ARTIKELEN

Skin Substitutes for wound healing and development of animal alternative assays

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INTRODUCTION

One of the most advanced fields of tissue engineering is in the area of skin. Already different types of skin constructs have been introduced into the clinic for healing difficult to close wounds (e.g.: chronic wounds and large traumatic wounds). Skin constructs are also being used as animal alternatives in compliance with the 3R's (reduction, refinement and replacement of test animals) e.g.: reduction/refinement when testing new therapeutics, and replacement when testing cosmetics (irritants). Skin tissue engineering is a multidisciplinary exercise that requires each construct to be specifically designed for its particular future application. Some factors which need to be taken into account are: i) is a bi-layered construct required or is a mono-layered construct sufficient or even preferred; ii) are autologous cells required or can allogeneic cells be used; iii) are cells required from diseased skin or healthy skin; iv) which cell types should be incorporated (keratinocytes, melanocytes, Langerhans cells, fibroblasts and/or endothelial cells); v) should the skin represent inflammatory skin or skin in homeostasis; vi) which inflammatory profile of mediators is preferred by the cells within the construct. In addition to these factors, the choice of dermal matrix is extremely important e.g.: should it be biodegradable or stable, contractile or not, dense or sponge-like, is it suitable for clinical use in an Advanced Therapy Medical Product (ATMP). The choices made are dependent on whether the construct is designed for clinical use or for use in in vitro assays. Below we discuss the choices made and current areas of research and development within the Department of Dermatology, VUmc and her spin-off company A-Skin BV.

SKIN SUBSTITUTES FOR ADVANCED WOUND HEALING THERAPIES

When developing a skin substitute for wide spread clinical use, it is not only important to consider the cell types and (bio-)physical properties of the construct but also to consider all of the logistics involved in its application as a future therapy. Therefore, the entire production process and clinical application needs to be taken into account. The production process needs to comply with the recently implemented European legislations for ATMPs (ATMP directive, Dec 2008) and culture has to be carried out according to Good Manufacturing Practise (GMP) in a cleanroom. The logistics of transport of biopsies (for autologous culture) to the cleanroom and transport of the skin substitute back to the wound care centre should follow simple standard procedures making it easy and realistic for the clinician to schedule the application. Also preferably, the time required should be similar to the time required for standard wound care treatments so as not to disrupt out-patient clinic and surgery time tables. The construct should be easy to handle by the clinician and importantly, the added burden to the patient should be kept to a minimum. Therefore, for future application of skin substitutes as advanced

wound healing therapies, not only the design of the construct needs to be carefully considered, but also whether its application is clinician and patient friendly. Taking all of these points into account we have developed two different constructs which are currently being investigated in clinical research studies for i) healing therapy-resistant chronic wounds and ii) improving scar formation in deep (3rd degree) burns. These constructs and their applications are discussed below.

AN AUTOLOGOUS FULL THICKNESS SKIN SUBSTITUTE FOR HEALING THERAPY-RESISTANT CHRONIC WOUNDS

Living skin substitutes provide an alternative advanced treatment for healing therapy-resistant ulcers (>12 weeks duration). They are easy to apply in the out-patient setting. No hospitalization is required. Patients are treated during their regular visit to the wound care specialist within the standard allowed time for each patient. Much less donor skin is required to construct an autologous skin substitute than is required for split-thickness autograft or punch biopsies to cover a wound. Therefore, treatment with a living skin substitute is 'user friendly' for both the patient and the clinician. The advantage of living skin substitutes above acellular dressings is now widely accepted: they provide an immediate cover but above all they continuously secrete a potent cocktail of cytokines, chemokines and growth factors which promote wound healing.[1] These factors improve wound healing by stimulating angiogenesis, granulation tissue formation and wound closure. We made an early decision to develop an autologous skin substitute comprised of the patient own cells rather than an allogeneic skin substitute comprised of donor cells for the following 2 major reasons: 1) there is no risk of rejection therefore a single application should be sufficient and large wounds can be treated; 2) there is no risk of transferring infections from the donor material to the patient. The reason for developing a bi-layered construct (living epidermis and dermis) for therapy resistant chronic wounds above a mono-layered construct (either living epidermis or dermis only) is that a bi-layered construct is much more potent in secreting factors which stimulate wound healing due to cross-talk between the cells within the epidermal and dermal compartments. We choose human acellular donor dermis, isolated from glycerol preserved donor skin (Euro Skin Bank, Beverwijk, the Netherlands) as a matrix since this donor skin is prepared for clinical use for the temporary coverage of large burns. Furthermore this acellular dermis is biodegradable. It is broken down over a few weeks and replaced by new dermis formed by the patients own fibroblasts during the natural turnover of the skin. The pre-clinical and clinical data described below are from our 1st generation skin substitute. This research was performed before the new EU legislation was introduced in December 2008 for ATMPs. We have now performed extensive change control procedures in order to adapt the 1st generation skin substitute into a 2nd generation skin substitute which now

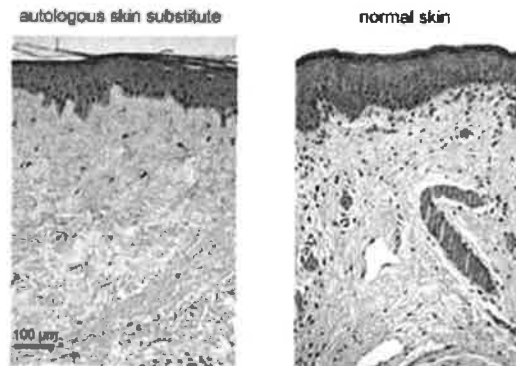

Figure 1. Histology of autologous skin substitute and normal skin.

fully complies with current EU legislation for ATMPs. This new construct has undergone the pre-clinical research and development phase and is now ready for testing in a phase 1 clinical study.

PRE-CLINICAL DATA

Histological characteristics - 1st generation skin substitute[2]
The bi-layered autologous skin substitute consists of keratinocytes in the epidermis and fibroblasts in the dermis. It closely resembles normal healthy skin (Figure 1). The epidermis consists of a basal layer, spinous layer, granular layer and stratum corneum. Epidermal differentiation in our autologous skin substitute resembles differentiation in native human epidermis as demonstrated by expression of multiple differentiation markers (e.g.: keratin 10, involucrin, loricrin). Proliferating keratinocytes are located in the basal layer. Fibroblasts populate the dermal matrix with a similar distribution to that observed in healthy skin. Alpha-smooth muscle actin (α-SMA) is not expressed by the fibroblasts, indicating that no differentiation to myofibroblasts has occurred which could lead to adverse scar formation in the patient to be treated. Analysis of the basement membrane shows a correct deposition of basement membrane proteins (e.g.: laminin, collagen IV). These proteins are essential for epidermal attachment and keratinocyte migration and importantly prevent blistering occurring between the epidermis and dermis after application.

Secretion of wound healing mediators
The autologous skin substitute continuously secretes a potent cocktail of cytokines, chemokines and growth factors which promote wound healing.[1] These were identified with the aid of a protein antibody array. The secreted proteins have inflammatory, angiogenic and granulation stimulatory properties and are secreted to a greater extent by the autologous skin substitute than by native human skin, an epidermal substitute or a dermal substitute. This importantly indicates that the bi-layered skin substitute has a more potent wound healing capacity than an autograft or mono-layered construct. That this is indeed the case is illustrated by our clinical data in which application of the skin substitute to chronic wounds resulted in healing of

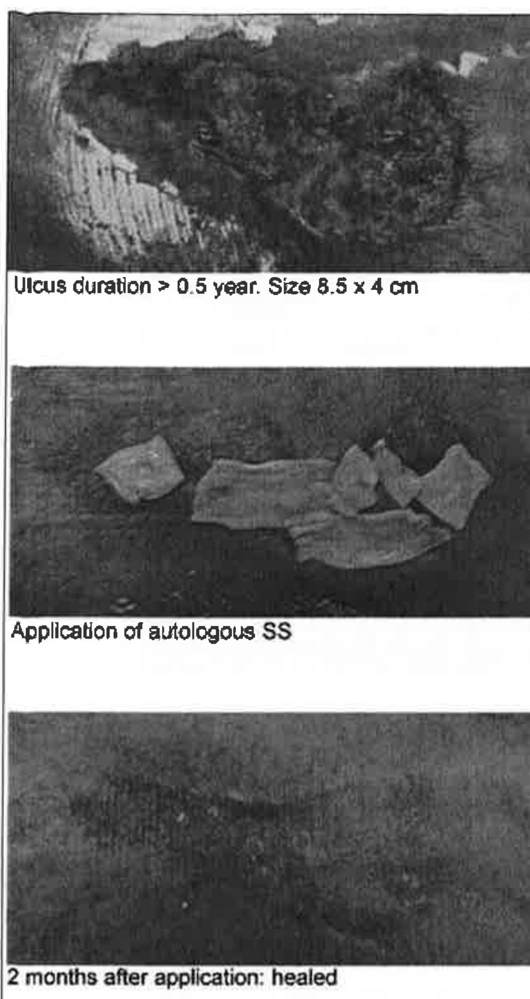

Figure 2. Skin substitute application: venous ulcer.

wounds that had previously been unresponsive to autograft.[2]

Stimulation of the inert wound bed
Conditioned medium collected from the skin substitute during culture accelerates migration of fibroblasts, keratinocytes and endothelial cells in vitro (data not published). Furthermore, wound exudate collected from the chronic wound bed, when added to the culture medium of the skin substitute greatly enhances the secretion of factors which stimulate granulation tissue formation e.g.: MCP-1, IL-6 and IL-8. Therefore a two-way cross talk can be proposed in which the skin substitute stimulates ingrowth of cells into the wound bed and the exudate secreted into the wound bed stimulates the skin substitute to produce factors responsible for this ingrowth. In this way, once the skin substitute comes into contact with the wound bed, it not only covers the wound immediately but is also extremely potent in stimulating wound healing. These properties are required to transfer an inert wound into a healing wound.

CLINICAL DATA

Since 2004 the 1st generation skin substitute has been applied to a variety of chronic wounds in the hospitalized and out-patient (multi-centre) setting.[2-6] The autologous skin substitute is placed directly onto the cleaned wound bed. No stitches are necessary. The skin substitute is held in place with wound dressings and bandages (Figure 2). First bandage change is 3-5 day after application, depending on the amount of wound exudate. Patients are requested to move the leg as little as possible during the 1st week in order to allow the skin substiute to attach to the wound bed and become vascularized. Hereafter patients are encouraged to become mobile again. Between 2004 and 2009, more than 80 patients with chronic wounds of different aetiology were treated with the 1st generation skin substitute. The majority of the study group consisted of (arterio-)venous ulcers, but also decubitus, diabetic foot,traumatic ulcers (major accidents, burns) or major surgery (abdominal). Size ranged from 1 cm^2 to 150 cm^2; ulcer duration was more than 12 weeks therapy resistant to 50 years recurrent. Many patients were treated in the regular out-patient setting during their standard weekly appointment with the dermatologist or wound care nurse. Others were hospitalized for up to 10 days. From this very broad group of patients with therapy resistant chronic wounds, the majority of the ulcers showed more than 50 % wound closure after a single skin substitute treatment and many ulcers completely healed (manuscript in preparation).[2]

CULTURED KERATINOCYTES FOR IMPROVED SCAR FORMATION IN 3RD DEGREE BURNS

Clearly, the potent wound healing potential of the skin substitute described above resides in the cross-talk between cells in the bi-layered construct which result in the continual release of mediators which in turn stimulate granulation tissue. These properties are required to transfer a chronic therapy-resistant ulcer into a healing wound. However, when designing a construct for treating large burns over-activation of the wound bed needs to be avoided. In these wounds, the quality of the scar, normotrophic instead of hypertrophic, is extremely important. From our pre-clinical data we could show that cultured keratinocytes have the potential to cover large wound areas whilst at the same time secreting very low amounts of inflammatory mediators compared to the bi-layered skin substitute.[1] This is confirmed in the literature which describes applications of cultured keratinocyte sheets for burn patients dating back as far as 1979, starting with the pioneer work of Green.[7] However, a number of limitations have prevented wide spread implementation of this keratinocyte therapy. Culture procedures depended on using lethally irradiated 3T3 murine fibroblasts as feeder cells in order to sufficiently amplify the yield of keratinocytes. Such co-culture is accompanied with the risk of viruses, prions or other macromolecules being transmitted from the feeder cells to the human cells during culture. Also high concentrations of bovine serum in the culture medium

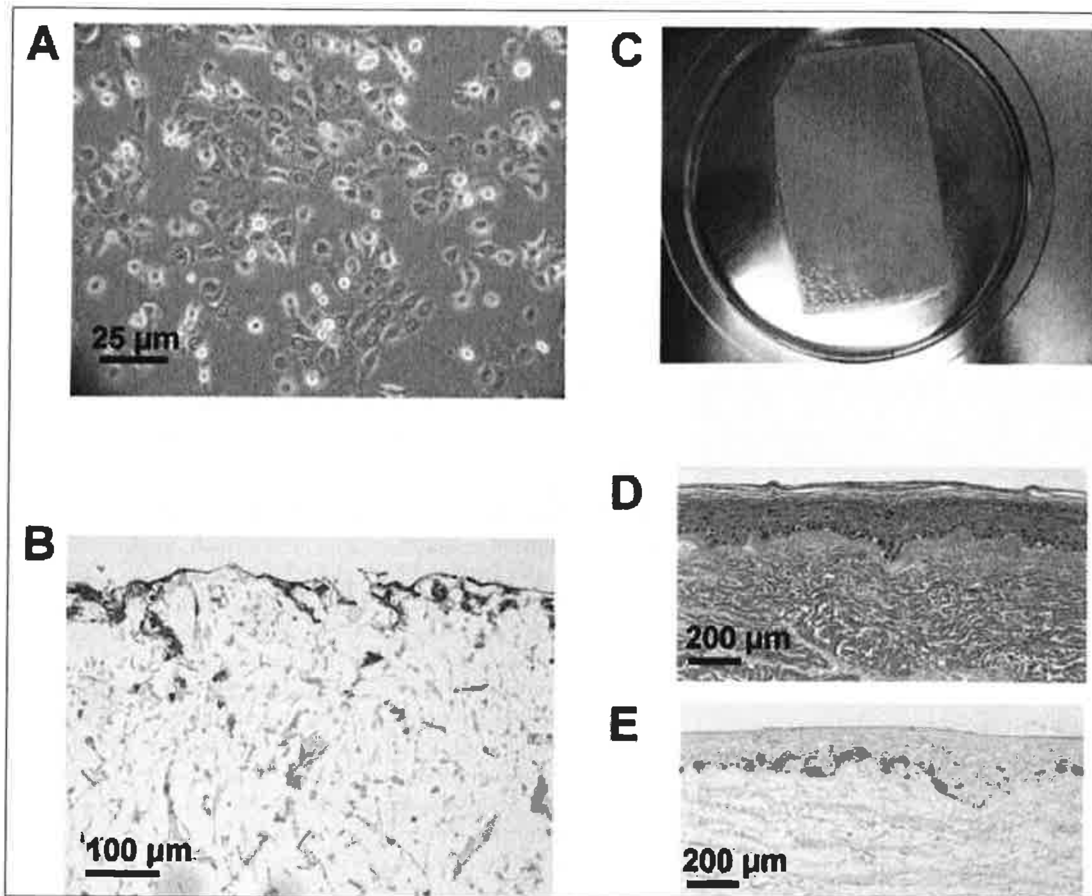

Figure 3. Use of a collagen/elastin matrix as transport carrier system to transfer proliferating epidermal cells. A) amplification of cultured keratinocytes and melanocytes in tissue culture plates; B) macroscopic view of epidermal cells seeded into Matriderm®, C) immunohistochemical staining (pan-keratin) of paraffin embedded section of Matriderm® containing epidermal cells; D) after transfer of epidermal cells to human dermis a fully differentiated epidermis forms (HE of paraffin embedded tissue section); E) the differentiated epidermis contains melanocytes (BTEB immuno-staining).

was used which results in non-compliance with current regulatory requirements. Confluent keratinocyte sheets are extreme fragile which makes transport logistics and application onto the wound surface very difficult. Importantly, the enzymatic (dispase) digestion required to remove the sheets from the tissue culture plastic results in poor and highly variable take, and frequent blistering of the sheets after application.[8-10]

Use of a collagen/elastin matrix as transport carrier system to transfer proliferating epidermal cells to large burns

When developing a construct for healing large trauma and burn wounds, we took all of these previous drawbacks into account. We developed a means to amplify large numbers of proliferating autologous epidermal cells (keratinocytes and melanocytes) and a means to easily transport and finally transfer the cells onto the wound bed, all in accordance with the regulations set by the Dutch Central Committee on Research Involving Human Subjects (CCMO).[11]
Large numbers of proliferating epidermal cells were generated within 10-14 days and seeded onto a three-dimensional matrix composed of elastin and collagen types I, III and V (Matriderm®, dr. Suwelack Skin & Health Care, Billerbeck, Germany) which enabled easy and stable transport of the epidermal cells for up to 24 hours under ambient conditions (Figure 3). Also importantly, the Matriderm matrix was used in order to make it very easy for clinicians to handle the cultured epidermal cells during surgery. As a pre-clinical model system for clinical *in vivo* transfer, the epidermal cells were transferred from Matriderm onto human acellular dermis during a period of 3 days. After transfer the epidermal cells maintained the ability to regenerate into a fully differentiated epidermis containing melanocytes on the human dermis. Proliferating keratinocytes were located in the basal layer and Keratin-10 expression was located in differentiating suprabasal layers similar to that found in human epidermis. No blistering was observed (separation of the epidermis from the basement membrane). Keratin-6 expression was strongly up-regulated in the regenerating epidermis similar to normal wound healing. This construct is currently being studied in a multi-centre trial in collaboration with the 3 Dutch Burns Centres. The construct is applied above a split thickness meshed autograft in order to determine whether the cultured

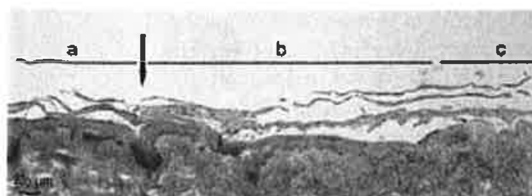

Figure 4. In vitro wound healing model. Cryo-burns were introduced into skin equivalents. Histology of the migrating front during re-epithelialization is shown. Cultures were harvested 2 days after wounding. arrow = wound margin; a = unwounded epidermis; b = re-epithelializing wound; c = still open wound area.

keratinocytes will transfer into the gaps in the mesh graft thus resulting in faster wound closure and improved scar formation.

SKIN EQUIVALENTS FOR USE IN ANIMAL ALTERNATIVE (IN VITRO) ASSAYS

Why are in vitro assays needed?
Since the 1980's efforts to achieve a reduction in animal testing have slowly been gaining momentum in Europe. This was initiated by pressure applied from animal welfare groups, public opinion and ethical debates. In 1986, an EU directive on the protection of animals used for experimental purposes (Directive 86/609/EEC) (12) banned the use of an animal experiment when a scientifically approved alternative exists. The 7th Amendment to the Cosmetics Directive (Directive 76/768/EEC) has resulted in a complete ban on animal testing for cosmetic ingredients from 2009 with the exception of repeated dose toxicity and sensitization for which the deadline is 2013. Finally, in addition to the above mentioned directives, the REACH legislation (Registration, Evaluation and Authorization of Chemicals) has been implemented,[13] requiring additional risk assessment of nearly 30,000 chemicals already marketed in the EU. Whereas a future ban will be implemented on the testing of cosmetic and industrial products in animals, this ban will not be implemented for the testing of new therapeutics. For therapeutics however, a reduction and refinement in the number of test animals is required. Currently it is realised by all parties concerned, that animal models may not always correctly represent humans, and in some cases no suitable animal model exists. Therefore, a validated human in-vitromodel may prove to be superior to an animal model. All in all, this has resulted in a huge stimulus to develop new in vitro techniques to replace animal testing for both the scientific and industrial community.[14] Here we discuss a number of in vitro models undergoing research and development, and also pre-validation in our department.

The phases leading to validation and implementation of an in vitro assay
In vitro assays can be divided into 2 main categories: those aimed at animal replacement and those aimed at reduction / refinement of the use of test animals. The former are generally more simple assays and the later are generally more complex assays.
The majority of skin equivalent based assays aimed at replacing animals completely generally require minimal expertise to perform since they make use of commercially available constructs and standardized (pre-)validated protocols e.g.: epidermal equivalent for determining corrosive or irritant potential of a substance (fully validated protocols are available on ECVAM website). These assays aim for widespread implementation in any laboratory with basic cell culture know-how. These assays currently address the area of risk assessment of hazardous chemicals. The assays involve extensive validation procedures and inter-laboratory studies in order to assess the transferability of the assay in addition to the robustness, specificity and sensitivity of the assay.
Assays aimed at animal refinement / reduction are often complex assays that require a high level of expertise to perform and /or require a regular supply of healthy or diseased skin e.g.: chronic and burn wound in-vitromodel,[15] and in vitro hypertrophic, keloid and normotrophic scar models (manuscripts in preparation). These assays generally aim to test novel therapeutics in the future. They are limited to a small number of specialized laboratories working in close collaboration with medical specialists. Below we will describe examples of our progress on i) a simple model aimed at widespread implementation and animal replacement for determining potency of contact sensitizers, and ii) more complex skin models aimed at testing novel therapeutics for enhanced wound closure and reducing adverse scar formation e.g.: hypertrophic scar formation resulting from trauma burn wounds.

A potential epidermal equivalent assay to determine sensitizer potency
The assay described here is part of a preventative medicine strategy since it aims at identifying the potency of a sensitizing substance. Such assays are required in order to prevent individuals being exposed to substances that may lead to them developing allergic contact dermatitis.
Many in vitro assays are under development aiming to distinguish sensitizers from non-sensitizers.[16] Currently very few assays address the problem of determining sensitizer potency, i.e. whether the sensitizer is a weak, intermediate or strong sensitizer. With this in mind we have developed an assay which may possibly be able to classify sensitizers according to their irritant potency with the aid of epidermal equivalents (EE). The assay's potential application is therefore in a tiered manner, where first tier 1 has previously identified the sensitizer (e.g.: dendritic cell based assay; see review Santos et al.[16]) and then tier 2, this assay then determines sensitizer potency. Thirteen well characterised skin sensitizers have been applied topically in a dose response manner to EE for 24 hours. The EE-EC_{50} value (effective chemical concentration required to reduce cell viability by 50%) and the EE-IL-$1\alpha_{10x}$ value (chemical concentration that increases IL-1α secretion by 10 fold) were calculated;[17] (Santos, manuscript submitted). From

13 skin sensitizers, EE-EC_{50} and/or EE-IL-1α_{1x} values were obtained from 12 chemicals. For the majority of the sensitizers, EE-EC_{50} and IL-1α_{10x} values decreased in proportion to increasing sensitizer potency. Having passed this first phase known as the 'Basic Research Phase 1', the assay entered Phase 2: Assay Optimization and Development. Here, the correlation of the in vitro EE potency assay with existing *in vivo* mouse and human sensitization data (LLNA, HRIPT) was determined and a standard operating procedure was developed. The assay showed good correlation with both *in vivo* mouse and human data. Also, importantly, it was determined whether the protocol which used until now our *in house* VUmc EE was transferable to commercially available EE. This is necessary for inter-laboratory studies in the future and wide spread implementation. Two sensitizers, DNCB and Resorcinol, were correctly assessed as extreme and moderate sensitizers using commercial EE (EST1000™ from Cell Systems and RHE™ from SkinEthic). These positive results enabled the assay to enter Phase 3: Pre-validation. Currently an interlaboratory study is underway with 5 European laboratories using the commercially available EST1000 EE. In this Phase 3, attention is paid to the degree of intra- and inter- laboratory variation. Variation should be as low as possible if the assay is indeed transferable and robust. Also it will be determined whether a small test panel of coded sensitizers can be correctly assessed in all laboratories. If this is the case, the assay may progress to full validation in the future. The robustness, sensitivity and specificity of the assay will then be tested in an inter-laboratory study with an extended panel of well characterised and coded test chemicals. After completion of the experiments, codes will be broken and results will be compared with data available from the Local Lymph Node Assay (LLNA) and with human data. If the assay passes this validation stage it may then be accepted as an animal replacement method for testing the sensitizing potency of a skin sensitizer. Currently it is thought that no single in vitro assay will fully accomplish the task of identifying sensitizers or predicting their sensitizing potency and therefore a battery of assays will be required which compliment each other and which mimic sensitization in vitro. Currently many assays are in Phase 1 (basic research) and Phase 2 (assay optimization and development). A few assays are entering Phase 3 (pre-validation) and currently no assays have yet entered Phase 4 (validation) with regards to in vitro sensitization assays as animal replacements. Therefore clearly, the process of complete animal replacement takes many years of research and collaboration between European scientists.

Development of tissue engineered wound healing and adverse scar models

The assays described in this section can be considered as specialized *in house* assays since they require advanced tissue culture techniques and / or a regular supply of diseased skin in order to construct the skin models. One of the main problems faced by scientists until know when trying to understanding the mechanisms involved in adverse scar formation (hypertrophic scar and keloid) and in developing novel treatment protocols, is that laboratory animals do not develop adverse scars and the fibrotic tissue which they do develop is very poorly representative of hypertrophic or keloid scars. Therefore scar management and development of new therapies are hindered by lack of physiologically relevant human test models. When developing an in vitro wound healing model a number of factors need to be taken into account. In addition to selecting the cells to be incorporated into the model (e.g.: keratinocytes, melanocytes, fibroblasts etc), it is important to consider whether cells derived from healthy tissue or diseased tissue are required. Hypertrophic scars develop in areas of extensive skin trauma to healthy skin and therefore healthy skin can be considered as an optimal tissue source. In contrast keloids only develop in predisposed individuals. These scars actively expand outside the margin of the (often superficial) wound site and readily recur after excision. Therefore diseased keloid skin can be considered as an optimal tissue source. Furthermore, the choice of dermal matrix is extremely important. Fibroblasts cultured under optimal conditions will synthesize there own dermis. Differences in the newly formed dermis can be expected when scar tissue is formed compared to healthy tissue. Therefore, the dermal matrix used to construct a fibrotic skin equivalent should only be a temporary sponge-like support enabling the cells within the construct to rapidly degrade and remodel it in order to form fibrotic tissue. In contrast, when a wound healing model is required to investigate the rate of wound closure (re-epithelialization), then a stable matrix with a low rate of biodegradability and which closely represents human dermis (with or without a basement membrane) should be considered. Examples of these models are described below:

Hypertrophic scar model

A major problem in burn wound care is the quality of the scar formation. Hypertrophic scars are raised, red and firm lesions which often form after healing of deep burn wounds. Since it is known that the chance of developing a hypertrophic scar is related to the degree of skin trauma and the depth of the wound (e.g: 3rd degree burn has a 90% chance in developing a hypertrophic scar), we hypothesized that i) the degree of scar formation is related to the depth of the wound and ii) skin repair arising from the subcutaneous layers (e.g.: adipose tissue) may be responsible for hypertrophic scar formation. Keeping this in mind we constructed full thickness skin equivalents consisting of a fully differentiated and pigmented epidermis on a dermal matrix containing either mesenchymal cells (also know as fibroblasts) derived from adipose tissue or the dermis (manuscript in preparation).[18] From our hypothesis, constructs containing adipose derived mesenchymal cells should develop more scar characteristics than constructs containing mesenchymal cells derived

from the dermis. This was indeed the case. The constructs were compared with native healthy skin, normotrophic scars and hypertrophic scars. Parameters studied were thickness of the regenerating dermis and epidermis, contraction, histological- and immunohistochemical stainings (e.g. vimentin, α-SMA), and degree of epithelialization. The constructs containing adipose derived mesenchymal cells developed a notably thicker dermis, more contraction, had α-SMA expression, more epidermal layers and delayed epithelialization compared to constructs containing dermis derived mesenchymal cells. Taken together the skin eqivalent containing adipose derived mesenchymal cells (instead of dermis derived mesenchymal cells) represented a hypertrophic scar constructed in vitro from healthy adult skin and adipose tissue. The model is currently being validated with known therapeutics before progressing to testing potential novel therapeutics.

Keloid scar model

Keloid scars result from an abnormal wound healing in pre-disposed individuals. A minor skin injury or irritation can trigger keloid formation. Keloids are distinguished from hypertrophic scars by the fact that they expand outside of the margin of the original skin lesion and recur at the same site after excision. Therefore, in order to construct a keloid scar in vitro it was decided to use the diseased skin obtained from routinely excised keloids rather than healhy skin as the source of tissue. Since keloids are continuously expanding scars we hypothesized that cells isolated from the peripheral regions would exhibit different scar forming characteristics to cells isolated from the central region. This was indeed the case. Differences were observed in the degree of contraction, keratinocyte activation, and secretion of inflammatory mediators (cytokines and chemokines) between skin equivalents constructed from peripheral and central derived tissue. Comparison of these keloid models with normal skin and normotrophic scars will enable us to understand the mechanisms of keloid formation. Once we understand this we will be able to develop new strategies aimed at preventing keloid formation and recurrence in addition to testing novel therapeutics.

Wound healing model

Tissue remodeling resulting in scar formation occurs after wound closure (re-epithelialization). However, repeated clinical observations note that in particular for large trauma wounds, the time taken for wound closure is inversely related to the quality of the final scar: the faster the wound closure, the better the quality of the final scar. Therefore it is important to understand the mechanisms involved in normal re-epithelialization and to develop strategies to enhance this. With this in mind we have developed an in-vitromodel to investigate re-epithelialization after cryo-wounding (freeze) where scar free healing occurs and after 3rd degree burn wounding where adverse scar formation occurs (figure 4).[15] Comparison of the mechanisms involved in wound closure between the two models showed that indeed freeze wounds closed rapidly and burn wounds closed significantly more slowly. This was found to be related to the degree of trauma to the basement membrane. Whereas after freezing the basement membrane remained intact, after burning the basement membrane was destroyed (most probably due to heat denatuaration of basement membrane proteins). These models are currently being used to test novel therapeutics such as salivary derived histatin peptides.[19]

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SAMENVATTING

In dit overzichtsartikel beschrijven we een aantal huidsubstituten, die ontwikkeld zijn voor de behandeling van moeilijk te genezen wonden en als alternatief voor proefdiergebruik (in-vitro-assays). Het multidisciplinaire karakter van huid *tissue-engineering* maakt het mogelijk om geschikte huidsubstituten te ontwikkelen voor specifieke toepassingen. In dit overzicht beschrijven wij de keuzes die gemaakt werden over celtype, dermale matrix, logistiek van productie en transport en ook de ontwikkeling van 'patiënt- en artsvriendelijke' richtlijnen voor de toepassing van deze nieuwe behandelingen voor wondgenezing. Er zijn twee huidsubstituten voor klinische toepassing ontwikkeld: één voor de genezing van therapieresistente chronische wonden en één voor verbetering van littekenvorming bij diepe derdegraads verbrandingen. We beschrijven ook een aantal huidsubstituten (in-vitro-assays) die ontwikkeld zijn in overeenstemming met het 3V-principe (vermindering, verfijning en vervanging van proefdiergebruik). Eén assay is geschikt voor het bepalen van de potentie van een sensibiliserende stof en zou proefdiergebruik volledig kunnen vervangen. Drie andere assays zijn gericht op vermindering en verfijning van het gebruik van proefdieren. Deze assays weerspiegelen fysiologisch relevante wondgenezing en abnormale littekenvorming bij de mens (hypertrofische littekens en keloïd). Desbetreffende assays zijn bedoeld om nieuwe behandelingen in de toekomst te testen. Deze assays zijn verreweg superieur aan de huidige testmodellen bij dieren, omdat abnormale littekenvorming – zoals bij de mens – bij dieren niet voorkomt.

TREFWOORDEN

wondgenezing – huidmodel – tissue-engineering – invitromodel – chronische wond – brandwond – litteken

SUMMARY

In this review we describe a number of skin substitutes which are under development for advanced wound healing therapies and for use as animal alternative (in vitro assays). Skin tissue engineering is a multi-disciplinary subject which requires each construct to be specifically designed for its particular future application. We describe our choices made with regards to cell types, dermal matrix, the logistics of production and transport as well as the development of "patient and clinician friendly" protocols for application of novel wound healing therapies. Two skin substitutes have been developed for clinical applications: i) healing of therapy-resisistant chronic wounds and ii) improved scar formation in deep 3rd degree burns. We also describe a number of skin substitutes being developed in compliance with the 3Rs (reduction, refinement and replacement of test animals). One assay is aimed at wide spread implementation as a complete animal replacement and is an assay which may determine the potency of a sensitizing substance. Three other assays are specialized assays aimed at reduction and refinement of the use of test animals. These three assays represent human physiologically relevant wound healing and adverse scar models (hypertrophic and keloid scar). Such assays are aimed at testing novel therapeutics in the future and are considered to be far superior to current animal models since animals do not form adverse scars which are comparable to humans.

KEYWORDS

wound healing – skin substitute – tissue engineering – in vitro assays – chronic wounds – burn – scar