20 year evolution of Glyaderm® dermal regeneration matrix

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CHAPTER 1

INTRODUCTION
Introduction and outline of this thesis

Introduction

Severe Burns remain a major cause of injury-related mortality. Advancements in burn care and surgical burn reconstruction have led to improved patient survival and rehabilitation. Currently, the primary focus of burn care has shifted from only survival to improving the quality of patient survival. The quality of life of burn patients is largely influenced by their ability to re-integrate into society which is associated with scar quality and appearance, and perception of their own appearance.

Split thickness skin graft (STSG) remains the gold standard for surgical reconstruction of deep dermal and full thickness burns. However, STSG is associated with challenges such as a paucity of donor sites, donor site morbidity, graft contracture, and/or unfavourable and unpredictable scarring.

The development of biological, synthetic skin substitutes, and human allografts has augmented the armamentarium of the burn clinician with alternatives to autologous STSG.

Dermal regenerative matrices (DRMs) are permanent skin substitutes that allow for a degree of regeneration of the dermal skin component in the management of major burns, traumatic contractures, and skin defects. The use of a DRM in burn surgery has shown to produce a more favourable functional and aesthetic results.

Drawbacks for the use of DRM include the need for a two-stage procedure, increased infection risk, and high cost.

Historical aspects and aim of our studies

Historical Aspects

At the start of the millennium our research group was faced with challenges in burn care reconstruction. The DRM Integra was gaining attention in literature and at conferences but the variability in take rate and high cost prevented its widespread application and routine use in the Gent Burn Unit.

Pirayesh who had witnessed the savage burn trauma in revolution and war as a child in Iran was grasped by Plastic Surgery & Burn Care as a Senior House Officer at the burn unit in East Grinstead where his mentor Philip Gilbert taught him the principles of burn care passed on by Sir Archibald McIndoe who had bravely treated the burns of RAF pilots from the Battle for Britain. He started research into keratinocyte culture and presented papers at burn conferences where he met Hans Hoekstra, the inventor of the glycerol preserved allograft (GPA). Hoekstra was active in experimental burn research in Amsterdam and taught Pirayesh the core principles of experimental burn research together with Dr Nelleke Richters who worked as an immunologist and researcher for the Dutch Burn Foundation.

Pirayesh was impressed by the research output from the Gent Plastic Surgical Unit and approached Prof Stan Monstrey at a conference who gave him the opportunity to
apply for a residency. Pirayesh was selected for residency and had to start with a pre-
residency year at the Gent Burns Unit. Henk Hoeksema, the principle burn care
coordinator taught him the principles of conservative burn care and surgical burn
care. They introduced and initiated studies with MEEK grafting and interactive honey
dressings in the Gent unit which was famous for the use of laser doppler imaging to
scientifically delineate burn depth and therefore the ideal place for clinical studies on
burns.

Their collaborative brainstorming sessions culminated in the idea of developing a
dermal substitute based on glycerol preserved allograft which would be a non-profit
and cost-effective DRM for widespread application and improvement of the quality
of life of the burn patients.

Pirayesh, Hoeksema, Richters, Hoekstra and Monstrey signed away their IP rights for
the DRM “Glyaderm” which was affirmed by notary to the EuroSkinBank, now
EuroTissueBank, Beverwijk, The Netherlands.

Pirayesh returned to the Netherlands to build his private practice, but propagated
Glyaderm® research and global application which resulted in the development of a
Colombian Glyaderm® for victims of acid attacks. The Two Faces
(https://twofacesfoundation.org) charity was set up to help these victims by his
partner Eva Velders.

Berend van der Lei, together with Prof Monstrey, have been inspiring forces
throughout Pirayesh’s career, and coached him to structure and submit this thesis
under their guidance. As a result of their efforts and supported by the newly available
long-term results, the place of Glyaderm® amongst other dermal regeneration
matrices is firmly attested.

**Aim and study set up.**

The initial studies comprised of development of a dermal matrix from glycerol
preserved allogeneic skin from conception to delineation of a prototype (Chapter 2).
The best prototype was named Glyaderm® (Glycerolised Acellular Dermis) and
compared with different dermal substitute matrices in a porcine wound model
(Chapter 3) and as dermal scaffold for closure of abdominal wall defects in a rat
model (Chapter 4).

Trial set-up and ethics approval for clinical trials necessitated us to investigate
literature concerning skin replacement in burns (Chapter 5).

We performed a systematic review of scar assessment scales (Chapter 6) as well as a
systematic review and critical appraisal of available scar assessment tools (Chapter 7)
to update protocols for our clinical trials.

The first clinical publication of Glyaderm® showed favorable long-term results in 55
patients in a two-stage procedure (Chapter 8).

Collaboration with researchers from Nijmegen University resulted in visualization of
newly synthetized collagen-elastin matrix in vitro and in vivo with Glyaderm®
engraftment (Chapter 9).
A prospective, controlled, randomized, intra-individual, comparative, single-blinded study in a monocenter setting investigating the simultaneous application of Glyaderm® DRM + Split thickness skin graft vs autologous split thickness skin graft alone in full thickness skin defects and burns was performed with enrollment of 64 patients in a one-stage procedure (Chapter 10).

We concluded our studies with an update on the evolution, scope, and future directives of Glyaderm® and its place amongst currently used DRM’s (Chapter 11).

Outline

In order to improve wound healing of deep burns, dermal substitutes can be utilized in conjunction with expanded, thin autologous skin grafts. These dermal matrices can be sourced from either xenogeneic or human tissue, but antigenic structures such as cells and hairs must be removed to prevent adverse inflammatory responses upon implantation.

In this study, a cost-effective method for de-cellularizing human donor skin preserved in 85% glycerol using low concentrations of NaOH is described. Donor skin was incubated in NaOH for varying time periods of 2, 4, 6, or 8 weeks, and the resulting dermal matrix prototypes were analyzed using standard histology techniques. Functional tests were conducted in both rat subcutaneous implant and porcine transplantation models, where the prototypes were placed in full thickness excision wounds covered with autologous skin grafts (Chapter 2).

A porcine wound model was used subsequently to compare already known acellular dermal substitutes with our new prototype (Glyaderm) prepared from glycerol preserved human skin. All donor cells are removed by incubation in a solution of 0.06M NaOH. The dermal substitutes were applied to full thickness wounds and covered with an STSG. As a control, wounds were covered with only an STSG. The wound healing response was analysed for 8 weeks, macroscopically and on biopsies (Chapter 3).

To further evaluate the efficacy and biocompatibility of the "Glyaderm" dermal regenerative matrix (DRM), we conducted a subsequent study to assess its ability to provide coverage for abdominal wall defects. Abdominal wall repair can be performed using either synthetic or biological materials, with the latter often preferred due to their reduced risk of infections and fibrosis. In this study, we aimed to compare two acellular human dermis products using a rat model. One material was prepared using low concentrations of NaOH, while the other was the commercially available SureDerm®. Full thickness defects were created in the abdominal wall and repaired with the two materials. Rats were sacrificed at either 1- or 4-months post-operation, and the number of adhesions to the bowels were scored. Samples were collected for histological analysis and to measure the breaking strength of the repaired area (Chapter 4).

We subsequently set out to review the literature on Skin Replacement in Burns. The aim of this study was to give an overview of which types of skin replacements have been developed and which problems still need to be faced. None of these
commercialized products can currently claim to be the optimal skin replacement, because clinical evidence is too scarce (several large multicenter trials are currently in process). The number of products becoming commercialized is nevertheless increasing steadily, which pleads for a certain overview, classification and clear comparison of the available products. (Chapter 5).

Due to the improvements in burn treatment as provided in highly specialized burn centers, more patients with deep and extended burn injuries do survive nowadays\textsuperscript{16–18}, resulting in a larger group of patients with more extensive scar formation\textsuperscript{19}. Scar formation depends on several variables, including the wound treatment, the depth of the burn, the skin type and age of the patient, the healing process (inflammation, infection, etc.) but also on the application of preventive measures\textsuperscript{20,21}. As a rule, wounds that are not healed within 2–3 weeks are considered most at risk for excessive scar formation\textsuperscript{22}.

Because of the relatively high prevalence of unfavorable scar formation after burns, most studies on scar assessment and scar treatment are focused on the burn scar\textsuperscript{22–25}. Surgical and dermatologic scars will rarely result in extensive scar formation, and since the impact of scar complications strongly correlates with the dimension of the scar (e.g., pain, itching, and fragility), the impact of these types of scars is usually more limited, although also less well studied\textsuperscript{26}. Therefore, burn scars are probably the scars with the highest impact on the quality of life\textsuperscript{27–35}. Both physical and psychologic effects related to excessive scarring may hamper the quality of life, including the often lengthy, painful treatment, often resulting in still a suboptimal result\textsuperscript{26,27,34,36–40}. Scars may cause pain, itching, and discomfort; and contractures may also constrict mobility. The integration of patients with hypertrophic scars in a society where well-being, individuality, and external appearance have become increasingly important might also be troublesome\textsuperscript{27}. It has been demonstrated by many authors that burn scars, because of their clearly visible and stigmatizing appearance, may have a major psychologic impact, comparable to other chronic (skin) diseases\textsuperscript{26,27,41–43}. A study of Balci et al. analyzed the quality of life in patients with hypertrophic scarring and keloids and found a similar impairment as in patients with psoriasis\textsuperscript{37}. Brown et al. identified five main areas of impact in patients with excessive scarring resulting in coping behavior to hide or compensate the scars: the physical comfort and functioning, confidence in the nature and management of the condition, acceptability to self and others, social functioning, and emotional well-being\textsuperscript{26}. They concluded that scarring has a major influence on a patient’s psychologic morbidity and behavior and has important implications for clinical practice. Van Loey et al. described how scars may contribute to social anxiety and post-traumatic stress syndromes, since pressure garments or red and disfiguring scars can attract a lot of attention from other people, which may induce feelings of shame\textsuperscript{27,44}. Several preventive measures and treatments have been proposed to decrease pathologic scar formation, and multiple invasive and noninvasive treatment modalities have been introduced\textsuperscript{20,45–49}. Although scar assessment seems essential, this is still a neglected area, and there is still no consensus on the ideal method of scar evaluation, despite the many scales and tools that have been developed during the last
decades. Adequate assessment of scars is, however, important in the clinical evaluation and follow-up, but it is also essential to compare different wound or scar treatment modalities. Moreover, for medico-legal reasons, an objective scar evaluation can be required, e.g., for reimbursement of treatment and proof of disability.

Scar evaluation can be performed by rather simple, paper-and-pencil scar scales assessing several variables, usually by purely subjective word-descriptions (red, elevated, etc.), but also by using technically advanced and objective devices (scar tools) analyzing one or more variables in a more reproducible way (spectrometry, ultrasound etc.). The objective of this study was to provide an analysis and critical overview as to which scar scales have been developed to assess the physical aspect of burn scars, and what their role is in burn assessment (Chapter 6).

The paucity of literature on scar tools available for scar assessment brought us to investigate the available scar tools which can be used in burn scar assessment and research. (Chapter 7).

We extensively reported on the various cellular, acellular, temporary, and permanent skin replacements available for burns and full thickness defects in a previous publication. 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. Glycerol preserved allogeneic skin (GPA) 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. 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 favourable results in terms of tissue integration and wound contraction and scar quality.

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 in 55 patients with long term results (Chapter 8).

In tissue engineering and regenerative medicine, type I collagen is a critical biomaterial due to its significant role in the organization of tissues and organs and its involvement in organogenesis. Conversely, collagen gels are widely used in 3D
studies, especially in cancer research, to investigate cellular migrational behavior. However, distinguishing between pre-existing collagen and newly synthesized collagen remains a significant challenge due to the highly conserved nature of collagens, which leads to cross-reactivity between different species. Current methods, including the use of antibodies, metabolic radiolabeling and mass spectrometry, are labour-intensive and do not provide topographical or organizational information about newly synthesized collagen fibers.

This study aimed to address this challenge by evaluating newly synthesized type I collagen using dermatan sulfate's intrinsic association with collagen fibrils. Proteoglycans decorin and biglycan, both collagen fibril-associated molecules that regulate collagen fibril diameter, contain dermatan sulfate, which remains associated with mature collagen fibrils. The study utilized single chain variable fragment antibody GD3A127 to selectively detect dermatan sulfate combined with the absence of dermatan sulfate in experimentally or commercially produced biomaterials. The technique was tested using several collagenous biomaterials, including gels cultured with human fibroblasts with or without keratinocytes (denovoSkin and denovoDerm respectively), experimental and commercially available scaffolds, and glycerol preserved acellular human dermis (Glyaderm®), both in vivo and in vitro (Chapter 9).

Although with the results of our previous studies it was concluded that Glyaderm® is a suitable replacement for the dermal layer in full thickness wounds, certain drawbacks limited a widespread application of this product. In the current era of universal budget restrictions, it is imperative to respect financial limitations when it comes to the implementation of new technologies. Burn care is already considered an expensive niche of our health care system. Costs are high because patients with burn injuries frequently require specialized treatment, prolonged hospitalization, intensive surgical and non-surgical treatment. The initial surgical regimen for using Glyaderm® often consisted of three consecutive operations (allografts, Glyaderm® and STSG). This protocol increases the financial burden due to additional surgical procedures and the obligatory extended hospital stay of 3 weeks after implantation of the dermal substitute. Animal studies showed that simultaneous application of Glyaderm® and STSG was not feasible. The dermal replacement should be able to supply nutrients to the STSG. This requires an adequate vascularization of Glyaderm® or the STSG will not survive. The research team attributed the impossibility of simultaneous application to the batch-to-batch inconsistencies and the proportions of Glyaderm®.

The Glyaderm® used in the previous studies proved to be too thick (thickness varying between 0.8mm to 1.3mm), obstructing rapid ingrowth of blood vessels, which is needed to vascularize the autograft. Glyaderm® with a more uniform and optimal thickness was needed. In the next phase selection by hand was performed, but as this was too labour intensive a laser tool had been developed. This purpose designed laser device can create Glyaderm® of a homogeneous thickness (0.30mm), resulting in a standardized Glyaderm® thus eliminating the batch-to-batch inconsistencies. A pilot study with simultaneous application of Glyaderm® and STSG has already been
conducted. This pilot study has shown comparable results between the bilayered reconstruction and the STSG alone in terms of vascularization, graft take rate and wound healing time. Stronger evidence, based on a comparative intra-individual trial, of this simultaneous application was needed in order to prove its validity. Our research team has conducted a study to further investigating the simultaneous application of Glyaderm® and STSG in providing a bilayered skin reconstruction. The aim of this study is to gather the evidence proving that by reducing and standardizing thickness of Glyaderm® to 0.30mm, a simultaneous application of Glyaderm® and STSG is possible. Reducing the number of surgical interventions would not only make the application of Glyaderm® more cost-effective, but it would also decrease the morbidity since wound closure will be achieved one week earlier compared to the two-step procedure. We mentioned before that Glyaderm® is comparable to other currently available dermal equivalents, but with the advantage of being low-priced. Power calculation determined that 75 wound comparisons are to be included.

As hypothesis we state that treating deep burns and other full thickness skin defects with a simultaneous application of Glyaderm® + STSG will result in superior scar quality compared to the application of STSG alone. This project started in October 2016. This study evaluates the results of the 80 wound comparisons of this study. This will include both short-term results (Glyaderm®/STSG take rate, microbial contamination, infection rate, length of hospitalization...) and long-term results gathered from follow-up (elasticity, erythema, water loss...). Patients are evaluated up to 12 months after complete wound closure. (Chapter 10).
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