicated a fast ingrowth of fibroblasts and blood vessels into the Glyaderm matrix, leading to significantly reduced wound contraction compared to the control wounds. Interestingly, the numbers of inflammatory cells were also lower in wounds treated with Glyaderm in the two-stage procedure compared to the one stage procedure, both early after wounding (day 7) and at day 56. In addition, the numbers of my fibroblasts in the scars at day 56 were significantly lower when compared to control wounds, covered with only autologous split skin. As expected, wounds treated with Integra also showed improved results compared to the controls. There were no significant differences in wound contraction and numbers of my fibroblasts between Integra or Glyaderm in the two stage procedure, only the presence of giant cell formation in wounds treated with Integra was observed. This may be caused by the glutaraldehyde crosslinking of the fibers in the Integra.

Ducked et al. Have described the use of Integra in a one stage procedure in a porcine model and did not observe differences in contraction between control and Integra-treated wounds. Thus, although two operations are needed, final results with respect to wound contraction and scar formation are much better if a dermal substitute like Integra or Glyaderm is used in a two-stage procedure. In this way, it is possible to apply a human tissue based dermal substitute with a thickness of 0.3-0.5 mm to the wound. Only thinner dermal substitutes with a more open structure that allow faster ingrowth of blood vessels may be used in a one-stage procedure.[4] These types of dermal substitutes lack the natural structures of collagen and elastin fibers present in Glyaderm that can modulate fibroblasts to produce more randomly organized collagen fibers. Open pore structure matrices are more vulnerable to early degradation by Matrix-Metallo-Proteinases (MMP's) produced by infiltrating fibroblasts and macrophages.

These good results obtained with Glyaderm in the two-stage lead to a pilot study with a group of 12 burn patients. Growth of blood vessels from the wound bed into Glyaderm was assessed using laser Doppler imaging. An interval of 5-7 days between the first operation and the second operation was sufficient to achieve a take rate > 95 %. Thereafter, an intra-individual comparative clinical study was performed to evaluate the long-term effects of the Glyaderm on quality of the scar. The elasticity of the scar was significantly improved when Glyaderm had been applied to the wound, at 1 year after treatment. In combination with negative wound pressure therapy, several layers of Glyaderm could be applied on exposed bone and the wound could be successfully closed with STSG. Biopsies taken 7 days after implantation clearly showed new collagen in the Glyaderm, confirming the observation in the porcine model.[5] The human donor derived, native elastin fibers serve as a scaffold for the autologous fibroblasts, resulting in scar tissue with improved elasticity.

RESULTS

Inflammatory response in the dermal matrices

In the sections of the biopsies from day 7 and 14, numerous immune cells (neutrophil granulocytes and macrophages) were seen in the dermal matrices compared to the control wounds. The highest numbers seemed to be present in the DED matrix, followed by Alloderm. The number of cells that could be observed in the Glyaderm matrix was relatively low but higher in comparison with the control wounds. In the sections from the biopsies taken at the end of the experiment (8 weeks after operation), the dermal matrices could be observed in the newly formed scar tissue. Elastin fibers surrounded with large collagen fibers were present that could be easily distinguished from the new thin fibers produced by fibroblasts migrated into the matrices. Macrophages and lymphocytes were also present in the dermal matrices, sometimes large accumulations were observed, especially in the DED matrix. Since the week 8 sections were from biopsies covering the whole wound area (the biopsies measured 5x 1 cm), it was possible to quantify the areas within the dermal matrices covered with inflammatory cells. As can be seen in the inflammatory response was significantly higher in the DED matrix and Alloderm compared to Glyaderm.

Two stage procedure

Effect of a two-stage procedure on the survival of the autologous skin: Since survival of the STSG on the dermal substitutes was reduced compared to the control wounds transplanted with autologous skin only, we decided to test Glyaderm in a two-stage procedure. In this procedure, the Glyaderm

was placed into the wound bed and covered with Surf soft and wound dressings during a week, allowing fibroblasts and newly sprouted blood vessels to grow in from the wound bed into the matrix. When the dressing was removed, the Glyaderm had a slightly red appearance if no infection or dehydration had taken place. The risk for bacterial contamination is higher compared to the one stage procedure but by using iso-Betadine on the Surf soft it could be controlled. The thickness of Glyaderm had to be < 0.6 mm to enable blood vessel ingrowth within one week.

The take rate of the autologous skin on the dermal substitute is much higher compared to the one stage procedure (Table 1) and comparable to control wounds and Integra in a two-stage procedure (Table 2).

Number of wounds		Mean take rate*	Range take rate
Control	12	92	75-100
Glyaderm	12	65	25-100
AlloDerm	6	75	35-100
DED†	8	50	20-100

Table 1. Take rate of autologous split skin in the one stage procedure

Number of wounds		Mean take rate *	Range take rate
Control	12	95	75-100
Glyaderm	8	90	60-100
Integra	6	96	55-100

Table 2. Take of the autologous skin in the two stage procedure

Inflammatory response in the dermal matrices

Compared to the one-stage procedure, the numbers of inflammatory cells in the wounds treated with Glyaderm were lower at day 7 and 14 after wounding. The influx of immune cells in the Integra matrix was comparable but some giant cells were present. At 8 weeks after wounding, elastin fibers of Glyaderm could still be observed in the newly formed scar tissue, surrounded by newly produced collagen fibers. Most of the collagen fibers of the Glyaderm matrix were already replaced by new collagen fibers. In the Glyaderm matrix only a few accumulations of macrophages and lymphocytes could be observed but around the Integra fibers still some giant cells were present at week 8 after operation.

CONCLUSION

In conclusion, a cellular dermal substitute such as Glyaderm can be successfully used to reduce wound contraction in the porcine wound model. Glyaderm with a thickness>0.5 mm should be used in a two-step procedure for optimal results. During the interval between the first step and second step, coverage with autologous STSG, blood vessels and fibroblasts will populate the Glyaderm. In this way, the survival of the STSG is improved, resulting in a better quality of the final scar.

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