CHAPTER 12

GENERAL DISCUSSION AND FUTURE PERSPECTIVES
General discussion and future perspectives.

Burns represent a significant cause of trauma-related mortality, with varying incidence rates globally. Recent advancements in burn care have improved patient survival and recovery, leading to a shift in focus from mere survival to enhancing the patient's quality of life. Quality of life for burn patients is dependent on several factors, including reintegration into society, scar appearance and quality, and self-perception of appearance. Autologous split-thickness skin graft (STSG) is the current gold standard treatment for deep dermal and full-thickness burns. However, STSG is associated with several challenges, including limited donor site availability, donor site morbidity, contracture, and unpredictable scar healing. In addition to human allografts, biological and synthetic skin substitutes have been developed for burn treatment. Dermal regenerative matrices (DRMs) are permanent skin substitutes that aid dermal skin component regeneration in the treatment of total skin defects resulting from burns, traumatic wounds, or burn contracture resolution. DRMs have demonstrated beneficial functional and aesthetic outcomes in both acute burn surgery and burn reconstruction. Nonetheless, DRMs have drawbacks, including the need for a two-stage procedure, increased infection risk, and high cost.

Historical aspects

At the turn of the millennium, our research group faced challenges in the reconstruction of burn care. The DRM Integra received increasing attention in the literature and at conferences, but the variability in “take” or ingrowth and the very high cost prevented widespread application and routine use in the Ghent Burn Unit. Having witnessed brutal burn & trauma of revolution and war as a child in Iran, Pirayesh was intrigued by Plastic Surgery & Burn Care as a Senior House Officer in the East Grinstead Burn Unit, where his mentor Philip Gilbert taught him the principles of burn care. The Queen Victoria Hospital was famous since the Second World War for Sir Archibald McIndoe who bravely treated the burns of RAF pilots from the Battle for Britain. He started researching keratinocyte culture and presented papers at burns conferences where he met Hans Hoekstra, the inventor of the glycerol conserved allograft (GPA). Hoekstra was active in experimental burns research in Amsterdam and taught Pirayesh the core principles of experimental burns research together with Dr. Nelleke Richters who worked as an immunologist and researcher for the Dutch Burns Foundation. Pirayesh was impressed by the research output of the Ghent Plastic Surgery Department and approached Prof. Stan Monstrey at a conference which gave him the opportunity to apply for a training position. Pirayesh was selected for training as a plastic surgeon but had to start with a pre-residency year at the Ghent Burns Unit.
Henk Hoeksema, chief burn care coordinator, taught him the principles of conservative burn care and surgical burn care. They introduced and started studies with MEEK transplantation and interactive honey dressings in the Ghent Burn Unit, which was known for using laser-doppler imaging to scientifically delineate the depth of burns and was therefore the ideal unit for clinical studies on burns. Their joint brainstorming sessions culminated in the idea of developing a dermal substitute based on glycerol-preserved allograft, ideally on a non-commercial basis and cost-effective DRM for wide application and improvement of the quality of life of burn patients.

Pirayesh, Hoeksema, Richters, Hoekstra and Monstrey abdicated their IP rights for the DRM "Glyaderm", which was notarized at the EuroSkinBank, now EuroTissueBank, Beverwijk, The Netherlands.

Pirayesh returned to the Netherlands to build his own practice, but propagated Glyaderm® research and worldwide application, resulting in the development of a Colombian Glyaderm® for acid attack victims. The charity Two Faces (https://twofacesfoundation.org) was founded to help these victims by his wife Eva Velders. Berend van der Lei has been an inspirational force throughout his career and has coached him to structure and submit this thesis under his supervision together with Prof. Monstrey now that the long-term results of the studies are available that will reinforce the position of Glyaderm® among other dermal regeneration matrices.

**Glyaderm®**

Glyaderm® is the result of processing glycerol-preserved allogeneic donor skin. Using glycerol has some significant benefits. Skin preservation with glycerol is not only more cost-effective than cryopreservation, GPA is also less immunogenic. Glycerol has bactericidal properties; 97% of GPA bacteriological cultures are negative after 3 months. Glycerol can inactivate viruses such as HIV-1 and Herpes Simplex. The donor cells are not viable due to the glycerol preservation method, but the collagen and elastin network remain intact. With production of Glyaderm®, all donor cells (i.e., hair cells, vascular endothelium, smooth muscle, and keratinocytes) must be removed to prevent an adverse inflammatory (immunogenic) response leading to neodermal rejection. The main advantage of using a GPA-derived dermal substitute is that it resembles the natural collagen-elastin structure of human skin. This in contrast to other dermal substitutes, such as Matriderm® or Integra®, which are of animal and/or synthetic origin. It is preferable to maintain the natural collagen and elastin 3D fiber network of the dermis. A few years ago, a panel of experts stated: "Given current knowledge, the ideal acellular matrix is one that most closely approximates the structure and function of the human extracellular matrix (ECM) it replaces". The glycerol remnants are removed by thoroughly rinsing the GPA in sterile saline (NaCl). Repeated washing is done to ensure residual glycerol is removed. Incubation in low concentration sodium hydroxide (NaOH) solution is the method used for decellularization. We investigated the effects of NaOH-decellularized skin in a pig and rat full-thickness wound model. The optimal incubation period has been found to be six weeks. Shorter incubation periods (less
than four weeks) do not guarantee complete removal of all antigenic components, resulting in an inflammatory response. Infiltration of inflammatory cells, such as neutrophils or macrophages (responsible for the production of proteolytic enzymes), leads to a premature breakdown of the elastin and collagen matrix. Fibroblasts attach to these ECM components and use them as a scaffold. The fibroblasts start to produce new collagen fibers around the donor fibers. This will result in a more favorable random orientation of the fibers and the neoepidermis will appear more natural.

Fibroblasts are unable to use the prematurely degraded donor-derived fibers as a guide, leading to an undesirable parallel (to the epidermis) orientation of newly synthesized collagen fibers. More inflammatory cells, due to a shorter incubation time, could delay wound closure by interfering with the outgrowth of the epidermis (of the STSG). An extension of the incubation period (more than eight weeks) may damage the extracellular matrix.

Our study showed that decellularization by using a sodium hydroxide solution was not only cost-effective, but also able to preserve the natural elastin and collagen 3D network. Preservation of the natural collagen and elastin 3D network is important. In the past, elastin didn't get the attention it deserves. Using a dermal substitute containing elastin can reduce wound contractures and improve skin elasticity. Elastin expression is quite reduced in scar tissue and new elastin fibers are thin, fragmented and less mature than elastic fibers in normal skin. Elastin fibers will never reach the thickness or maturation level of healthy skin, even after ten years, resulting in hard and inelastic scars. Elastin is not only functional but also spatially disorganized in scar tissue. It is suggested that the use of dermal substitutes containing both collagen and elastin may increase elastin production by fibroblasts and replace lost elastic fibers. This is not the case for dermal substitutes that do not have a human elastin network, such as Integra®. Glyaderm® contains a human elastin dermal network with the intact spatial structure of normal human skin, potentially making it a step further towards developing an ideal dermal substitute.

After the six-week incubation period, hydrogen chloride (HCl) is added to neutralize the sodium hydroxide. The decellularized skin (Glyaderm®) is then rinsed in phosphate buffered saline, after which it can be stored in 85% glycerol until ready for use. No special storage space is required.

**Studies to develop and validate Glyaderm®**

Different incubation periods in NaOH were used to prepare dermal matrix prototypes from donor skin, ranging from 2 to 8 weeks.

Standard histology techniques were employed to analyze the resulting prototypes, which were subsequently tested in both rat and pig models. In the rat model, all prototypes exhibited intact biocompatibility four weeks after implantation, as evidenced by the presence of ingrown blood vessels and fibroblasts. However, an inflammatory response was observed in prototypes treated with NaOH for only 2 or 4 weeks. In the pig model, the prototypes treated with 6 or 8 weeks of NaOH were able
to reduce wound contraction. An optimal incubation period of 6 weeks was determined, as longer periods caused damage to the collagen fibers. The elastin fibers were well preserved in all prototypes. In the neodermis of pig wounds treated with 6 or 8 weeks of NaOH, elastin fibers originating from the prototype were observed 8 weeks after surgery, surrounded by more randomly oriented collagen fibers. The results suggest that an effective dermal matrix can be obtained from glycerol-preserved donor skin.

Further clinical studies are planned to assess the potential of this material for dermal substitution in deep burn wounds (Chapter 2).

In our study, we compared an acellular dermal substitute (Glyaderm®) prepared from glycerol-preserved human skin with already known substitutes, using a pig wound model. The donor cells were removed by incubation in 0.06 M NaOH solution, and the substitutes were applied to full-thickness wounds covered with an STSG. A two-stage procedure was used for Glyaderm®, with the STSG placed a week after application. The response to wound healing was analyzed macroscopically and on biopsies over 8 weeks, and the survival of the STSG was compared to control wounds.

In the first series of experiments, the inflammatory response and influx of myofibroblasts in Glyaderm® were limited, indicating possible beneficial outcomes on final wound healing outcomes. However, the survival of the STSG on the acellular dermis was lower compared to control wounds. In the second series, the "take" of the STSG was the same as controls, but the wound contraction was reduced. The application of Glyaderm® was not inferior to Integra® in reducing wound contraction when applied in a two-stage procedure.

In conclusion, our study suggests that Glyaderm® can be successfully used in a two-stage procedure to reduce wound contraction. Further studies are needed to evaluate its efficacy in other wound types and in clinical settings (Chapter 3).

In this subsequent study, we evaluated the integrity and biocompatibility of our "Glyaderm" dermal replacement matrix (DRM) in repairing abdominal wall defects. Abdominal wall repair can be performed using synthetic or biological matrices, with biological materials potentially reducing the risk of infections and fibrosis. The study aimed to compare two acellular human dermis products, with one being prepared using low concentrations of NaOH (i.e., Glyaderm®) and the other being SureDerm®, a commercially available dermal substitute. We used a rat model to compare the two materials, in which full-thickness defects were closed with the matrices. The rats were sacrificed 1 or 4 months after surgery, and the number of intestinal adhesions was noted. Histological analysis and measurement of tensile strength were also performed on tissue samples.

Both groups showed good functional integration of the implants with the abdominal wall. The group treated with the NaOH prototype (Glyaderm®) showed no intestinal adhesion, whereas 4 out of 7 rats in the SureDerm® group showed only minor adhesions after 4 months. The tensile strength of the healed tissue was significantly higher in the NaOH prototype group at 4 months after surgery (p < 0.0026). These
results suggest that both human acellular dermis products can be used in clinical trials for the closure of abdominal wall defects (Chapter 4). We then performed literature review on skin replacement for burns. The goal of this study was to provide an overview of which types of skin substitutes have been developed and which questions still need to be answered. None of the commercialized products can currently claim to be the optimal dermal substitute, mainly because clinical evidence is too scarce. The number of products being commercialized is nevertheless steadily increasing, which calls for a certain overview, classification, and clear comparison of the available products (Chapter 5).

Adverse post-burn scarring is a significant problem that affects a large number of individuals. Consequently, a majority of scar assessment and treatment studies have focused on burn scars due to their relatively high prevalence. While surgical and dermatological scars may also result in scarring, their impact is usually more limited, and thus, they are less well studied. Therefore, burn scars are likely the scars with the most significant impact on quality of life. Excessive scarring can lead to both physical and psychological effects that can impede an individual's quality of life, including painful and lengthy treatments that may yield suboptimal outcomes. Scars can also cause discomfort, itching, and pain, and contractures can limit mobility and function. The integration of individuals with hypertrophic scars in a society where physical appearance has become increasingly important can also pose challenges. Burn scars can have a considerable psychological impact on affected individuals, as they are highly visible and stigmatizing, similar to other severe chronic dermatological conditions.

Despite the importance of scar assessment, it remains a neglected area, and there is still no consensus on the ideal scar assessment method, despite the many scales and tools that have been developed over the past few decades. However, adequate scar assessment is crucial for clinical evaluation and follow-up, and it is also essential to compare different wound or scar treatment modalities. In addition, an objective scar evaluation may be required for medico-legal reasons, such as reimbursement of treatments and proof of disability.

Scar evaluation can be performed using simple paper and pencil scar scales that rate several variables, often through subjective word descriptions (such as red or raised). However, more technically sophisticated and objective devices, such as spectrometry or ultrasound, can analyze one or more variables in a more reproducible manner. The aim of our research was to provide an analysis and critical overview of the scar scales developed to assess the aesthetic and physical aspects of burn scars and their role in burn assessment.

To achieve this, we investigated the available scar tools that can be used in burn assessment and scar research. Unfortunately, there is a paucity of literature on scar tools available for scar assessment. Therefore, our research focused on identifying the available scar scales that can be used to assess burn scars. We analyzed the various scales and tools that have been developed to assess burn scars, focusing on their advantages, disadvantages, and validity. We also reviewed the evidence on the
correlation between scar assessment scores and clinical outcomes, such as pain, itching, and mobility.

Our study has several implications for scar assessment and research. Firstly, our findings underscore the importance of using a standardized and objective approach to scar assessment to improve the comparability and reliability of results. Secondly, our study highlights the need for further research into the development and validation of scar scales and tools. Finally, our study emphasizes the need for scar assessment to be an integral part of burn assessment and treatment to optimize clinical outcomes and improve patients' quality of life (Chapters 6 and 7).

The development of an effective and affordable skin substitute for burn, cancer, and trauma victims has been a long-standing goal of medical researchers. In this regard, glycerol-preserved allografts have been identified as a promising material for developing a dermal substitute due to their human collagen and elastin matrix, ease of storage and handling, inactivation of viruses and microorganisms, and non-profit availability. The most favorable prototype of this substitute, Glyaderm®, has been tested in animal studies and a pilot study on humans. This paper aims to provide an overview of the results of the first clinical publication of Glyaderm® and discuss the key findings of the study.

The study was conducted on 55 patients who underwent a two-stage procedure for skin restoration with Glyaderm®. The pilot study involved wound bed preparation with allografts for five days followed by simultaneous application of Glyaderm® and autograft for wound closure. Objective scar assessment was performed at regular intervals up to six years post-treatment. The study compared the outcomes of patients treated with Glyaderm® and skin graft with those treated with skin graft alone. The study demonstrated that Glyaderm® is a cost-effective and non-commercial dermal replacement that is comparable to currently available dermal equivalents. The long-term results of the study showed consistent and stable outcomes, with patients exhibiting supple skin after six years of treatment. Objective scar assessment showed that patients treated with Glyaderm® and skin graft had significantly improved skin elasticity compared to those treated with skin graft alone (p = 0.003). The study also highlighted the benefits of dermal replacement in surgical burn care and its added value in long-term patient outcomes.

The study's findings are significant in that they demonstrate the effectiveness of Glyaderm® as a viable dermal substitute. The study's results confirm the earlier promising results seen in animal studies and the pilot study. The use of allografts for wound bed preparation was found to be necessary for successful application of Glyaderm®. Direct application of Glyaderm® to the wound bed without wound bed preparation was not a viable option. The study also identified the optimal thickness of glycerol-preserved dermis for processing into Glyaderm® (0.2-0.4mm). The study showed that simultaneous application of Glyaderm® and autograft after wound bed preparation with allografts was an effective procedure for wound closure, reducing morbidity and costs.

The first clinical publication of Glyaderm® demonstrated its favorable long-term results in 55 patients in a two-stage procedure. The study confirms the effectiveness
of Glyaderm® as a cost-effective and non-commercial dermal replacement that can be compared to currently available dermal equivalents. The study also highlighted the benefits of dermal replacement in surgical burn care and long-term patient outcomes. The study's findings have advanced our understanding of the use of glycerol-preserved allografts for developing a dermal substitute and identified the optimal thickness of glycerol-preserved dermis for processing into Glyaderm®. The study's findings will be useful for clinicians in the field in developing practical and affordable skin replacements for victims of burns, cancer, and trauma (Chapter 8).

We were surprised to read that De Hennau et al recently (2021) reported this simultaneous transplant reported in our early clinical trial as “the first”, but pleased to see that our findings are reproducible by other centers, which is also our intention. This center, which has been using Glyaderm® as a DRM since 2017, found, similar to our results, that this procedure resulted in an excellent average absorption rate of 98%. In contrast to our protocol, the bilayer skin reconstruction was performed with and without Negative Pressure Wound Therapy (NPWT), both of which resulted in favorable results.

Collaboration with researchers from the University of Nijmegen resulted in visualization with histochemical techniques of newly synthesized collagen-elastin matrix in vitro and in vivo with Glyaderm® implantation reported in Scientific Reports (Nature) (Chapter 9).

Finally, we conducted "A prospective, controlled, randomized, intra-subject comparative, single-blind study in a monocentric setting, investigating the concomitant application of Glyaderm® + autologous skin grafts (STSG) versus autologous skin grafts (STSG) alone in complete thickness skin defects full and deep burns.

A total of 66 patients were included in this intra-individual study, corresponding to 82 wound comparisons.

The simultaneous application of Glyaderm® and autologous skin proved non-inferior to the previous protocol in terms of graft uptake, subjective scar scales and scar color. The two-step procedure proved to be superior in terms of elasticity. The experts' visual scar evaluation one year after wound closure clearly favored Glyaderm® using the two-step procedure. This was also the case when using the simultaneous application, but not as distinctly as with the two-step procedure. Although we cannot give unequivocal figures, the costs are undoubtedly in favor of the simultaneous application of Glyaderm® with STSG in 1 operation.

Commercially available dermal substitutes often suffer from reduced tissue vascularization and integration. Budding capillaries have difficulty penetrating the DRM when they are too dense. Adequate vascularization requires valuable time, preventing immediate autotransplantation. Commonly used DRMs such as Integra® Bilayer and Matriderm® Bilayer have an autotransplant interval of three weeks. This results in a prolonged inflammatory phase, increasing the risk of fibrosis and scar retraction. The autotransplant interval carries an increased risk of infection, and this has been proven with Integra® Bilayer. Both Integra® and Matriderm® have developed a single-layer 1.00 mm product that allows for a one-stage procedure.
Glyaderm® has a unique human collagen-elastin matrix with a thickness of 0.30 mm and is easily vascularized, allowing immediate autotransplantation. None of the Glyaderm® or autologous skin was lost due to complications. Skin graft survival was excellent and consistent, indicating the formation of a dermoepidermic junction. The biopsies showed adequate vascularization through numerous capillaries. In conclusion, Glyaderm® is easily and adequately vascularized, allowing simultaneous STSG autotransplantation.

Many of the biopsies of the wound sites treated with Glyaderm® showed the presence of elastic fibers and most of these fibers were organized according to a preserved network pattern of natural fibers. Even in the biopsies taken one year after wound closure, donor elastin fibers could be detected. This suggests that the lifespan of the donor elastic fibers is longer than 3 months and probably even longer than a year. We estimate that the donor collagen would have been removed by the time the first biopsy was obtained. Even though it is still an important element in the 3D collagen-elastin network, as stated in the introduction. If the fibroblasts can use this elastic network as a matrix, this would result in a much more favorable orientation of the scar tissue.

The microbiological analysis of the wound swabs taken during the study showed no increase in bacterial load. This suggests that the risk of infection is not increased. The objective evaluation of the scar color at long-term follow-up has shown that erythema and pigmentation are not comparable to those of normal skin. The skin of the intervention group shows slight hypopigmentation and increased erythema. The transepidermal water loss and skin hydration of the Glyaderm® treated scars were comparable to normal skin. These are desirable features of a functional skin replacement, from which it can be concluded that the simultaneous bilayer reconstruction of the skin using Glyaderm® has resulted in the restoration of the skin's natural barrier, protecting the patient from danger i.e., hypothermia, infection, and dehydration.

Skin substitutes have been used in the treatment of a variety of medical conditions. However, burns are a special kind of indication. Acute burns often involve large areas, resulting in a limited supply of viable autologous donor skin. In addition, the situation is complicated by intense local and systemic inflammation and there is only a small window for intervention to minimize scar formation. However, this was the result of using the two-step procedure. This study is the first large randomized clinical trial to investigate simultaneous bilayer reconstruction of the skin using Glyaderm®.

This study investigated the applicability of Glyaderm® in burns in an acute setting. Several dermal substitutes, such as Integra®, have been successfully used in the reconstruction of chronic burn contractures. Matriderm®, Integra® and Renoskin® can be used to treat patients with exposed bone or tendons. In these severe cases, applying STSG is insufficient. We also reported on the successful use of Glyaderm® in a case of burns complicated by tibial bone exposure following the failure of free flap surgery. In selected cases, Glyaderm®, combined with negative wound pressure therapy and skin grafting, can be used as an alternative to lap surgery.
The variety of dermal substitutes is huge, and research is done in different ways, resulting in different results with each product. Whether the simultaneous application can be improved by using dermal substitution with Glyaderm® with a thickness greater than 0.30 mm needs to be addressed in future research.

Very interestingly, the single most important parameter, the patient's overall experience and feeling as scored by using the POSAS, was in favor of Glyaderm® and the difference in favor of Glyaderm® group increased with each follow-up time. Our research group believes that scar quality should be assessed even up to two years after complete wound closure. We believe that the final result in scar quality should be at least 1.5 years after wound closure and preferably even up to 2 years when tissue remodeling and our standard scar therapy treatments are complete (Chapter 10).

Conclusion

Over the past two decades, we have focused on the treatment of major skin defects by using Glyaderm® as a dermal substitute. Dermal substitutes face particular challenges, such as the inability to provide adequate temperature control or pressure sensation, reduced vascularization due to prolonged survival of the substitute, inadequate immune regulation, failed integration, high cost, slow wound healing, infection, pain, and unaesthetic scarring. Currently available cellular skin substitutes consist of only two cell types: fibroblasts and keratinocytes. These skin replacements are therefore unable to form specialized structures such as glands or hair follicles. A gammut of innovative research has been published in recent years. A recent study yielded the first LGR6+ stem cell-based skin substitute capable of epithelization, hair growth and angiogenesis in wound beds. To illustrate a good example of innovative discovery.

Another recent study defined the ideal skin replacement as follows: “However, an ideal skin replacement would be a durable bilayer reconstruction that is morphologically and biochemically similar to the original skin, mimicking its texture, structure and ability to regenerate”.

At present day there is no product that can meet all these high requirements. However, Glyaderm® contains a natural dermal network that has the intact spatial structure of normal human skin, making it the closest theoretical approach to the ideal skin substitute. In the first part of this thesis, the clear advantages of using donor skin of human origin have been mentioned. The use of human-derived dermal substitutes has drawbacks such as a limited supply of donor skin, potential ethical issues, slower penetration of endothelial cells, and the lack of skin appendages. Research has been going on for many years and significant progress has been made. Technologies once considered "the future" are making their appearance. Electrospinning, recombinant proteins, small molecule engineering, autologous cultured skin substitutes using stem cells and three-dimensional bioprinting are just a few examples of the modern approach in burn care not to mention the promise of artificial intelligence.
As mentioned earlier, burn injuries contribute significantly to the mortality and morbidity of the population worldwide. In 2004, nearly 11 million people were severely burned and required clinical medical treatment. Post-trauma burn victims are left with cosmetic deformities, impaired functions, psychological trauma, difficulty with daily activities, and social dysfunction. The goal of burn care, as always, is not just the reconstruction of the damaged tissue, but the full recovery of the patient as a whole. Just one of many possible solutions to achieve this is to use a dermal substitute. The results of our studies have provided us with interesting data. Not only did we find that the simultaneous application of Glyaderm® and STSG was possible, but that the donor's elastin fibers were histologically detectable even one year after the wound had completely closed. The double-layer reconstruction with Glyaderm® was in many ways equal to both the gold standard and the two-step procedure. In addition, the tissue reconstructed with Glyaderm® had many features similar to those of healthy human skin.

It is our intention to educate and propagate the application scope of Glyaderm® as a DRM for plastic surgeons and burn surgeons.

Glyaderm® has been successfully used for indications other than deep burns and burn scars, i.e., oncologic resections, free flap donor site reconstructions, giant melanocytic naevi and reconstructions of post-necrotizing fasciitis.

We remain committed to our original goal and intent to make Glyaderm® DRM available for widespread application in burns. To this end, a strong collaboration with plastic surgeons in Colombia has led to Glyaderm® being successfully produced at the Bogota Skin Bank and applied in one stage and two procedures for severe (facial) burns. Also, royalties from book and charitable organizations provide funding to make Glyaderm® available to patients with severe burns and full-thickness traumatic defects.

A plethora of research needs to be done now and in the future until the perfect ready-to-use skin replacement and acellular matrix becomes available.

Glyaderm® can be a viable DRM to bridge this gap to improve the quality of life of many victims of trauma and burns now and in the near future. In addition, Glyaderm® can serve as a biological dermal matrix for further cell regeneration and tissue engineering research in the quest for continuing tissue regeneration.