Review Article

Conservative procedures in skin reconstitution

Uwe Wollina[1*]

[1]Krankenhaus Dresden-Friedrichstadt, Städtisches Klinikum, Akademisches Lehrkrankenhaus der TU Dresden

GMS Curr Top Otorhinolaryngol Head Neck Surg 2005;4:Doc17

Abstract

Skin exerts a number of essential protective functions ensuring homeostasis of the whole body. In the present review barrier function of skin and its expression of antimicrobial peptides are discussed. Barrier function is provided by the dynamic stratum corneum structure composed of lipids and corneocytes. Stratum corneum is a *conditio sine qua non* for terrestrial life. Impairment of barrier function can be due to injury and inflammatory skin diseases. Therapeutic options are discussed with special emphasis of radiodermatitis and irritant contact dermatitis in patients with hearing device. The use of antimicrobial peptides is illustrated by facial inflammatory skin diseases. In wound healing new developments include biotechnological developments of matrix- and growth factors and tissue-engineered skin substitutes. In everyday wound care of chronic wounds the concept of wound bed preparation (TIME) constitutes the base of successful treatment.

Keywords: stratum corneum barrier, lamellar bodies, antimicrobial peptides, irritant contact dermatitis, radiodermatitis, acne, atopic dermatitis, wound healing, moisturizer, dexpanthenol, calcineurin inhibitors, hyaluronic acid, growth factors, tissue engineering, wound bed preparation

1. Introduction

A critical structure in interaction of the human body with the environment represents the skin surface with the stratum corneum. Without the horny layer a terrestrial life would be impossible. On the other hand the stratum corneum becomes impaired in any kind of superficial or deep injuries. To retain body homeostasis recovery of stratum corneum is necessary.

Other important protective functions of skin are protection against infection and irradiation, in particular ultraviolet (UV) irradiation. In the following chapters major protective functions and correlated conservative therapeutic measures are discussed.

1.1 Embryology

The epidermis derives from the ectoderm. Initially the epidermis comes as a monolayer. In the first trimenon the epidermis is covered by single layered periderm. Epidermal stratification starts after 8

[*]**Corresponding Author:**
Prof. Dr. med. Uwe Wollina, Chefarzt der Hautklinik, Krankenhaus Dresden-Friedrichstadt, Städtisches Klinikum, Akademisches Lehrkrankenhaus der TU Dresden, Friedrichstraße 41, 01067 Dresden, eMail: wollina-uw@khdf.de

weeks. During the second trimester cornification is realized. Then the periderm disappears and becomes a constituent of vernix caseosa. The epidermal thickness of an -immature newborn is about 29 µm, in mature newborns and adults the thickness is about 50 µm. The skin surface of the newborn is covered by protective gelatinous vernix caseosa whereas the skin surface of adults is rather dry [1].

1.2 Stratum corneum barrier and skin surface

The stratum corneum is the essential structure of skin barrier. The major constituents of stratum corneum are lipids and proteins. In a typical case about 20 layers of nucleus-free corneocytes densely packed with keratin filaments are surrounded by a matrix. The matrix is composed of filaggrin and -derivates, and lipid-rich lamellar bodies. Lamellar bodies fuse end-to-end thereby forming lipid double layers [2].

The lipid constitutes of cholesterin, ceramides, and free fatty acids. Ceramides stand for about 50% of horny layer lipids and are essential for the lamellar structure of the epidermis. Cholesterol regulates the phase behaviour of the stratum corneum. The free fatty acids are mostly long chained molecules with more than 20 C atoms. Lipids are responsible for the hydrophobicity of the horny layer [3]. The water exchange is realized by migrating pores, i.e. polar transport pathways within the lipid mosaic [4]. The matrix develops under influences of pH gradients, sodium ions and enzymes (synthetases, reductases, hydrolases, lipases) [3]. In a simplistic was stratum corneum can be described by the bricks-in-mortar model [2]. More recent studies discovered subunits of octaeders of corneocytes within the horny layer which may migrate [4].

The horny layer is covered on its surface by a thin amorphous film contributing to stratum corneum structure and function. In newborns there is an almost neutral skin surface pH of 6.6 that changes within days or weeks into an acidic pH of 5.9 (acid skin surface film). This leads to activation of pH-dependent hydrolytic enzymes like β-glucocerebrosidase and stratum corneum secretory phospholipase A2 [5], [6]. Skin surface pH is modulated by microbial harvest, eccrine and sebaceous gland secretions, and endogenous catabolic pathways. The acidification of the horny layer is necessary for barrier function [7]. Exposure of horny layer to neutral buffers (i.e. wet work) or blocking of acidification increases the pH. Hereby, serine proteases become activated that digest desmoglein 1. Desmoglein 1 is a major constituent of corneosomes. Metabolization of desmoglein 1 decreases cohesivity of corneocytes and enforces horny layer permeability [8].

The horny layer impairment as subclinical dryness of skin is quite common. It may have substantial impact on the whole body. Prolonged exposure of human skin to wetness (water) and/ or occlusion leads to measurable disturbances of barrier function. The transepidermal water loss (TEWL) increases in relation to duration of exposure and temperature [9], [10].

2. Therapeutic aspects

2.1 Reconstitution of barrier function

2.1.1 Dry skin - xerosis cutis

Xerosis cutis is a consequence in epidermal water content reduction (<10% of stratum corneum). An increased transepidermal water loss (TEWL) leads to itch, scaling, roughness and fissuring [11]. Xerosis might be a symptom of irritant contact dermatitis, atopic dermatitis or ichthyosis.

The basic principle of treatment of dry skin is the use of emollients and moisturizers. Emollients reduce the TEWL and smooth the skin surface. They practically fill the space in between corneocytes of dry skin. Moisturizer cause a prolonged increase in epidermal water content. Simple moisturizers combine occlusive and water binding components. Occlusive substances are for instance vaseline, mineral oils, paraffin, silicon, oils and fatty acids from both animals or vegetables. Ceramides are of particular interest in cosmeceutical since they represent a major physiological constituent of stratum corneum

barrier [12]. Hydrogenated polydecenes are chemically better defined substances as mineral mixtures like vaseline without their stickiness [13]. The more and more popular vegetable oils and fatty acids are not without their own problems. Some like coconut butter exert a comedogenic potential, others like peanut butter can cause allergic contact dermatitis. Among water binding substances, known as humectants, are urea, glycerine and α-hydroxy acids. Urea that is also a normal components of the natural moisturizing factor (NMF) of human skin may be released from oil in water and water in oil emulsions and than penetrate into the horny layer. On average, about 150 nmol cm^{-2} urea are found within human stratum corneum. A six-fold increase of urea concentration by topical application doubles the water content of the horny layer [14].

Vehicles are used as drug carriers as well as skincare products without a specific drug constituent. For a long time lipid combinations are designed to specifically modulate the horny layer and support barrier recovery. In recent times new pharmaceutical technologies were introduced like liposomes, nano particles, cyclodextrines, and microemulsions [15].

Vitamins used in cosmetics are popular among consumers. However, their percutaneous penetration is limited. Because of solubility problems, UV- and oxygen-instability vitamins are a challenge for topical use. Vitamin C - a popular antioxidans - is not taken up percutaneously. Tocopherol shows antioxydative and photoprotective effects. Percutaneous resorption of tocopheral has been demonstrated. In most cases of vitamins, however, scientific investigations of topical application are missing [16].

A rather extensive data file, scientific and clinical, is available in case of dexpanthenol that is transformed in skin into panthothenic acid also known as vitamin B5. Panthothenic acid is a component of coenzyme A involved in the synthesis of fatty acids, sterols, proteins etc. Dexpanthenol is a humectant as well [17]. In models of skin irritation effects of dexpanthenol has been studied in detail. In donor sites of split thickness skin grafts dexpanthenol improved non-inflammatory microcirculation [18]. Dexpanthenol restored skin hydration more rapidly than the vehicle alone. Itch relief was fates and pain was reduced [19]. In randomized, placebo-controlled studies among healthy subjects dexpanthenol significantly increased the horny layer water binding and reduced transepidermal water loss [20]. On experimental damaged skin dexpanthenol ointment improved the stratum corneum water content more rapid and significant compared to its vehicle. TEWL and inflammation were reduced in relation to the pharmaceutical formulation [21]. The anti-inflammatory potency of dexpanthenol is comparable with hydrocortisone [22].

2.1.2 Inflammation - radiodermatitis

Radiodermatitis is defined as the whole range of cutaneous reactions due ionizing irradiation. Exposure of skin to ionizing irradiation activates transcription factor NF-κB by formation of intracellular oxygen radicals. Major mediators of irradiation stress in skin are ceramides and sphingomyelins. Before a radiodermatitis becomes obvious vascular irritation leads to epidermal barrier function impairment. Transepidermal water loss is increased and reaches a peak earlier than radiodermatitis. Patients with an early barrier dysfunction run a prolonged course of radiodermatitis [23]. Fractioning improves tolerability of irradiation. Xerosis cutis is a hallmark of radiodermatitis and needs treatment [24]. Soaps should be avoided for skin cleansing since they may change skin surface pH into the basic range. The rather popular powdering of skin is not able to improve barrier function. Non-erosive radiodermatitis can be treated with water-in-oil emulsions [25]. In open trials with breast cancer patients and radiotherapy after tumour excision topical treatment with moisturizers improves quality of life [26]. Moisturizers are not capable to prevent radiodermatitis [27], [28].

Comparing skin cleansing and skin care with the traditional method to keep skin dry during radiotherapy of breast cancer cleansing with water was superior. In particular wet desquamation occurred less often when washing was allowed [29]. In a study on skin care during radiation therapy a mild *Aloe vera*-containing soap alone was compared with the soap combined with moisturizer. At cumulative dosages >27 Gy the combination was more effective than the soap alone [30].

A protective effective has been described for panthothenol in models of oxygen radical-induced cell and tissue damage such as lipid oxidation of cell membranes treated by gamma irradiation [31]. Using a moisturizing concept with dexpanthenol topically in more than 1,000 radiotherapy patients both tolerance to treatment and acceptance of treatment by patients was improved compared to dry powdering. Allergic reactions, super infections, epidermolysis, and xerosis cutis were either less frequent or even completely prevented [32]. In contrast, a randomized study including 86 patients with larynx or breast cancer and radiotherapy dexpanthenol ointment alone had no significant effect on EORTC/RTOG-score of radiodermatitis, pain and itch [33]. In this study quality of life, super infection and tolerability of irradiation have not been investigated.

In acute radiodermatitis topical momethasone furoate was compared with vehicle in a randomized study including 49 breast cancer patients. The topical steroid was significantly more effective than the vehicle in the reduction of inflammation [34]. There is no such data available for the use of other topical steroids. In erosive radiodermatitis wet compressions with black tee or eosin 1-2% and a short course of hydrocortisone foam have been recommended [35].

2.1.3 Protection against skin infection and antimicrobial peptides

There is a close relationship between horny layer barrier function and the risk of skin infection [36]. A Cochrane group analysis has shown that in newborns emollients alone are capable to stabilize the stratum corneum barrier thereby preventing skin infections [37].

Topical antiseptics are at use to prepare skin and mucous membranes for surgery. They may also be used as an adjuvant in management of chronic wounds that are at risk of infection or are infected without replacement of systemic antibiotics when necessary. Chlorhexidine has a longer half life than alcohol what makes it more suitable. Iodine compounds like povidone iodine are also used quite often. Iodine is contraindicated during pregnancy, in newborns and patients with thyroid disease since there is a significant percutaneous absorption of iodine.

Among topical antibiotics for the head and neck region mupirocine is of particular importance. There is no cross-reaction with other antibiotics. The drug exerts an excellent activity against *Staphylococcus aureus* that makes it the first choice for eradication of a nasal *staphyloccous* reservoir [38]. The use of other topical antibiotics like neomycine, bacitracine, polymyxine etc. is better avoided since contact-allergic reactions are not uncommon and they are not effective in deeper infections. In these cases systemic treatment may be necessary [39]. Interestingly enough, the new calls of topical calcineurin-inhibitors used for atopic dermatitis reduces the microbial settlement of skin by *staphylococci* and restores the barrier function [40], [41].

In recent years research has identified several families of antimicrobial peptides in vertebrae including humans [42]. Many of these peptides are multifunctional. They are not only natural antibiotics but chemotaxins as well. The human cathelicidin LL-37 is chemotactic for neutrophils, monocytes, mast cells, and T-lymphocytes, causes mast cell degranulation and supports vascularization and re-epithelialization of wounds [43].

In human skin appendages genes like *DCD* and *CAMP* are expressed encoding antimicrobial peptides cathelicidin LL-37 and dermicidin. By the action of serine proteases new antimicrobial are produced with their own antimicrobial profile [44], [45]. β-2 defensine has been idientified in lamellar bodies of the huma skin [46]. There seems to be some antimicrobial activity of the lipid phase in stratum corneum. In addition, such substances are secreted together with the sweat and spread on the skin surface [45], [47].

In newborns there is a 10- to 100-fold increase of expression of cathelicidin LL-37 and β-defensin-2 compared with adult skin [48]. This explains as a compensatory mechanism of a still immature immune system in adapting to post-uterine life. The antimicrobial peptides are concentrated in the vernix caseosa. Both α-defensin-1 to -3 and cathelicidin LL-37 have been detected there [49].

During the recovery of barrier function after wounding or inflammatory disease (eczema, psoriasis) there is a close interaction between growth factors and antimicrobial peptides [50]. The microbial settlement induces antimicrobial control mechanisms: For instance saprophytic yeast *Malasezzia furfur*, but bacteria as well, induce the expression of β-defensin-2 in human epidermal keratinocytes [51], [52].

2.2 Wound healing - TIME concept

In acute wounds surgery is the treatment of choice. In the management of chronic wounds conservative treatments may be successful as well. In chronic non-infected wounds and chronic wounds not at risk of infection the principle of moist wound healing has become the standard.

The use of wound dressings is a part of the concept of wound bed preparation - TIME. TIME includes the following aspects [53]:

T = Tissue

Is the tissue viable or necrotic? In cases of necrosis debridement is necessary either with the by surgery, application of maggots (biosurgery), or enzymatic. However, the beneficial effect of regular debridement has been proven scientifically only in the treatment of diabetic foot ulcers yet [54].

I = Infection, inflammation

In any case of infection and / or inflammation infection control is need either by antibiosis, removal of the bacterial biofilm or disinfection. In addition the basic pathogenic factors like hypoxia, impaired circulation, metabolic disease and oedema have to be treated as well. In chronic wounds at risk of infection silver-containing wound dressings are useful and effective [55], [56].

M = Moisture balance

Moist wound healing is physiological wound healing. In particular re-epithelialization of skin is supported by a moist but not wet wound milieu. This is by no doubts a clinical routine in mucous membrane wounds.

The aim of moisture balance is protection against secondary infection and balancing fluid in the wound bed. For this purpose wound dressings with a semi-occlusive surface membrane combined with polyurethane foams, alginates or hydro fibres are useful. They can also prevent maceration of surrounding skin. The major effects are improvement of granulation and re-epithelialization, pain reduction, reduction of dressing change frequency and thereby decreasing the nursing time [57], [58].

In recent years more and more interactive dressings have been developed, that balance the content and activity of matrix metalloproteinases in the wound fluid [59], [60].

E = Edge of wound

The wound edge can be undermined like in pyoderma gangraenosum, hyperplastic like in plantar ulcers or growing onto the wound bed like in healing wounds (edge effect). The edge effect can also be observed after skin transplant (i.e. in full thickness Reverdin islands). Platelet-derived growth factor (PDGF-BB) increases the speed of re-epithelialization in diabetic and varicose ulcers. The basic mechanisms of action are neoangiogenesis and increased fibroblast activity [61]. On the other hand, dermal matrix components like hyaluronic acid, oxygen partial pressure and other growth factors may stimulate keratinocyte proliferation and migration [62], [63]. Inter linked hyaluronic acid has been used as wound dressing and as a keratinocyte delivery system in tissue-engineered skin [64].

References

1. Loomis CA, Birge MB. Fetal skin development. In: Eichenfield LF, Frieden IJ, Esterly NB

(Hrsg). Textbook of Neonatal Dermatology. Philadelphia: WB Saunders 2001: 1-17.
2. Elias PM. Epidermal lipids, barrier function, and desquamation. J Invest Dermatol 1993; 80 (Suppl.): 44S-49S
3. Feingold KR. The regulation and role of epidermal lipid synthesis. Advan Lipid Res 1991; 24: 57-82
4. Forslind B. A domain mosaic model of the skin barrier. Acta Derm Venerol 1999; 74: 72-77
5. Behne MJ, Barry NP, Hanson KM, Aronchik I, Clegg RW, Gratton E, Feingold K, Molleran WM, Elias PM, Mauro TM. Neonatal development of the stratum corneum pH gradient: localization and mechanisms leading to emergence of optimal barrier function. J Invest Dermatol 2003; 120: 998-1006.
6. Fluhr JW, Behne MJ, Brown BE, Moskowitz DG, Selden C, Mao-Qiang M, Mauro TM, Elias PM, Feingold KR. Stratum corneum acidification in neonatal skin: secretory phospholipase A2 and the sodium/hydrogen antiporter-1 acidify neonatal rat stratum corneum. J Invest Dermatol 2004; 122: 320-329
7. Chapman SJ, Walsh A. Membrane-coating granules are acidic organelles which posses proton pumps. J Invest Dermatol 1989; 93: 466-470
8. Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM. pH directly regulates epidermal barrier homeostasis, and stratum corneum integrity/ cohesion. J Invest Dermatol 2003; 121: 345-353.
9. Hildebrandt D, Ziegler K, Wollina U. Electrical impedance and transepidermal water loss of healthy human skin under different conditions. Skin Res Technol 1998; 4: 130-134.
10. Kligman AM. Hydration injury to human skin: a view from the horny layer. In: Handbook of Occupational Contact Dermatitis. Kanerva L, Elsner P, Wahlberg JE, Maibach HI (Hrsg). Berlin - Heidelberg - New York: Springer 2000, 76-80.
11. Grubauer G, Elias PM, Feingold KR: Transepidermal water loss: The signal for recovery of barrier structure and function. J Lipid Res 1989; 30: 323-333.
12. Draelos Z. Therapeutic moisturisers. Dermatol Clin 2000; 18: 597-606.
13. Motta S, Monti M, Rigano L. Polydecene oligomers versus mineral oils: The rationale of use in dermatological preparations. In: Yazan Y (Hrsg). Skin Care and Aesthetics in the Millenium. Istanbul: ESCAD & TCOS 2003: 101-104.
14. Gabard B, Chantelain E. Concentration of urea in the stratum corneum and moisturizing effect. Trends Clin Exp Dermatol 2003; 2: 27-36
15. Loftsson T. Cyclodextrins in skin delivery. Cosmet Toilet 2000; 115: 59-66
16. Chiu A, Kimball AB. Topical vitamins, minerals and botanical ingredients as modulators of environmental and chronological skin damage. Br J Dermatol 2003; 149: 681-691.
17. Ebner F, Heller A, Rippke F, Tausch I. Topical use of dexpanthenol in skin disorders. Am J Clin Dermatol 2002; 3: 427-433.
18. Girard P, Berand A, Goujou C, Sirvent A, Foyatier J-L, Alleaume B, de Bony R. Effect of Bepanthen® Ointment on the graft-donor site wound-healing model: double-blind biometrical and clinical study, with assessment by the patient, versus the vehicle. Nouv Dermatol 1998; 17: 559-570.
19. Girard P, Goujou C, Violin L, de Bony R, Foyatier JL. A double-blind biometrical, histological, clinical and patient evaluation pilot study of the effect of Bepanthen® Ointment versus excipient using a skin graft donor site as a wound healing model. Nouv Dermatol 1997; 16: 266-275.
20. Gehring W, Gloor M. Die Bedeutung von topisch appliziertem Dexpanthenol für die epidermale Barrierefunktion und die Hydratation des Stratum corneum. Arzneim-Forsch/ Drug Res 2000; 50(II): 659-663.
21. Gehring W, Gloor M. Der Effekt von Dexpanthenol bei experimentell geschädigter Haut. Z Hautkrankh 2001: 76: 212-218.
22. Wollina U, Nissen H-P, Kubicki J. Antientzündliche Wirkungen von Dexpanthenol. Akt Dermatol 2004; 30: 478-482.
23. Schmuth M, Sztankay A, Weinlich G, Linder DM, Wimmer MA, Fritsch PO, Fritsch E. Permeability barrier function of skin exposed to ionizing radiation. Arch Dermatol 2001; 137:1019-1023.
24. Ratliff C. Impaired skin integrity related to radiation therapy. J Enterostomal Ther 1990; 17: 193-198.
25. Zimmermann JS, Budach W, Dörr W. Individual skin care during radiation therapy. Strahlenther Onkol 1998; 174 [Suppl 3]: 74-77.
26. Porock D, Kristjanson L. Skin reactions during radiotherapy for breast cancer: the use and impact of topical agents and dressings. Eur J Cancer Care (Engl) 1999; 8: 143-153.
27. Szumacher E, Wighton A, Franssen E, Chow E, Tsao M, Ackerman I, Andersson L, Kim J, Wojcicka A, Ung Y, Sixel K, Hayter C. Phase II study assessing the effectiveness of Bifine cream as prophylactic agent for ration-induced acute skin toxocity to the breast in women

undergoing radiotherapy with concomitant CMF chemotherapy. Int J Radiol Oncol Biol Phys 2001; 51: 81-86.
28. Fisher J, Scott C, Stevens R, Marconi B, Champion L, Freedman GM, Asrari F, Pilepich MV, Gagnon JD, Wong G. Randomized phase III study comparing Best Supportive Care to Biafine as a prophylactic agent for radiation-induced skin toxicity for women undergoing breast irradiation: Radiation Therapy Oncology Group (RTOG) 97-13. Int J Radiat Oncol Biol Phys 2000; 48: 1307-1310.
29. Roy I, Fortin A, Larochelle M. The impact of skin washing with water and soap during breast irradiation: a randomized study. Radiother Oncol 2001; 58: 333-339.
30. Olsen DL, Raub Jr W, Bradley C, Johnson M, Macias JL, Love V, Markoe A. The effect of aloe vera gel/ mild soap versus mild soap alone in preventing skin reactions in patients undergoing radiation therapy. Oncol Nurs Forum 2001; 28: 543-547.
31. Slyshenkov VS, Omelyanchik SN, Moiseenok AG, Petushok NE, Wojtczack L. Protection by panthothenol and beta-carotene against liver damage produced by low-gamma radiation. Acta Biochim Pol 1999; 46: 239-248.
32. Schratter-Sehn AU, Brinda K, Kahrer M, Novak M. Improvement of skin care during radiotherapy. Onkologie 2001; 24: 44-46.
33. Lokkevik E, Skovlund E, Reitan JB, Hannisdal E, Tanum G. Skin treatment with bepanthen cream versus no cream during radiotherapy - a randomized controlled trial. Acta Oncol 1996; 35: 1021-1026.
34. Bostrom A, Lindman H, Swartling C, Berne B, Bergh J. Potent corticosteroid cream (mometasone furoate) significantly reduces radiation dermatitis: results from a double-blind, randomized study. Radiother Oncol 2001; 59: 257-265.
35. Panizzon RG, Mirimanoff R-O. Hautpflege bei Tiefenbestrahlung. Hautarzt 2001; 52: 150.
36. Roth RR, James WD. Microbial ecology of the skin. Annu Rev Microbiol 1988; 42: 441-464.
37. Soll RF, Edwards WH: Emollient ointment for preventing infection in preterm infants. Cochrane Database 2000; Syst Rev CD 001150
38. Rhody C. Bacterial infections of the skin. Prim Care 2000; 27: 459-473.
39. Wollina U. Contact allergy associated with topical treatment. In: Gebhardt M, Elsner P, Marks Jr JG (Hrsg). Handbook of Contact Dermatitis. London: Martin Dunitz: 2000:71-81.
40. Lazarous MC, Kerdel FA. Topical tacrolimus Protopic. Drugs Today (Barc) 2002; 38: 7-15.
41. Wolff K, Stuetz A. Pimecrolimus for the treatment of inflammatory skin disease. Expert Opin Pharmacother 2004; 5: 643-655.
42. Nicolas P, Vanhoye D, Amiche M. Molecular strategies in biological evolution of antimicrobial peptides. Peptides 2003; 24: 1669-1680.
43. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol 2004; 75: 39-48
44. Mangoni ML, Papo N, Mignogna G, Andreu D, Shai Y, Barra D, Simmaco M. Ranacyclins, a new family of short cyclic antimicrobial peptides: biological function, mode of action, and parameters involved in target specificity. Biochemistry 2003; 42: 14023-14035.
45. Murakami M, Ohtake T, Dorschner RA, Schittek B, Garbe C, Gallo RL. Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. J Invest Dermatol 2002; 119: 1090-1095
46. Oren A, Ganz T, Liu L, Meerloo T. In human epidermis, beta-defensin 2 is packed in lamellar bodies. Exp Mol Pathol 2003; 74: 180-182.
47. Schittek B, Hipfel R, Sauer B, Bauer J, Kallenbacher H, Stevanovic S, Schirle M, Schroeder K, Blin N, Meier F, Rassner G, Garbe C. Dermicidin: a novel human antibiotic peptide secreted by sweat glands. Nat Immunol 2001; 2: 1133-1137.
48. Dorschner RA; Lin KH, Murakami M, Gallo RL. Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. Pediatr Res 2003; 53: 566-572.
49. Yoshio H, Tollin M, Gudmundsson GH, Lagercrantz H, Jornvall H, Marchini G, Agerberth B. Antimicrobial polypeptides of human vernix caseosa and amniotic fluid: implications for newborn innate defense. Pediatr Res 2003; 53: 211-216.
50. Sorensen OE, Cowland JB, Theilgaard-Mönch K, Liu L, Ganz T, Borregaard N. Wound healing and expression of antimicrobial peptides/ polypeptides in human keratinocytes, a consequence of common growth factors. J Immunol 2003; 170: 5583-5589.
51. Donnarumma G. Paoletti I, Biommino E, Orlando M, Tufano MA, Baroni A. Malassezia furfur induces the expression of beta-defensin-2 in human keratinocytes in a protein C-dependent manner. Arch Dermatol Res 2004; 295: 474-481.
52. Chung WO, Dale BA. Innate immune response of oral and foreskin keratinocytes: utilization of different signalling pathways by various bacterial species. Infect Immun 2004; 72: 352-358.
53. European Wound Management Association (EWMA). Position Document: Wound Bed Preparation in Practise. London: MEP Ltd, 2004.

54. Brehm H, Jacobs T, Vileikyte L, Weinberger S, Gibber M, Gill K, Tarnovskaya A, Entero H, Boulton AJ. Wound-healing protocols for diabetic foot and pressure ulcers. Surg Technol Int 2003; 11: 85-92.
55. Verdú Soriano J, Rueda López J, Martínez Cuervo F, Soldevilla Agreda J. Effect of an activated charcoal silver dressing on chronic wounds with no clinical signs of infection. J Wound Care 2004; 13: 419-423.
56. Thomas S, McCubbin P. A comparision of the antimicrobial effects of 10 silver-containing dressings. J Wound Care 2003; 12: 305-308.
57. Meaume S, Téot L, Lazareth I, Martini J, Bohbot S. The importance of pain reduction through dressing selection in routine wound management: the MAPP study. J Wound Care 2004; 13 : 409-413.
58. Wollina U. A hydropolymer dressing for chronic wounds: clinical experiences with 478 patients in an open multicenter trial. Anais Brasil Dermatol (Rio de Janeiro) 1997; 72: 527-532.
59. Cullen B, Watt PW, Lundqvist C, Silcock D, Schmidt RJ, Bogan D, Light ND. The role of oxidised regenerated cellulose/collagen in chronic wound repair and its potential mechanism of action. Int J Biochem Cell Biol 2002; 34: 1544-1556.
60. Veves A, Sheehan P, Pham HT. A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. Arch Surg 2002; 137: 822-827.
61. Agren MS, Steenfos HH, Dabelsteen S, Hansen JB, Dabelsteen E. Proliferation and mitogenic response to PDGF-BB of fibroblasts isolated from chronic venous leg ulcers is ulcer-age dependent. J Invest Dermatol 1999; 112: 463-469.
62. Weindl G, Schaller M, Schäfer-Korting M, Korting HC. Hyaluronic acid in the treatment and prevention of skin diseases: molecular biological, pharmaceutical and clinical aspects. Skin Pharmacol Physiol 2004; 17: 207-213.
63. Wollina U, Konrad H, Karamfilov T. Recessive epidermolysis bullosa dystrophicans (Hallopeau-Siemens) - improvement of wound healing by autologous epidermal grafts on an esterified hyaluronic acid membrane. J Dermatol (Tokyo) 2001; 28: 217-220.
64. Wollina U, Heinig B, Uhlemann C. Transdermal CO2 application in chronic wounds. International J Lower Extremity Wounds 2004; 3:103-106.
65. Yoipovitch G. Dry skin and impairment of barrier function associated with itch - new insights. Int J Cosmetic Sci 2004; 26: 1-7.