New Strategies in Clinical Care of Skin Wound Healing

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Introduction

The skin is the largest organ of the human body. For a long time, however, it was merely seen as a simple coat just covering the body. Nowadays we know more about the multitude of physiological organic functions it has to fulfill. These tasks include mechanistic, metabolic, regenerative, energetic and immunological aspects.

Skin tissue was the first tissue to have been successfully tissue-engineered in vitro and to have also been successfully translated back into clinical application. Not only due to its easy accessibility but also because of the fact that the skin is one of the most active and continuously regenerating organs, it is a prime target for regenerative therapies and, in addition, a fascinating model from which to learn more about the human body's intrinsic regenerative mechanisms.

Modern wound care starts to integrate and support the body's own regenerative capacities more systematically. Methods of 'regenerative medicine' are in the scientific focus, including the use of stem cells, growth factors and new bioactive materials. These tools are experimentally well described but clinically poorly performed. The main reasons for this are both legislative and economic. This review describes state-of-the-art techniques, up-to-date research projects, innovative preclinical and clinical approaches in wound care, and activities to translate these innovative techniques into clinical routine.

Key Words
Erythropoietin • Growth factors • Tissue engineering • Gene therapy • Stem cells

Abstract

The prevalence of chronic wounds is closely correlated to the aging population and so-called civilizational diseases. Therefore, they are causing morbidity and mortality of millions of patients worldwide, with an unbroken upward trend. As a consequence, chronic wounds induce enormous and rapidly growing costs for our health care systems and society in general. Thus, medically effective and cost-efficient treatment methods are urgently needed. Methods of 'regenerative medicine' might offer innovative scientific solutions, including the use of stem cells, growth factors and new bioactive materials. These tools are experimentally well described but clinically poorly performed. The main reasons for this are both legislative and economic. This review describes state-of-the-art techniques, up-to-date research projects, innovative preclinical and clinical approaches in wound care, and activities to translate these innovative techniques into clinical routine.
medicinal products’ and for ‘tissue/cell transplantation’, production processes often need good manufacturing/ laboratory practice facilities and major financial support, especially in the conduct of clinical trials. For the near future, the processes which will be able to pass through this ‘bottleneck’ of legislative and economic burden will be mainly protein- or matrix-based therapies and intra-operative cell processing, including autologous (stem) cell transplantation.

Skin Wound Therapies

As one cannot talk about the skin wound, there does not exist the wound therapy. Fortunately, the majority of all skin wounds heal spontaneously without or with only minimal medical aid. This is due to the fact that the human body, like all other organisms, adapted its regenerative capacity during evolution. The lack of wound healing capacity is not compatible with life. Therefore, we find more or less complex regenerative capacities in all organisms. As a result, special care is only needed if the wound gets more complex, or if it affects deeper structures or the organism itself is in a suppressed condition.

Loss of Epidermis (Superficial)

An isolated loss of the epidermis, leaving the dermis intact, heals completely and without scar formation within 4–8 days. A typical example would be sunburn. In a situation like this basically no specialized therapy is needed. Nevertheless, a variety of therapeutic options exist. Most available products just have a cooling affect, some also have a local analgesic affect and others just help to keep the wound moist.

Loss of Superficial Dermis (Superficial Dermal)

Superficial dermal thermal injuries display blister formation. If the blister ground is exposed to air it is extremely painful. Today’s state-of-the-art treatment option is the occlusive method. It includes the removal of the blisters and occlusive local therapy.

There exist several treatment options and products (silver nitrate, mafenide, vinegar, iodine, silver sulfadiazine, etc.) which enable microbiological control, but some of these lack enough moisturizing capacity for the wound surface. A widely used product is Flammacine® (silver sulfadiazine), which is simple to handle and has a favorable cost-effectiveness ratio. The disadvantage of this is the (painful) daily dressing change and the drying out of the wound area.

In the occlusive method, closure of the wound surface is realized with synthetic membranes under strictly sterile conditions (e.g. Biobrane® or Suprathel). The advantage of these products is that they stay in place until complete wound healing, thus painful dressing changes are no longer needed; nevertheless, frequent wound controls are necessary.

Loss of Deep Dermis (Deep Dermal)

After a deep dermal injury (a deep second-degree burn), the necrotic superficial layers of the skin have to be removed either biologically or surgically until vital layers are exposed.

After bleeding control, keratinocytes (as solution or as sheets) can be transplanted if enough dermal tissue is preserved and the regenerative capacity of the patient is postulated to be sufficient. Split-skin grafts are used if deeper layers of the dermis are involved. If, after extensive thermal trauma, the remaining nondamaged body surface does not allow for sufficient amounts of split-skin grafts to be taken, temporary skin substitutes such as heterologous or xeno split-skin grafts, or amnion, can be used for a short period of time to prevent both infection and hypertrophic granulation and later scar tissue formation.

Loss of Full Skin (Deep)

If an acute full-thickness skin defect has occurred (e.g. a third-degree burn), the wound has to be cleaned carefully and all remnants of necrotic skin or foreign bodies have to be removed. In a condition like this the underlying tissue is subjected to infection and trauma since the protecting barriers, dermis and epidermis are lost. Therefore, wound closure is the most important aim. This can be achieved with split-skin graft transplantation or, after pretreatment with a dermis substitute (e.g. Integra®) and neodermis formation, keratinocytes may be transplanted. If a chronic full-thickness skin defect exists we need a different therapeutic strategy. In chronic wounds an ‘antihealing environment’ exists and a still not fully understood combination of inhibitory factors prevents healing. In addition, chronic wounds are usually colonized with a multitude of microorganisms and are sometimes even infected. These microorganisms have to be, at least grossly, removed before a wound closure attempt can be made. After cleaning the wound and removing necrotic tissue remnants the wound environment has to be changed from antiproliferate to proproliferate.

Therefore, antiproliferative factors such as metalloproteinases and TNF-α have to be antagonized and the
concentrations and effectiveness of proproliferative factors such as erythropoietin (EPO) or transforming growth factor TGF-β3 have to be increased. Granulation tissue formation can then take over or a neo dermis can be grown using a dermis substitute. Later split-skin grafts can be transplanted on the prepared new wound bed if necessary. If a proproliferative environment cannot be created, for example due to advanced loss of vital and vascularized tissue, plastic surgical techniques have to be employed by using local or free tissue transfers to substitute the previous tissue loss in an adequate manner.

Figure 1 shows the four stages of thermal injury to the skin.

**Innovative Approaches and Clinical Trials**

This section will focus on innovative treatment approaches which are at the stage of clinical phase I–III trials or even only in the preclinical phase, but which seem to have a special promising potential.

**Scar-free Healing**

One very interesting fact is the scar-free healing of mammalian embryos. So far, several investigations have been carried out to investigate adult and embryonic wound healing and scarring reaction in adults. Nowa-

days, many factors involved in adult and embryonic skin regeneration are being described. In the embryo, the immune system and the inflammatory cascade are not sufficiently developed. Therefore, the resulting inflammatory reaction in the embryo is much smaller and of a shorter period of time than in more advanced developmental stages and adults. Transforming growth factors TGF-β1–3 and platelet-derived growth factor (PDGF) seem to play prominent roles. Embryonic scar-free healing can be achieved if PDGF and TGF-β1 and 2 are neutralized, and TGF-β3 is added to adult wounds [1]. This has already been successfully demonstrated in rodents, pigs and healthy human volunteers [2].

Thus, new drugs for the prevention of scarring are being developed and phases I/II clinical trials have been carried out [3]. Locally administered TGF-β3 is well tolerated and improves skin regeneration and thus reduces scarring after trauma [4]. Unfortunately, a multinational, multicenter, double-blind clinical phase III trial testing two different dosing regimens against a placebo was interrupted after 350 patients had been enrolled, and neither the primary nor the secondary study end-points could be met [5].

**Regenerative Approaches**

Very few clinical trials with satisfying high evidence levels are to be found in this area of research. This is actually surprising in view of the fact that chronic wounds are the cause of suffering for millions of patients worldwide and cause billions of dollars of costs to the health care systems [6]. One reason might be the difficulty in obtaining standardized and comparable wound conditions in patients, which are needed for proper scientific work.

The only routinely standardized wound in clinical practice is the surgically induced split-skin graft donor site. Therefore, this wound type has already been used as a study target in a multitude of studies to compare different strategies of locally applied therapeutics. None of these, however, has focused on the biological regenerative effects on a cellular level.

If it were possible to activate and deactivate all the tools necessary for wound healing and regeneration, exactly as needed in the particular situation, we would have a universal tool for the acceleration of normal regeneration and wound healing in our hands. However, it has to be taken into consideration that many, especially chronic, wounds are biologically seen far from a normal wound-healing situation. In these instances, therefore, pathological healing processes have to be reduced in favor of biological normalization of the wound milieu.

Fig. 1. Four stages of thermal injury to the skin. First-degree burn (spontaneous healing in 5–10 days, no scar formation); Second-degree burn, superficial (spontaneous healing in 10–21 days, no scar formation); Second-degree burn, deep (spontaneous healing in >21 days, scar formation); Third-degree burn (no spontaneous healing, scar formation).
**Proregenerative Agents**

There are several publications investigating the effects of proregenerative agents on skin regeneration, but few report about their use in humans. One proregenerative agent which gained increasing attention within the last number of years is EPO. Several proregenerative effects, like anti-inflammatory and anti-apoptotic effects, stem cell activation and angiogenesis, could be demonstrated for systemic EPO application in acute and chronic, ischemic and diabetic environments [7–9], as well as for local application in diabetic environments [10]. In a full-thickness-defect mouse model treated with EPO, the healing process clearly improved in a dose-dependent manner [11].

In a standardized murine scalding injury model, the authors could demonstrate statistically significant faster wound healing and reepithelialization after topical EPO application. In addition, the extracellular matrix proliferation was much faster and an increased angiogenesis could be shown with increased CD31, VEGF and eNOS levels. [12].

In the same murine scalding injury model, the combined existence of the EPO receptor and the EPO-β1 heteroreceptor in the injured and the noninjured mouse skin could be demonstrated. In the noninjured skin, the receptors were downregulated after EPO treatment, but in the injured skin the receptor expression was stable under EPO treatment. In addition, a faster skin regeneration which was of higher quality could be shown [13].

Even sclerodermic ulcers improved statistically significantly in patients under EPO therapy [14]. Keast and Fraser [15] reported about 4 paraplegic patients whose decubital ulcers improved significantly under systemic EPO treatment.

At present, the first large, prospective, randomized, double-blind, multicenter trial, founded by the German Federal Ministry of Education and Research, is being carried out to investigate the wound-healing effects of EPO in severely burned patients (EudraCT No. 2006-002886-38, protocol No. 0506 and ISRCT No. ISRCTN95777824).

Table 1 shows EPO effects on different growth factors and their most important functions.

**Table 1. EPO effects on different growth factors and their most important functions**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect</th>
<th>Effected cells</th>
<th>EPO action</th>
</tr>
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<tbody>
<tr>
<td>TNF-α</td>
<td>primary injury response</td>
<td>ubiquitary</td>
<td>antagonism</td>
</tr>
<tr>
<td>IL-2, -6, -8</td>
<td>proinflammatory cytokines</td>
<td>ubiquitary</td>
<td>inhibition</td>
</tr>
<tr>
<td>γ-Interferon</td>
<td>proinflammatory cytokine</td>
<td>ubiquitary</td>
<td>inhibition</td>
</tr>
<tr>
<td>Macrophage inflammatory proteins 1 and 2</td>
<td>proinflammatory protein</td>
<td>ubiquitary</td>
<td>inhibition</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>invasion marker for macrophages</td>
<td>ubiquitary</td>
<td>inhibition</td>
</tr>
<tr>
<td>IL-1β</td>
<td>proinflammatory cytokine</td>
<td>ubiquitary</td>
<td>inhibition</td>
</tr>
<tr>
<td>Interferon</td>
<td>proinflammatory cytokine</td>
<td>ubiquitary</td>
<td>inhibition</td>
</tr>
<tr>
<td>VEGF</td>
<td>angiogenesis</td>
<td>vessels</td>
<td>stimulation</td>
</tr>
<tr>
<td>eNOS</td>
<td>recruitment of EPCs</td>
<td>endothelium</td>
<td>stimulation</td>
</tr>
<tr>
<td>Brain-derived neurototic factor</td>
<td>neuronal plasticity</td>
<td>neurones in the CNS</td>
<td>stimulation</td>
</tr>
<tr>
<td>Thrombopoietin</td>
<td>differentiation</td>
<td>megakaryocytes into thrombocytes</td>
<td>synergism</td>
</tr>
<tr>
<td>EGF</td>
<td>angiogenesis</td>
<td>endothelium</td>
<td>stimulation</td>
</tr>
<tr>
<td>Endothelial PAS domain-containing protein 1</td>
<td>adaption to hypoxia, stimulation of EPO and VEGF</td>
<td>ubiquitary</td>
<td>stimulation</td>
</tr>
<tr>
<td>Inducible nitric oxide synthase</td>
<td>dilatation</td>
<td>vessels</td>
<td>stimulation</td>
</tr>
<tr>
<td>BCL-XI</td>
<td>antiapoptotic</td>
<td>CNS</td>
<td>stimulation</td>
</tr>
<tr>
<td>Insulin-like growth factor 1</td>
<td>antiapoptotic cell proliferation</td>
<td>ubiquitary</td>
<td>stimulation</td>
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<tr>
<td>Caspase-3</td>
<td>apoptosis</td>
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<td>inhibition</td>
</tr>
<tr>
<td>B cell lymphoma 2</td>
<td>antiapoptotic proliferation</td>
<td>ubiquitary</td>
<td>stimulation</td>
</tr>
<tr>
<td>Heat shock protein 70</td>
<td>intracellular processes</td>
<td>ubiquitary</td>
<td></td>
</tr>
</tbody>
</table>

Another promising approach is the treatment with platelet-rich plasma (PRP) [16–19]. PRP is a biomimetic, highly potential mixture of platelets and multiple growth factors with chemotactic and promitotic qualities [20–22]. PRP suppresses proinflammatory cytokines and their actions; it interacts with macrophages, acts proan-
giogenically and triggers an improved reepithelialization of chronic wounds [23, 24]. So far, PRP is not part of clinical routine treatment. One reason for this is probably that a certain amount of technical prerequisites are necessary to prepare and use PRP [25]. In addition, the evidence contains lots of contradictory study results and, therefore, it needs further investigation.

**Growth Factors**

The use of single or combination growth factors has been investigated concerning their potential for the treatment of chronic wounds. Promising reports in humans were found with epithelial growth factor for the treatment of ulcers cruris [26], and with keratinocyte growth factor [27], fibroblast growth factor [28] and PDGF for the treatment of decubital ulcers [29].

So far, only PDGF has been examined in clinical trials, thus it was used in the treatment of diabetic, neuropathic ulcers. In these trials a significant improvement of wound healing could be demonstrated [30–32]. So far, treatments with growth factors have not reached the clinical routine. The reasons for this are probably of diverse origin, including cost considerations and insufficient scientific evidence; further investigation is, therefore, necessary.

**Gene Therapy**

Gene therapy is a possible alternative to the direct application of growth factors. This is because of a continuous or a temporary production and, thus, the effects of the necessary factors can be achieved. In former times, when the biological impact of keratinocytes for creating a stable wound closure was still overestimated, it was demonstrated that transfected keratinocytes are able to survive in a wound and synthesize the respected proteins [33]. Transfected keratinocytes transplanted onto athymic nude mice evoke the desired positive proregenerative effects, but no tendency for malignant degeneration was observed [34]. Nowadays, we know that the prime target for stable wound healing is a sufficiently perfused and stable integrated dermis. Therefore, more scientific attention has recently been directed towards the biological improvement of dermis regeneration and dermal scaffolds (see Tissue Engineering).

There are very few clinical trials being published in the field of gene therapy. In a recently published article the amputation rate of diabetic feet was statistically significantly reduced by the injection of modified endothelial cells into the effected extremity [35].

One of the reasons for the poor evidence situation might be the fact that many gene vectors, especially the viral ones, cause an inflammatory reaction which makes their use in humans highly questionable.

**Stem Cells**

The Regulation on Advanced Therapies [regulation (EC) 1394/2007] defines stem cells as advanced therapy medicinal products. Therefore, newly developed production processes and quality procedures have to comply with pharmaceutical standards and good manufacturing practice regulations defined by the European Union, US-FDA and ICH. This represents new challenges, and both scientists and the cell-based therapy industry will have to deal with these obstacles in the near future.

Today, dermal stem cells have been identified in the skin, and in skin appendages like hair follicles and sweat glands, which showed the same phenotype as adult mesenchymal stem cells [36–38]. Mesenchymal stem cells, when grown under hypoxic conditions and with the addition of IL-6 to the culture medium, showed decreased proliferation rates, but when EPO was also added this changed to increased proliferation rates [39].

The first clinical trials were carried out using autologous mesenchymal stem cells (bone marrow). Chronic ischemic wounds treated with bone marrow-derived stem cells revealed complete wound closure in more than 50% of the patients. Pain reduction could also be achieved as well as a prolongation of walking distance from 0 to 40 m [40].

CD34+ cells could be successfully applied on diabetic ulcers. After the treatment, the patients revealed improved blood circulation and higher values in transcutaneous oxygen partial pressure [41].

So far, stem cells are not routinely used in the clinical setting, which is at least partially explainable by the above-mentioned regulatory restrictions.

**Tissue Engineering**

As mentioned before, skin was the first tissue to be successfully tissue-engineered and implemented back into the clinical routine. The first commercially available tissue-engineered products to be found on the market were keratinocyte sheets.

As already stated, keratinocyte sheets are less commonly used nowadays and serve only as an additive in the therapy of severe full-thickness skin defects. During the first enthusiastic phase, we learned that for the application of keratinocytes an existing dermis (rest) is an obligatory prerequisite, otherwise they cannot expel their bio-
logical effects, which causes impaired healing and scar- ring. Actually, the most commonly used variety is keratinocyte fibrin spray, which is used especially in der- mis-preserved pediatric superficial thermal injuries. Heterologous keratinocytes have additional disadvantag- es in that they are not only expensive, but they might also transmit infective diseases.

Ideally, a tissue-engineered skin substitute is a multi- layered, structurally fully functional skin substitute, which might fulfill all the different functions of the der- mis and epidermis.

An interesting multilayer tissue-engineering approach to cover large full-thickness defects has already been de- scribed a few years ago. Autologous keratinocyte and fi- broblast primer cell cultures were established, the cells were grown on special hyaluronic acid matrices, and the construct was transplanted in a single-step procedure. A completely autologous and biological fully active epider- mal-dermal substitute was realized, and in addition a thin split-skin graft could be transplanted if necessary [42].

Aplikely® is a tissue-engineered multilayered skin equivalent which is commercially available and approved by the legal authorities (FDA and EMEA). It is a bilayered skin substitute consisting of allogenic neonatal fibro- blasts and keratinocytes grown in and on a bovine col-
lagen matrix. Phase III clinical trials testing the product in different chronic wounds (venous ulcers and diabetic foot ulcers) showed very promising results with an 80% take rate and statistically significant higher healing rates of the chronic wounds. Nevertheless, disadvantages are the allogenic origin of the cells and, therefore, the unclear infectiological status, the bovine origin of the collagen, the lack of dermal structures like sweat glands and hair follicles and the unclear fate of the graft after transplantation in general [43].

See table 2 for an overview of the literature, including preclinical and clinical trials.

**Conclusions and Future Perspectives on Skin Regenerative Therapies**

Regenerative therapies after skin injuries, especially with local topical approaches, have been studied for a long time, but in the majority of cases without focusing on the underlying biological processes taking place. Only recently, with a better molecular biological understanding of stem cell and protein-based principles, are we able to customize regenerative therapeutic strategies which respect such fundamental biological principles. Perhaps the therapeutic use of prorregenerative agents like EPO, which selectively triggers cell-protective and prorregenerative effects, or stem cells or combinations of these methods, may play a key role in future developments of new therapeutics to enable and improve regeneration after skin injuries. Full skin loss will still remain a therapeutic challenge for clinicians, since total skin loss necessitates skin transplantation or bioartificial generation of skin substitutes in such situations. Three key problems need to be solved in the future to optimize skin tissue engineering and tissue regeneration: (1) creating a stable epidermo-dermal junction between the two major compartments (dermis and epidermis); (2) implementing a vascular supply in the dermal layer and (3) supporting the construct with its functional cells and appendices (e.g. melanocytes, sweat and sebaceous glands, hair bulges, etc.).

Today, evidence-based treatments for these patients are hardly possible because of insufficient scientific evidence due to the lack of a sufficient number of high-quality clinical trials. To offer evidence-based, cost-effective and up-to-date therapies we need more high-quality clinical trials following the rules of good medical practice and high ethical, moral and scientific standards to be able to identify the most promising new therapeutic methods.

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