

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/burns

Glyaderm[®] dermal substitute: Clinical application and long-term results in 55 patients



Ali Pirayesh^a, Henk Hoeksema^a, Cornelia Richters^{b,c}, Jozef Verbelen^a, Stan Monstrey^{a,*}

^a Department of Plastic and Reconstructive Surgery, Burn Center, Ghent University Hospital, Ghent, Belgium

^b Department of Molecular Cell Biology and Immunology, Medical Faculty, Vrije Universiteit Medical Center, Amsterdam, The Netherlands

^c Euro Skin Bank, Beverwijk, The Netherlands

ARTICLE INFO

Article history:

Accepted 16 May 2014

Keywords:

Glyaderm[®]
Dermal substitute
Acellular dermal matrix
Full thickness burn
Skin substitute

ABSTRACT

Introduction: Glycerol preserved acellular dermis (Glyaderm[®]) consists of collagen and elastin fibers and is the first non-profit dermal substitute derived from glycerol-preserved, human allogeneic skin. It is indicated for bi-layered skin reconstruction of full thickness wounds.

Methods: A protocol for clinical application and optimal interval before autografting with split thickness skin graft (STSG) was developed in a pilot study.

A phase III randomized, controlled, paired, intra-individual study compared full thickness defects grafted with Glyaderm[®] and STSG versus STSG alone.

Outcome measures included percentage of Glyaderm[®] take, STSG take, and scar quality assessment.

Results: Pilot study (27 patients): Mean take rates equaled 91.55% for Glyaderm[®] and 96.67% for STSG. The optimal autografting interval was 6 days (± 1 day).

Randomized trial (28 patients): Mean Glyaderm[®] take rate was 88.17%. STSG take rates were comparable for both research groups ($p = 0.588$). One year after wound closure, Glyaderm[®] + STSG was significantly more elastic ($p = 0.003$) than STSG alone. Blinded observers scored Glyaderm[®] treated wounds better in terms of scar quality.

Discussion: The efficacy of Glyaderm[®] as a suitable dermal substitute for full thickness wounds is attested. Currently a procedure for simultaneous application of Glyaderm[®] and STSG is adopted, allowing for further widespread use of Glyaderm[®].

© 2014 Elsevier Ltd and ISBI. All rights reserved.

1. Introduction

Dermal substitution has become an integral part of surgical burn care and many commercial dermal equivalents have emerged on the market since the introduction of Integra[®] dermal substitute (Integra LifeSciences Corporation) some two decades ago [1–3].

We extensively reported on the various cellular, acellular, temporary and permanent skin replacements available for burns and full thickness defects in a previous publication [4].

Glycerol preserved acellular dermis (Glyaderm[®] – Euro Skin Bank, Beverwijk, The Netherlands) is the first non-profit dermal substitute derived from glycerol preserved, human allogeneic skin [4–6]. Glycerol preserved allogeneic skin (GPA)

* Corresponding author at: Department of Plastic Surgery, Burn Center, Ghent University Hospital, B-9000 Gent, Belgium. Tel.: +32 93 32 32 26; fax: +32 93 32 38 99.

E-mail addresses: Stan.Monstrey@UGent.be, vp15vnj@hotmail.com (S.).

<http://dx.doi.org/10.1016/j.burns.2014.05.013>

0305-4179/© 2014 Elsevier Ltd and ISBI. All rights reserved.

is routinely utilized as a temporary biologic dressing on partial thickness burns and as a means of wound bed preparation on excised burns. Allograft coverage prevents dehydration and infection of the wound and stimulates granulation formation to prepare the wound for closure with autologous skin [5,6]. Allografts contain donor cells, which are ultimately rejected and can therefore only be used as temporary wound coverage. Glyaderm[®], which is decellularized by treatment with sodium hydroxide (NaOH), can be used to replace lost dermis in full thickness wounds serving as a dermal substitute. Glyaderm[®] consists of a collagen and elastin fiber network with native collagen and can ensure a bilayered skin restoration in combination with a thin autologous split skin graft. It is intended to be cost-effective and easy to use for widespread application in full thickness wounds such as full thickness burns. Glyaderm[®] is placed in a wound bed prepared with allografts, after which, a thin autologous split thickness skin graft (STSG) will close the wound following Glyaderm[®] ingrowth. Animal studies showed favorable results in terms of tissue integration and wound contraction and scar quality [6].

We first initiated a phase I pilot study to elucidate the most practical protocol for Glyaderm[®] application and to further investigate the scope of use of the dermal matrix in the clinical setting.

The second study was a phase III randomized, controlled, paired, intra-individual comparison of full thickness skin defects engrafted with Glyaderm[®] and STSG versus STSG alone.

2. Materials and methods

2.1. Enrollment

Between September 2005 and October 2010 27 patients were recruited for the pilot study and 28 patients met the criteria for inclusion in the randomized controlled, paired, intraindividual trial.

Study protocols were approved by the Ghent University Hospital Ethics Committee.

Glyaderm[®] was produced and provided by Euro Skin Bank, Beverwijk, The Netherlands. The preparation steps of Glyaderm[®] have been described previously [6].

2.2. Phase I pilot study

The pilot study was initially performed to assess the scope of clinical applications of Glyaderm[®] as a dermal substitute and to optimize usage protocol. Patients with full thickness burns, but also other full thickness skin defects were considered eligible for this study.

All burn wounds that were not clearly full thickness on clinical assessment were treated during the first 48 h with an enzyme alginogel (Flaminal[®] Forte – Flen Pharma) [7] and covered with a paraffin gauze dressing (Jelonet[®] – Smith & Nephew). Flaminal[®] Forte combined with Jelonet[®] ensured maintenance of a moist wound environment [7] for the first 48 h prior to assessment by laser Doppler imaging (LDI). This is the standard treatment for all burns admitted to the Ghent Burn Center.

In our burn center we use the MoorLDI2-BI imager (Moor Instruments Ltd., Axminster, UK) to objectively determine the healing potential of the burn [8]. LDI is now becoming a standard of care for early diagnosis of healing potential, which is a main determinant of subsequent treatment policy. In clinical trials LDI ensures exact comparison between two burns without depth difference bias.

In this study, besides clinical observation, LDI was also intended to monitor the rate of vascularization into the dermal substitute and thereby to delineate the optimal time between the application of Glyaderm[®] and the final coverage with an autologous STSG. Ingrowth of blood vessels into Glyaderm[®], resulting in increased blood flow through the dermal substitute, was assessed by means of LDI at day 1, 3, 5 after the application of Glyaderm[®] to the wound. An increase in flux values over the measurement period was interpreted as increased blood vessel ingrowth. Biopsies were harvested before autografting to support this hypothesis. In order to visualize blood vessel ingrowth into Glyaderm[®] the sections taken from the biopsies were colored with antibodies against alpha-smooth muscle actin (ASMA) in order to demonstrate the presence of myofibroblasts and pericytes, which are supporting cells for blood vessels.

Efficacy of the protective open pore structure polyamide dressing (Surfasoft[®] – MediProf) and finally the coverage with a 10% povidone iodine (PVP-I) gel (iso-Betadine[®] Gel – Meda-Pharma Belgium) in combination with Jelonet[®] was tested.

Outcome measures were percentage of Glyaderm[®] take and percentage of STSG take.

Patients were invited for a long-term follow-up after complete scar maturation. The long-term scar assessment included objective measurement of elasticity with the DermaLab[®] (Cortex Technology, Denmark) and measurement of scar erythema and pigmentation with the DermaSpectrometer[®] (Cortex Technology, Denmark), as well subjective scar evaluation by means of the adapted Vancouver Scar Scale (aVSS) and the Patient and Observer Scar Assessment Scale (POSAS). The aVSS, besides scar color, pigmentation, pliability and scar height also takes into account scar itching and the presence of defects.

In 4 patients biopsies were taken at 1 month and sent for histological analysis. Biopsies were fixed in 4% formalin and were further processed into paraffin. Sections were prepared and stained with Haematoxylin-Eosin and Elastica von Giesson to study the presence of Glyaderm[®].

3. Phase III study

3.1. Study design

This was a randomized, controlled, paired, intra-individual comparison of full thickness skin defects engrafted with Glyaderm[®] and STSG (experimental treatment) versus STSG alone (conventional treatment).

3.2. Study objective

Primary outcome measure was comparison of autograft survival at one week between full thickness defects treated with Glyaderm[®] plus STSG versus STSG alone.

Secondary outcome measures were the functional and cosmetic outcome of skin restoration of full thickness defects treated with Glyaderm[®] plus STSG versus STSG alone 1, 3, 6 and 12 months post wound closure.

3.3. Patient selection

Patients up to 80 years of age with full thickness burns or full thickness lower arm defects after free flap harvesting were considered eligible.

Burn wounds had to be either clearly full thickness burns as clinically assessed by two plastic surgeons, or flux values measured by LDI had to be below 200, corresponding with a healing time clearly longer than 21 days.

Eligible patients with the possibility to follow the complete treatment schedule were consented for the trial.

Patients with one or more serious medical conditions that, in the opinion of the investigator, made the patient an inappropriate candidate for the study, or any condition that seriously compromised the patient's ability to complete this study, were excluded. Patients with TBSA of over 40% and patients who had participated in another study utilizing an investigational drug within 30 days prior to study inclusion were also excluded.

3.4. Randomization

The experimental and conventional treatments were confined to anatomically related areas to allow a paired, intra-individual comparison. Preferably a right/left comparison was made; if not feasible, a superior/inferior or medial/lateral comparison within a wound surface area was performed.

To exclude any bias due to selection of the surgeon or the researcher, investigators received pre-sealed envelopes containing individual patient's treatment assignments according to a predetermined scheme randomizing the experimental treatment.

Randomization was performed in the operation theater after the plastic surgeons had removed the allografts used for wound bed preparation and assessed the wound to be ready for STSG application. Usually this would be at the second operation, unless further wound bed preparation with allografts was necessary at that stage.

3.5. Surgical regimen (Table 1)

The first operation consisted of either full thickness removal of the burn scar performed as soon as possible after burn depth diagnosis, or the harvesting of the free radial forearm flap resulting in an almost circumferential (16 cm × 13 cm) defect (Table 1).

In both cases this was followed by application of glycerol preserved allografts meshed 1:2 for wound bed preparation.

The second operation was performed 5–10 days after the first operation and the surgery to be performed depended upon the quality of wound bed preparation with the allografts.

If wound bed preparation was not satisfactory, allograft application would be repeated.

If wound bed preparation was satisfactory the experimental (Glyaderm[®] + STSG) and conventional (STSG) treatments were confined to anatomically related areas to allow a paired, intra-individual comparison according to the randomization scheme.

After removal of the allografts and scrubbing with a PVP-I 10% solution (iso-Betadine[®] Dermicum – MedaPharma Belgium) and saline, and hemostasis with adrenaline soaked gauzes, the wounds were treated with sutured or stapled application of Glyaderm[®], perforated 1:1, on the treatment side and renewed application of allograft on the conventional treatment side. Both wounds were covered with Surfasoft[®].

Final operation, also performed 5–7 days after treatment confinement, as guided by clinical assessment and supported by LDI, consisted of the removal of the allografts at the conventional side and gentle scrubbing of the Glyaderm[®] dermal matrix and the application of a STSG (0.010 in) on top of both study treatment areas. Mesh ratio was always similar for the experimental side as well as for the conventional treatment side. Autografts were covered with Surfasoft[®].

3.6. Wound treatment regimen

All burn wounds that were not clearly full thickness on initial clinical assessment were treated once daily during the first 48 h with iso-Betadine[®] Dermicum for decontamination followed by application of Flaminal[®] Forte covered with a Jelonet[®] dressing and a dry sterile gauze dressing. Clearly full thickness burns were treated with cerium nitrate–silver sulphadiazine (Flammacerium[®] – Sinclair Pharmaceuticals Ltd.) until the first operation.

Allografts were covered daily with iso-Betadine[®] gel and Jelonet[®] until the next operation. The same applies to Glyaderm[®].

Autografts were dressed with Jelonet[®], iso-Betadine[®] gel and a covering dry sterile gauze dressing until day one post application after which the wounds were dressed daily with, iso-Betadine[®] Dermicum soaked gauzes, Jelonet[®] and dry sterile gauze until removal of the Surfasoft[®] layer at day 6–7.

Donor sites were dressed with Hydrofiber[®] silver dressings.

3.7. Study assessments

All data were recorded in a purpose designed database.

Table 1 – Phase III randomized trial patient treatment scheme.

	Glyaderm [®] + STSG (experimental treatment)	STSG alone (conventional treatment)	
Wound bed preparation	1st operation: Allograft	1st operation: Allograft	Wound bed preparation
Dermal substitute	2nd operation: Glyaderm [®]	2nd operation: Allograft	
Autografting	3rd operation: STSG	3rd operation: STSG	Autografting

3.7.1. Baseline research group characteristics

Patient demographics were recorded at study inclusion. Patient gender, age, burn cause, total body surface area (TBSA) that was burned in %, burn body location, TBSA represented by the target wounds in % were noted.

3.7.2. Wound evolution

Clinical wound assessments were conducted twice weekly from inclusion to full wound closure. Wounds were photographed, if possible, the day of, or after admission and also the day of LDI and thereafter twice weekly and at every surgical procedure.

Wound swabs were harvested for semi-quantitative and qualitative microbiological investigation on admission, on the day of LDI and then repeatedly on a weekly basis from the region of interest as well as other burn areas according to a standard microbiology swab procurement regimen which exists as an integral part of the Ghent Burn Center wound care policy.

3.7.3. Take rates

Glyaderm[®] was evaluated with LDI at postoperative day 1, 3 and 5 for vascular ingrowth. Glyaderm[®] take rates were scored at day 6–7 post Glyaderm[®] application, during the autograft procedure. STSG take rates were scored at day 6–7 post autograft application and after Surfsoft[®] removal.

3.8. Treatment after wound closure

3.8.1. Pressure garments and silicones

Scar treatment was the same for both groups and consisted of custom made pressure garments and/or silicone garments.

There was an individual and especially adapted schedule worked out for every patient, regarding the silicone pressure garments.

3.8.2. Hydration of the scar

Hydration of the dry skin is necessary at least three times a day. All patients were using the same product Alhydran[®] (BAP-Medical) [9] during the complete follow-up period of 1 year.

3.9. Follow-up assessments

At regular follow-up of 1, 3, 6 and 12 months objective and subjective scar assessment was performed.

Objective evaluation of elasticity was performed using the DermaLab[®]. For color and pigmentation assessment of the scar, the DermaSpectrometer[®] was used.

For subjective measurements of quality of scar formation as for example the degree of hypertrophic scarring the aVSS as well as a subjective 5 Point Contour Scale, grading from severe contour deformity to normal anatomical contour, were used.

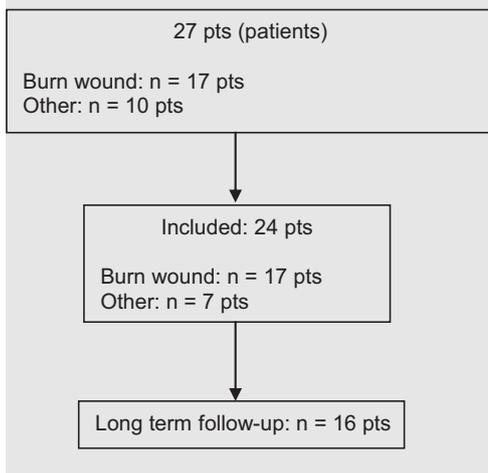
3.10. Statistical analysis

Statistical analysis was performed with SPSS 21.0 for Windows. Besides descriptive statistics, non-parametric statistical analysis of the groups was performed using Mann-Whitney *U*-test.

Statistical significance was declared if $p \leq 0.05$.

Table 2 – Patients pilot study.

Pat no.	Gender	Age (years)	Etiology	Localization	LDI
1	Male	33	Burn wound	Face right	No
2	Female	32	Burn wound	Arm right	No
3	Male	47	Burn wound	Hand right	Yes
4	Female	74	Burn wound	Breast right	Yes
5	Female	29	Burn wound	Thorax/abdomen	No
6	Male	3	Giant naevus	Lower leg right	Yes
7	Male	1	Burn wound	Hand left & right	No
8	Female	56	Burn wound	Neck	Yes
9	Male	20	Burn wound	Hand right	Yes
10	Female	6	Burn wound	Arm right	Yes
11	Male	34	Deglovement	Foot right	Yes
12	Male	2	Giant naevus	Upper leg right	Yes
13	Female	58	Skin tear	Lower leg left	Yes
14	Female	8	Burn wound	Arm right	Yes
15	Male	2	Burn wound	Arm left	Yes
16	Female	47	Burn wound	Neck	Yes
17	Male	24	Radial forearm flap	Forearm left	Yes
18	Male	25	Radial forearm flap	Forearm left	Yes
19	Male	40	Radial forearm flap	Forearm left	Yes
20	Female	54	Burn wound	Face	Yes
21	Female	51	Fasciotomy	Lower leg left	No
22	Female	60	Burn wound	Upper leg right	Yes
23	Female	51	Burn wound	Upper arm left	Yes
24	Male	28	Burn wound	Upper arm right	Yes
25	Male	27	Radial forearm flap	Forearm left, thigh left	Yes
26	Male	10	Burn wound	Thorax/abdomen	Yes
27	Male	50	Radial forearm flap	Forearm left	Yes

Table 3 – Phase I pilot study patient enrollment.

4. Results

4.1. Phase I pilot study

4.1.1. Baseline group characteristics

Twenty seven patients, with a mean age of 32.30 years (± 21.02), were recruited for the pilot study (Tables 2 and 3). In one patient who received Glyderm[®] after excision of a giant naevus, Glyderm[®] was lost due to infection with *Pseudomonas aeruginosa*. After removal of the Glyderm[®], control of infection and renewed wound bed preparation, the wound was re-grafted with Glyderm[®] and STSG with full take. In 3 patients with a full thickness skin defect after radial forearm flap harvest and immediate application of Glyderm[®], there was no ingrowth of Glyderm[®]. The protocol was changed to application of allografts to allow adequate wound bed preparation prior to application of Glyderm[®]. After this change the Glyderm[®] ingrowth in patients with radial forearm flap defects was satisfactory.

4.1.2. Take rates

Mean Glyderm[®] take rate in the patients with Glyderm[®] ingrowth was 91.55% (± 14.59) and 75% of those patients had a

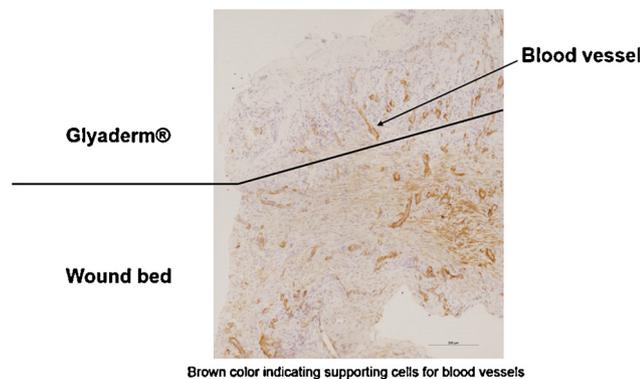


Fig. 1 – Histological analysis (ASMA staining) visualizing blood vessel ingrowth into Glyderm[®] 1 week after its application to the wound bed.

Glyderm[®] take rate of 95% or higher. Mean STSG take rate after Glyderm[®] ingrowth was 96.67% (± 4.75).

LDI demonstrated enhanced vascularization from day 1 to day 7, corresponding with both ASMA stained sections from biopsies (Fig. 1), harvested before autografting, and clinical observation of the dermal substitute starting at day of Glyderm[®] application until day of autografting. The color coded map on the computer, created by the measured flux values, allowed us to delineate the optimal engraftment interval. The optimal time before application of a STSG on top of the Glyderm[®] was 6 days with a 1 day standard deviation as shown in Fig. 2.

All patients responded well to a dressing regimen of Surfasoft[®] for protection of the Glyderm[®] combined with iso-Betadine[®] Gel and Jelonet[®] in terms of bacterial control and prevention from dehydration and desiccation of the Glyderm[®].

Histological analysis with Elastica von Giesson staining, of the biopsies taken at 1 month post wound healing, confirmed the presence of a native and vascularized collagen-elastin matrix embedded between the epidermis and the subcutaneous layer, thus recreating a neodermis as shown in Fig. 3.

4.1.3. Long-term follow-up (Table 4)

In total 16 patients participated in the long-term follow-up after Glyderm[®] scar maturation (Table 4).

Elasticity measurements with the DermaLab[®] resulted in an average young modulus of 8.51 (± 4.12) for Glyderm[®] + STSG and 6.77 (± 3.78) for normal skin. Statistics using the Mann-Whitney test demonstrated that, within this group of 16 patients, elasticity of Glyderm[®] + STSG is not significantly different from the elasticity of normal skin ($p = 0.319$).

DermaSpectrometer[®] measurements for erythema were on average 15.21 (± 5.31) for Glyderm[®] + STSG and 11.66 (± 3.14) for normal skin. Erythema measured in Glyderm[®] did not differ significantly from erythema measured in normal skin (Mann-Whitney test, $p = 0.052$).

DermaSpectrometer[®] measurements for pigmentation were on average 31.69 (± 4.67) for Glyderm[®] + STSG and 33.34 (± 2.90) for normal skin. Pigmentation measured in Glyderm[®] did not differ significantly from pigmentation measured in normal skin (Mann-Whitney test, $p = 0.120$).

POSAS score for general impression of the Glyderm[®] + STSG was on average 4.25 (± 1.81) for the investigators and 3.77 (± 2.62) for the patients. The POSAS score varies between 1 and 10 with 1 meaning the scar equals normal skin and 10 equaling the worst imaginable scar. From a statistical point of view there was no difference between the scores of investigators and patients (Mann-Whitney test, $p = 0.288$). Adapted Vancouver Scar Scale equalled 3.81 (± 2.26) on average, where the values for aVSS can vary between 0 (best score) and 18 (worst score).

In the absence of statistically significant differences between Glyderm[®] + STSG and normal skin we therefore concluded that long-term results of the phase I pilot study proved Glyderm[®] to be a suitable dermal matrix for full thickness burns and large soft tissue defects as also illustrated in Figs. 4–6.

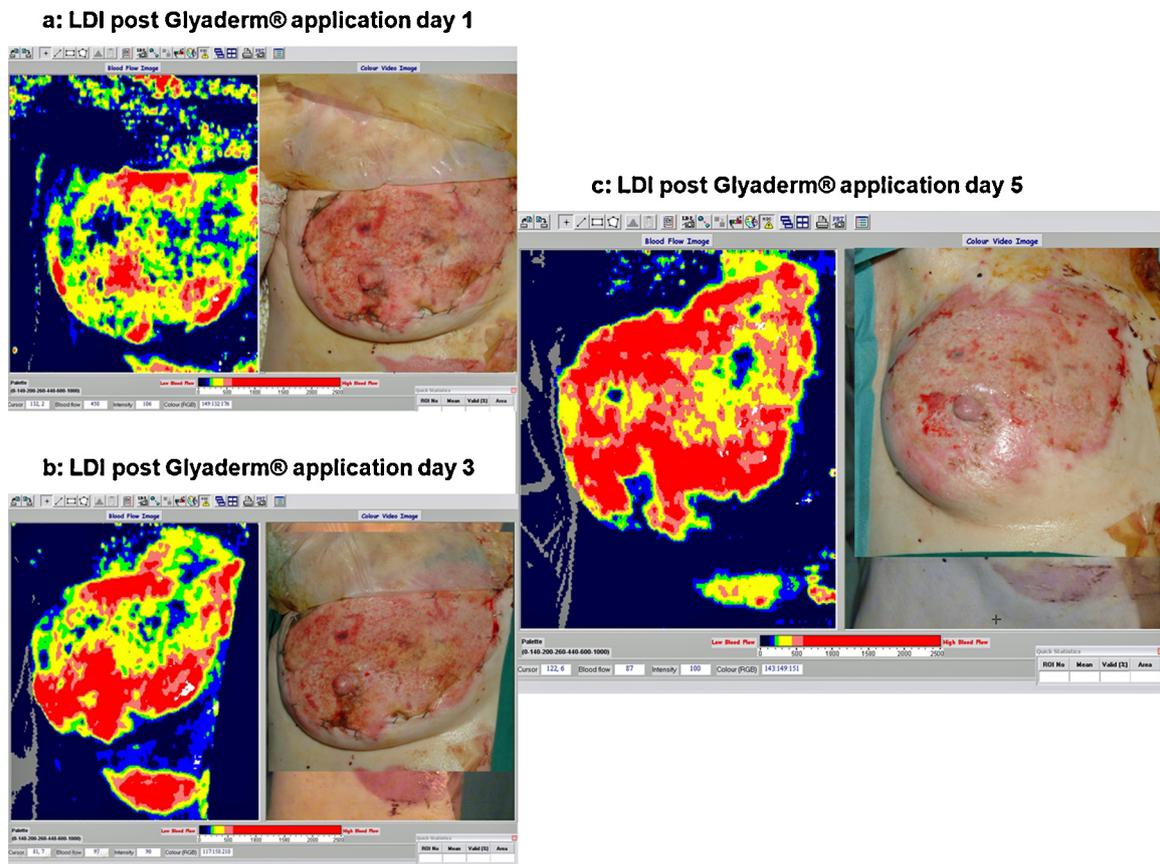
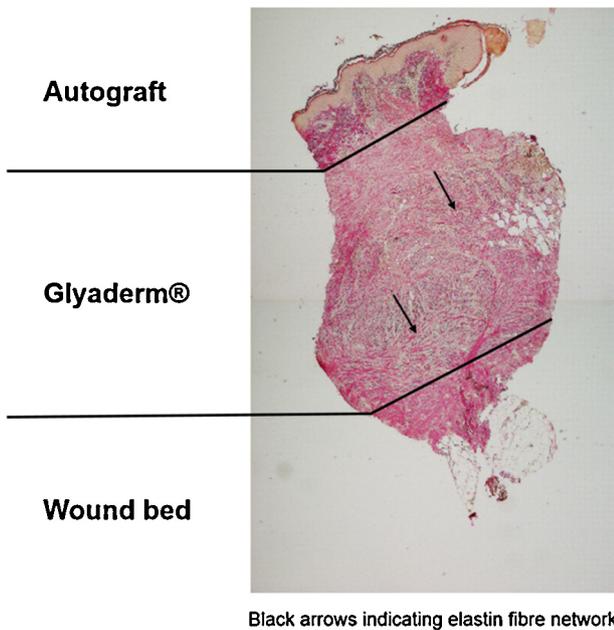


Fig. 2 – Laser Doppler imaging of Glyderm® vascularization.



Black arrows indicating elastin fibre network

Fig. 3 – Histological analysis (Elastica von Giesson staining) 1 month post wound healing of pilot study area treated with Glyderm®.

4.2. Phase III randomized trial

4.2.1. Baseline group characteristics

Thirty patients (34 sites) were eligible for inclusion in the study (Table 5). Two patients (two sites) were excluded prior to the Glyderm® procedure. Twenty-eight patients with a mean age of 33.07 years (± 10.35) and representing 32 sites met the inclusion criteria and were included in the study.

There were 9 patients with full thickness burns (13 sites) and 19 patients (19 sites) with full thickness defects after radial forearm flap harvest (Figs. 7 and 8). Two sites (one in each group) were lost during the procedure due to no Glyderm® ingrowth. Subsequent regrafting with Glyderm® and skin graft showed good take but these were excluded from the study.

4.2.2. Primary outcome measures (Table 6)

Mean wound surface area of the wounds treated with Glyderm® + STSG was 186.84 cm² (± 165.20) and mean wound surface area of the wounds treated with STSG alone was 184.33 cm² (± 175.87). Both procedures, as compared in this study, were comparable for treated wound surface area (Mann-Whitney test, $p = 0.536$) (Table 6).

Mean Glyderm® take rate in the included patients was 88.17% (± 18.34). Mean STSG take rate after Glyderm® ingrowth was 92.47% (± 23.19). STSG take rate in the wounds not treated with Glyderm® was 97.68% (± 4.99). The take rates

Table 4 – Phase I pilot study overview of statistical results (long-term follow up).

Variable	Statistical analysis	p-Value	Advantage
Elasticity (long term follow up) Glyaderm [®] + STSG versus normal skin	Mann–Whitney test	0.319	Glyaderm [®] + STSG compares to normal skin
Erythema (long term follow up) Glyaderm [®] + STSG versus normal skin	Mann–Whitney test	0.052	Glyaderm [®] + STSG compares to normal skin
Pigmentation (long-term follow up) Glyaderm [®] + STSG versus normal skin	Mann–Whitney test	0.120	Glyaderm [®] + STSG compares to normal skin

of STSG in the STSG + Glyaderm[®] group were not significantly different from the STSG take rates in the group with a STSG alone (Mann–Whitney test, $p = 0.588$). Non-parametric statistical analysis in the subgroups based on wound etiology also resulted in comparable STSG take rates for burn wounds (Mann–Whitney test, $p = 0.671$) and for full thickness skin defects after radial forearm flap harvesting (Mann–Whitney test, $p = 0.845$).

4.2.3. Secondary outcome measures (Table 6)

4.2.3.1. Elasticity. On average, elasticity (Young modulus) measured 1 month after wound healing was 8.81 (± 1.50) for Glyaderm[®] + STSG and 10.31 (± 0.84) for STSG alone. 12 months after wound healing the Young modulus values averaged 8.89 (± 1.10) for Glyaderm[®] + STSG and 9.29 (± 0.99) for STSG alone. Comparing the DermaLab[®] measurements of “Glyaderm[®] + STSG” versus “STSG alone” statistics indicate that: Glyaderm[®] + STSG” has significantly more elasticity when compared to “STSG alone” 1 month (Mann–Whitney test, $p = 0.001$) and 12 months (Mann–Whitney test, $p = 0.003$) after wound closure.

One year after wound closure we measured a mean Young modulus of 6.71 (± 0.16) on normal skin which was significantly more elastic than both Glyaderm[®] + STSG (Mann–Whitney test, $p < 0.0001$) and STSG alone (Mann–Whitney test, $p < 0.0001$).

4.2.3.2. Color and pigmentation. Measurements with the DermaSpectrometer[®] for erythema and pigmentation performed at 1 month and 12 months after wound closure did not result in statistically significant differences between “Glyaderm[®] + STSG” and “STSG alone”.

4.2.3.3. Scar scales. When looking at the aVSS at 12 months, with a mean score of 3.27 (± 2.76) for Glyaderm[®] + STSG and 4.73 (± 2.01) for STSG alone, scoring is on average better for Glyaderm[®] although there is no significant difference from a

statistical point of view (Mann–Whitney test, $p = 0.682$). aVSS scores for Glyaderm[®] noted in this study were comparable to aVSS scores for Glyaderm[®] observed in the pilot study. Independent blinded expert observers were asked to designate which of the intra-individual compared areas, according to their personal opinion, demonstrated best scar quality. According to these blinded expert observers best scar quality is mainly observed in Glyaderm[®] treated wounds (82%) as shown in Fig. 9.

5. Discussion

Excessive scar formation accounts for major morbidity and a continuing challenge in burn treatment [10]. Elasticity, flexibility, and strength of the normal dermis is compromised in scar tissue which can limit movement, causes pain, and is cosmetically undesirable [11,12]. The pivotal role of an adequate amount of dermis in surgical skin resurfacing is being increasingly understood and embraced [4]. The emphasis in surgical burn care has shifted from pure survival to quality of life after survival with increased interest in improvement of functional and esthetic scar outcomes. Dermal substitution is becoming more and more a standard procedure in surgical burn reconstruction. Dermal substitutes are also being used for bi-layered skin resurfacing after trauma or (oncological) resections and in the field of breast reconstruction and hernia repair [13,14].

Elastin is historically underrepresented in commercial dermal substitutes, yet it serves a fundamental role in skin structure and function. The dermal elastic network determines skin resilience, texture, and quality but is poorly regenerated following burn [15]. In addition to its structural and mechanical functions, elastin has inherent cell signaling properties that promote a diverse range of cellular responses including chemotaxis, cell attachment, proliferation, and



Fig. 4 – Full thickness burn in a 1 year old boy.



Fig. 5 – Full thickness burn in a 54 year old woman.

differentiation. Matrix elasticity and regeneration of the elastic fiber system is important for the development of functional dermal substitutes [15].

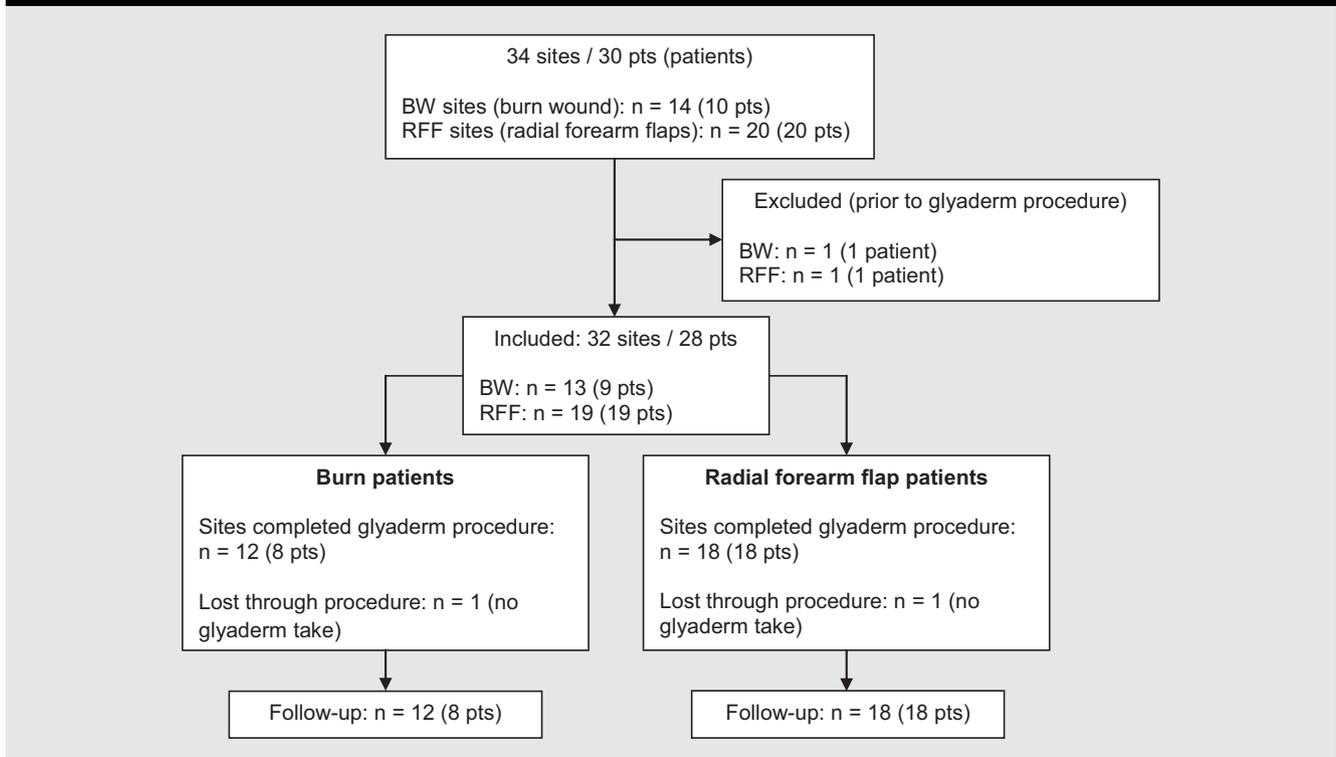
Collagen has been used in most dermal substitutes as it makes up the largest portion of the dermis, is biologically tolerated, and has well-defined structural, physical, and biological properties.

One of the earliest and still most widely used commercial collagen-based dermal substitutes is Integra® [1–3]. It consists of a porous dermal layer made from bovine collagen and chondroitin-6-sulfate and a temporary silicone layer that acts as a barrier between the body and the environment. The silicone layer is replaced with a thin skin autograft following the substitute vascularization.



Fig. 6 – Giant Naevus in a 4 year old boy.

Table 5 – Phase III randomized trial patient enrollment.



During the wound healing process, bovine collagen is degraded and replaced by native collagen deposited by host fibroblasts.

Collagen-based scaffolds currently dominate the dermal substitute field but are restricted by their lack of elasticity and impaired by scaffold contraction during repair [16,17].

Scaffold elasticity and regeneration of the elastic fiber system are now recognized as integral to the development of functional dermal substitutes [18-23].

The presence of elastin in collagen-based scaffolds has been shown to decrease scaffold stiffness [24] and modulate collagen contraction [25,26]. There is evidence suggesting that elastin can suppress the differentiation of proliferating fibroblasts into contractile myofibroblasts [27], thereby reducing wound contraction and modulating scar tissue formation.

Elastin does not adequately regenerate during severe wound healing and its distribution is disrupted in cutaneous scars [15]. It takes 4-5 years for elastin expression to rise following cultured

**a: 20-09-2007:
Full thickness burns**



b: Follow-up 1 year



c: Follow-up 4 years



Fig. 7 – Full thickness burn in a 3 year old boy (left foot = Glyaderm® + STSG/right foot = STSG alone).

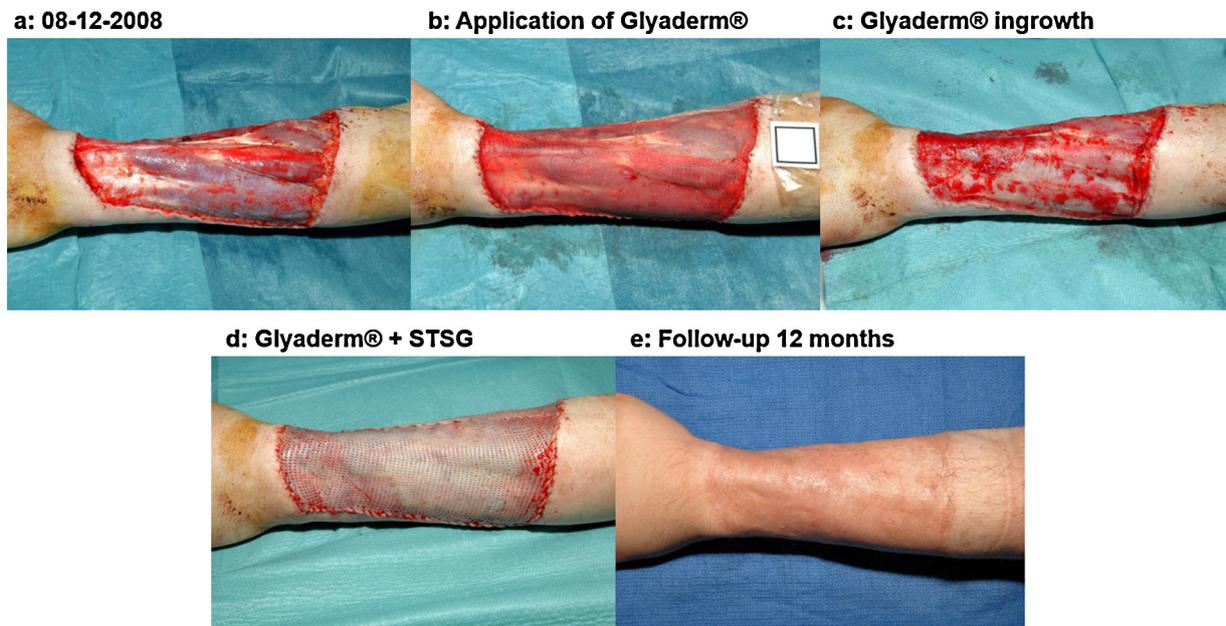


Fig. 8 – Radial forearm flap (Glyaderm® + STSG side).

epithelial autograft (CEA) treatment of burn wounds. Elastin is functionally and spatially disorganized in scar tissue [28,29].

Expression of both elastin and fibrillin-1 are reduced in scar tissue with a particularly prominent reduction in hypertrophic scars [15]. Newly synthesized, elastic fibers in scar tissue always appear thin, fragmented, and less mature than elastic fibers in normal skin [15,29,30]. Even in scars older than 10 years, elastic fibers never reach the size and maturity of healthy skin [30], which attributes to the fact that hypertrophic scars are usually hard and inelastic [29].

The disruption of the elastic fiber system in healing wounds and scar tissue is well documented, but the mechanism behind this phenomenon is not clear. It is possible that elastin upregulation in healing wounds is not sufficient to regenerate robust elastin fibers.

Elastin-containing dermal substitutes may improve the elasticity and functionality of severe scars by replacing the missing elastic network or by signaling the upregulation of elastic tissue biosynthesis. Consistent with this signaling role, dermal fibroblasts display increased elastin expression when

Table 6 – Phase III randomized trial overview of statistical results.

Variable	Statistical analysis	p-Value	Advantage
Mean wound surface area treated Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.536	Glyaderm® + STSG compares to STSG alone
Mean STSG take rate (%) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.588	Glyaderm® + STSG compares to STSG alone
Elasticity (1 month after wound closure) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.001	Glyaderm® + STSG
Elasticity (1 year after wound closure) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.003	Glyaderm® + STSG
Elasticity (1 year after wound closure) Glyaderm® + STSG versus normal skin	Mann-Whitney test	< 0.0001	Normal skin
Elasticity (1 year after wound closure) STSG alone versus normal skin	Mann-Whitney test	< 0.0001	Normal skin
Erythema (1 month after wound closure) Glyaderm® + STSG versus normal skin	Mann-Whitney test	0.072	Glyaderm® + STSG compares to STSG alone
Erythema (1 year after wound closure) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.786	Glyaderm® + STSG compares to STSG alone
Pigmentation (1 month after wound closure) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.581	Glyaderm® + STSG compares to STSG alone
Pigmentation (1 year after wound closure) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.828	Glyaderm® + STSG compares to STSG alone
Adapted Vancouver scar scale (1 year after wound closure) Glyaderm® + STSG versus STSG alone	Mann-Whitney test	0.682	Glyaderm® + STSG compares to STSG alone

The bold p-values indicate statistical significance.

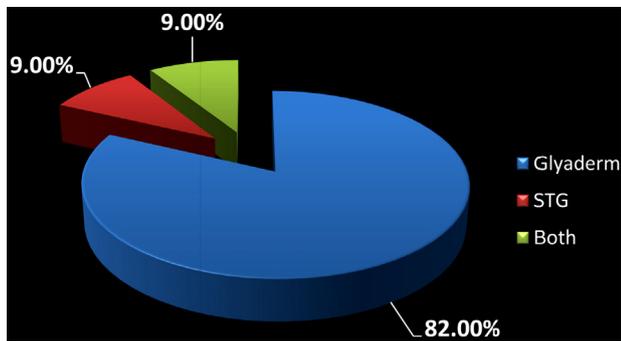


Fig. 9 – Best scar quality as subjectively attributed by independent blinded expert observers (phase III randomized trial).

they are stimulated with proteolytic digests of bovine elastin introduced into the skin of nude mice or into human skin explants [31].

Collagen–elastin composite scaffolds induce elastin deposition when implanted subcutaneously in rats, compared with collagen-only scaffolds that do not promote elastin synthesis [32,33].

The clinically best known human decellularized, collagen–elastin dermis is sodium chloride–sodium dodecyl sulfate-treated cadaver skin marketed as AlloDerm[®] [34]. AlloDerm[®] has been applied to human burns in a range of different procedures [34–37]. AlloDerm[®]-grafted sites often show good cosmetic and functional results, with limited contractures observed on relatively small burn areas (<20% TBSA) [35]. Case studies also report increased skin elasticity and improved cosmetic appearance when AlloDerm[®] is grafted with split thickness autografts, compared with split-thickness autografts alone [34,35]. When applied to burned joints, AlloDerm[®] can minimize wound contraction and allow joint movement [37]. Because of its high cost and limited quantity, AlloDerm[®] is mostly used in reconstructive surgery to release skin contractures and hypertrophic scars [37]. The cost of AlloDerm[®] as mentioned by Butterfield in a 2013 review article was 21.7 Euro/cm² [38].

Another dermal matrix consisting of native bovine collagen (type I, III and V) fibers was coated with 3% (w/w) α -elastin derived from bovine ligamentum nuchae, marketed as MatriDerm[®]. MatriDerm[®] in combination with a split-thickness mesh graft showed improved skin pliability and elasticity compared with split-thickness mesh grafts alone in scar reconstruction wounds. However, these benefits were not seen in burn wounds after 3 months [39]. In a scar follow-up study, no difference in scar elasticity was observed between MatriDerm[®]-grafted and control scars in the burn wounds at 12 years post grafting. However, there was a perceived improvement for MatriDerm[®]-grafted wounds compared with control wounds in subjective scar assessment conducted by patients and clinicians [38]. MatriDerm[®] has proved particularly useful in the treatment of hand burns, which are reported in 60–90% of burn cases [40,41]. A long-term follow-up of upper-extremity wounds treated with this scaffold in combination with a sheet autograft reveals good skin pliability, scar height, and ultimately, hand function [41]. Radu et al. found that MatriDerm[®] when used in combination with a split-thickness autograft improved the range of motion and the

quality of scars compared with split-thickness grafts alone [42]. The beneficial effects of MatriDerm[®], including the reduction of wound contraction and stimulation of dermal regeneration, are believed to be conveyed in the early healing stages (within the first 2 weeks) through the inhibition of dermal fibroblast differentiation into contractile myofibroblasts [25].

MatriDerm[®] is a first step toward incorporation of soluble elastin derivatives in dermal substitute scaffolds. MatriDerm[®], however, consists of a collagen scaffold coated with elastin, and its benefits are therefore not derived from the presence of an elastin fiber network or elasticity of the scaffold. The porous nature of the matrix may support a more rapid vascularization of the matrix, however the absence of elastin fibers and thus a network of elastin may also diminish its long-term beneficial effect in terms of elasticity. Further, the scaffold is composed of animal derived proteins, which carry risks of immune rejection and pathogen transfer as well as suffer from potential heterogeneity because of their batch-to-batch inconsistencies. The cost of MatriDerm[®] as mentioned by Lamy et al. in a 2013 article is on average 5.30 Euro/cm² [43].

Increasing understanding of the importance of elastin in tissue-engineered scaffolds has resulted in research into the elastin- and tropoelastin-based scaffolds. These scaffolds are currently undergoing in vitro and early in vivo testing [44]. In the clinical setting often logistic, financial and temporal issues continue to challenge the burn surgeon to use dermal substitutes on a more larger scale.

We set out to develop a dermal substitute from glycerol preserved allografts more than a decade ago, which was intended to have the following key advantages: native collagen and elastin matrix, easy storage and handling, inactivation of virus and micro-organisms [45,46] and most importantly, a non-profit product that could be available to a larger number of patients. The extreme high cost of dermal substitutes today impedes their widespread application and benefit for those who need it the most. As clinicians in the field our chief aim was to develop a practical and affordable dermal substitute for burn, cancer and trauma victims.

The most favorable prototype Glyaderm[®] was tested in animal studies, which showed favorable results in a three stage procedure, allograft, Glyaderm[®], autograft (manuscript in preparation). These promising results prompted the current pilot study and randomized comparison.

There have been many reports attesting the benefits of various dermal substitutes. However, to our knowledge there has been no conclusive randomized trial which demonstrates a superior outcome of skin resurfacing with a dermal substitute and split skin graft over skin resurfacing with a skin graft alone. Most burn experts do not question the value of dermal substitution in surgical burn care and long-term results of patients attest the added value.

Objective scar assessment and longer follow-up is elucidating this advantage, which is already clinically apparent. Our pilot study shows consistent, stable long-term results after 6 years with pliable skin after bi-layered skin restoration with Glyaderm[®].

Objective scar assessment showed a significantly improved elasticity of the skin in patients treated with Glyaderm[®] and skin graft compared to skin graft alone ($p = 0.003$).

Glyaderm[®] is the first cost-effective, non-profit, dermal substitute that can be compared with currently available dermal equivalents.

To our knowledge we are the first to show that laser Doppler imaging allows monitoring of vascular ingrowth in dermal substitutes such as Glyaderm[®]. Although most burn experts advocate the use of dermal substitutes, the challenge remains to objectively show the perceived benefit over split skin grafting alone. The evolving evaluation with objective scar assessment tools within these studies may help to further demonstrate this benefit in the near future.

A disadvantage in our initial studies with Glyaderm[®] was the necessity for three procedures to full wound closure. Direct application of Glyaderm[®] onto the wound bed without allograft wound bed preparation did not seem to be a viable option in either the animal studies nor the phase I pilot study as demonstrated by the 3 patients with a full thickness skin defect after radial forearm flap harvest where, following immediate application of Glyaderm[®], we expected no problems in view of the healthy wound bed, but in the end there was no ingrowth of the dermal substitute. The animal studies had also pointed out that simultaneous application of Glyaderm[®] and autograft was not feasible. In Glyaderm[®] processing a relative dense elastin-collagen network is preserved. Budding capillaries need to penetrate this network before they can nourish the overlying autograft. In addition, the earlier Glyaderm[®] prototypes were relatively too thick and suffered from batch to batch inconsistencies inherent to variation in selection. Continuous research, monitoring of selection and development improved this process of graft selection and standardization.

A purpose designed laser tool is now used to ensure selection of dermis of uniform thickness. The laser accurately scans the distance between the optic and the table and the optic and the Glyaderm[®] subsequently placed upon the table, allowing the difference in height to be the thickness.

The optimal 0.2–0.4 mm thickness glycerol preserved dermis is now selected for processing into Glyaderm[®].

Glyaderm[®] is currently applied with simultaneous skin grafting after wound bed preparation with allografts for 5 days. This improvement has a distinct favorable impact on morbidity and cost [47]. We have now modified the study protocol of a recent ongoing multicentre Glyaderm[®] study to allow for recruitment of patients with this shorter surgical procedure.

Glyaderm[®] is produced by the Euro Skin Bank, Beverwijk, The Netherlands, a non-profit tissue bank that also monitors Glyaderm[®] commercial distribution for burn care and reconstructive procedures.

Euro Tissue Bank ensures the quality and non-profit distribution of the product backed by a clinical specialist advisory group to facilitate and promote clinical use.

Conflict of interest statement

The Euro Skin Bank, The Netherlands, and the Dutch Burns Foundation, The Netherlands, both non-profit organisations, provided an unrestricted research grant for the realization of this research project.

REFERENCES

- [1] Yannas IV, Burke JF. Design of an artificial skin. I. Basic design principles. *J Biomed Mater Res* 1980;14(1):65–81.
- [2] Yannas IV, Burke JF, Gordon PL, Huang C, Rubenstein RH. Design of an artificial skin. II. Control of chemical composition. *J Biomed Mater Res* 1980;14(2):107–32.
- [3] Yannas IV, Burke JF, Warpehoski M, Stasikelis P, Skrabut EM, Orgill D, et al. Prompt, long-term functional replacement of skin. *Trans Am Soc Artif Intern Org* 1981;27:19–23.
- [4] Brusselaers N, Pirayesh A, Hoeksema H, Richters CD, Verbelen J, Beele H, et al. Skin replacement in burn wounds. *J Trauma* 2010;68:490–501.
- [5] Mackie D. Postal survey on the use of glycerol-preserved allografts in clinical practice. *Burns* 2002;28(Suppl. 1):S40–4.
- [6] Richters CD, Pirayesh A, Hoeksema H, Kamperdijk EWA, Kreis RW, Dutrieux RP, et al. Development of a dermal matrix from glycerol preserved allogeneic skin. *Cell Tissue Bank* 2008;9(4):309–15.
- [7] Beele H, Durante C, Kerihuel JC, Rice J, Rondas A, Stryja J, et al. Expert consensus on a new enzyme alginogel. *Wounds Int* 2012;3(2):42–50.
- [8] Hoeksema H, Van de Sijpe K, Tondu T, Hamdi M, Van Landuyt K, Blondeel P, et al. Accuracy of early burn depth assessment by laser Doppler imaging on different days post burn. *Burns* 2009;35(1):36–45.
- [9] Hoeksema H, De Vos M, Verbelen J, Pirayesh A, Monstrey S. Scar management by means of occlusion and hydration: a comparative study of silicones versus a hydrating gel-cream. *Burns* 2013;39:1437–48.
- [10] Engrav LH, Garner WL, Tredget EE. Hypertrophic scar, wound contraction and hyper-hypopigmentation. *J Burn Care Res* 2007;28:593.
- [11] Parenteau N, Hardin-Young J, Ross R. Skin. In: Lanza R, Langer R, Vacanti J, editors. *Principles of tissue engineering*, vol. 2. San Diego: Academic Press; 2000. p. 879–87.
- [12] Clark R, Singer A. Wound repair: basic biology to tissue engineering. In: Lanza R, Langer R, Vacanti J, editors. *Principles of Tissue Engineering*, vol. 2. San Diego: Academic Press; 2000. p. 855–78.
- [13] Bellows CF, Smith A, Malsbury J, Scott Helston W. Repair of incisional hernias with biological prosthesis: a systematic review of current evidence. *Am J Surg* 2013;205:88–101.
- [14] Vardanian AJ, Clayton JL, Roostaiean J, Shirvanian V, Da Lio A, Lipa JE, et al. Comparison of implant-based immediate breast reconstruction with and without acellular dermal matrix. *Plast Reconstr Surg* 2011;128:403e–10e.
- [15] Amadeu TP, Braune AS, Porto LC, Desmouliere A, Costa AM. Fibrillin-1 and elastin are differentially expressed in hypertrophic scars and keloids. *Wound Repair Regen* 2004;12:169.
- [16] Bell E, Ivarsson B, Merrill C. Production of a tissue like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc Natl Acad Sci USA* 1979;76:1274.
- [17] Dallon JC, Ehrlich HP. A review of fibroblast populated collagen lattices. *Wound Repair Regen* 2008;16:472.
- [18] Raghunath M, Bachi T, Meuli M, Altermatt S, Gobet R, Bruckner-Tuderman L, et al. Fibrillin and elastin expression in skin regenerating from cultured keratinocyte autografts: morphogenesis of microfibrils begins at the dermo-epidermal junction and precedes elastic fiber formation. *J Invest Dermatol* 1996;106:1090.
- [19] Balasubramani M, Kumar TR, Babu M. Skin substitutes: a review. *Burns* 2001;27:534.

- [20] Hafemann B, Ensslen S, Erdmann C, Niedballa R, Zuhlke A, Ghofrani K, et al. Use of a collagen/elastin-membrane for the tissue engineering of dermis. *Burns* 1999;25:373.
- [21] Haslik W, Kamolz LP, Nathschlager G, Andel H, Meissl G, Frey M. First experiences with the collagen elastin matrix Matriderm as a dermal substitute in severe burn injuries of the hand. *Burns* 2007;33:364.
- [22] Compton CC, Gill JM, Bradford DA, Regauer S, Gallico GG, O'Connor NE. Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting. A light, electron microscopic and immunohistochemical study. *Lab Invest* 1989;60:600.
- [23] Berthod F, Germain L, Li H, Xu W, Damour O, Auger FA. Collagen fibril network and elastic system remodeling in a reconstructed skin transplanted on nude mice. *Matrix Biol* 2001;20:463.
- [24] Daamen WF, van Moerkerk HT, Hafmans T, Buttafoco L, Poot AA, Veerkamp JH, et al. Preparation and evaluation of molecularly-defined collagen-elastin-glycosaminoglycan scaffolds for tissue engineering. *Biomaterials* 2003;24:4001.
- [25] Lamme EN, de Vries HJ, van Veen H, Gabbiani G, Westerhof W, Middelkoop E. Extracellular matrix characterization during healing of full-thickness wounds treated with a collagen/elastin dermal substitute shows improved skin regeneration in pigs. *J Histochem Cytochem* 1996;44:1311.
- [26] Middelkoop E, de Vries HJ, Ruuls L, Everts V, Wildevuur CH, Westerhof W. Adherence, proliferation and collagen turnover by human fibroblasts seeded into different types of collagen sponges. *Cell Tissue Res* 1995;280:447.
- [27] De Vries HJ, Middelkoop E, Mekkes JR, Dutrieux RP, Wildevuur CH, Westerhof W. Dermal regeneration in native non-cross-linked collagen sponges with different extracellular matrix molecules. *Wound Repair Regen* 1994;2:37.
- [28] Jones I, Currie L, Martin R. A guide to biological skin substitutes. *Br J Plast Surg* 2002;55:185.
- [29] Chen G, Chen J, Zhuo S, Xiong S, Zeng H, Jiang X, et al. Xie S. Nonlinear spectral imaging of human hypertrophic scar based on two-photon excited fluorescence and second-harmonic generation. *Br J Dermatol* 2009;161:48.
- [30] Roten SV, Bhat S, Bhawan J. Elastic fibers in scar tissue. *J Cutan Pathol* 1996;23:37.
- [31] Hinek A, Wang Y, Liu K, Mitts TF, Jimenez F. Proteolytic digest derived from bovine Ligamentum Nuchae stimulates deposition of new elastin-enriched matrix in cultures and transplants of human dermal fibroblasts. *J Dermatol Sci* 2005;39:155.
- [32] Daamen WF, Nillesen ST, Wismans R, Reinhardt D, Hafmans T, Veerkamp JH, et al. Depots of solubilised elastin promote the formation of blood vessels and elastic fibres in rat. *J Controlled Release* 2006;116:e84.
- [33] Daamen WF, Nillesen ST, Wismans RG, Reinhardt DP, Hafmans T, Veerkamp JH, et al. A biomaterial composed of collagen and solubilized elastin enhances angiogenesis and elastic fiber formation without calcification. *Tissue Eng Part A* 2008;14:349.
- [34] Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. *Burns* 1995;21:243.
- [35] Callcut RA, Schurr MJ, Sloan M, Faucher LD. Clinical experience with AlloDerm: a one-staged composite dermal/epidermal replacement utilizing processed cadaverdermis and thin autografts. *Burns* 2006;32:583.
- [36] Wainwright D, Madden M, Luterman A, Hunt J, Monafu W, Heimbach D, et al. Clinical evaluation of an acellular allograft dermal matrix in full-thickness burns. *J Burn Care Rehabil* 1996;17:124.
- [37] Yim H, Cho YS, Seo CH, Lee BC, Ko JH, Kim D, et al. The use of AlloDerm on major burn patients: AlloDerm prevents post-burn joint contracture. *Burns* 2010;36:322.
- [38] Butterfield JL. 440 Consecutive immediate, implant-based, single-surgeon breast reconstructions in 281 patients: a comparison of early outcomes and costs between SurgiMend fetal bovine and AlloDerm human cadaveric acellular dermal matrices. *Plast Reconstr Surg* 2013;131(5):940-51.
- [39] van Zuijlen PP, van Trier AJ, Vloemans JF, Groenevelt F, Kreis RW, Middelkoop E. Graft survival and effectiveness of dermal substitution in burns and reconstructive surgery in a one-stage grafting model. *Plast Reconstr Surg* 2000;106:615.
- [40] Bloemen MC, van Leeuwen MC, van Vucht NE, van Zuijlen PP, Middelkoop E. Dermal substitution in acute burns and reconstructive surgery: a 12-year followup. *Plast Reconstr Surg* 2010;125:1450.
- [41] Ryssel H, Gazyakan E, Germann G, Ohlbauer M. The use of Matriderm in early excision and simultaneous autologous skin grafting in burns – a pilot study. *Burns* 2008;34:93.
- [42] Radu CA, Germann G, Kloeters O, Gazyakan E, Ryssel H. Dermal substitution with Matriderm(®) in burns on the dorsum of the hand. *Burns* 2010;36:1248.
- [43] Lamy J, Gourari A, Atlan M, Zakine G. Utilisation de Matriderm® 1 mm en chirurgie reconstructrice. Série de 31 cas.[Use of Matriderm® 1 mm in reconstructive surgery. Series of 31 cases]. *Ann Chir Plast Esthet* 2013;58(3):235-42.
- [44] Rnjak J, Li Z, Maitz PK, Wise SG, Weiss AS. Primary human dermal fibroblast interactions with open weave three-dimensional scaffolds prepared from synthetic human elastin. *Biomaterials* 2009;30:6469.
- [45] Camerom PU, Pagnon JC, Van Baare J, Reece JC, Vardaxis NJ, Crowe SM. Efficacy and kinetics of glycerol inactivation of HIV-1 in split skin grafts. *J Med Virol* 2000;60:182-8.
- [46] Van Baare J, Ligtoet EE, Middelkoop E. Microbiological evaluation of glycerolized cadaveric donor skin. *Transplantation* 1998;65:966-70.
- [47] Hoeksema H, Pirayesh A, De Meyere K, Verbelen J, Richters C, Monstrey S. Towards a simultaneous application of a split skin graft and a new dermal substitute based on human collagen and elastin (Glyaderm). In: Kamolz LP, Andel H, editors. 15th European burns association congress. Book of abstracts. 2013. p. 60.