WOUND HEALING IN A SUCTION BLISTER MODEL
An experimental study with special reference to healing in patients with diabetes and patients with obstructive jaundice

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Abstract

The expression intensities of cytokeratins and tight junction proteins were determined on re-epithelization. Experimental blister wound healing was studied in patients with diabetes mellitus and in patients with obstructive jaundice.

Suction blisters were induced on healthy volunteers, and the healing blisters were biopsied at different time points. Cytokeratin expression and the tight junction proteins ZO-1 and occludin were studied immunohistochemically.

Blisters were induced on 17 patients with diabetes and 11 control subjects, and the healing process was followed indirectly by measuring water evaporation and blood flow in the wounds. Microvascular reactivity in the diabetic patients was also studied by using non-immunologic contact irritants.

Wound healing, skin collagen synthesis and serum levels of procollagen propeptides were studied in 24 patients with obstructive jaundice caused by neoplastic pancreaticobiliary obstruction and in 17 control patients with the corresponding condition without jaundice.

Cytokeratin expression was altered in healing epidermis. In the suprabasal layer, K10 was replaced by K14 and, most likely, by K16. K18 keratin, which is not present in normal epidermis, was found in the basal and suprabasal layers. Thus, there was a shift towards lower molecular weight cytokeratins, which is a reflection of immaturity, and probably towards motility. The tight junction proteins ZO-1 and occludin were expressed in the migrating epidermal sheet, where they apparently form an early barrier. Enhanced expression was seen in the hyperproliferative zone of the wound edge.

The diabetic patients showed slower restoration of the epidermal barrier and a weaker initial inflammatory response. Obstructive jaundice and its resolution had no effect on healing.

Skin collagen synthesis was decreased in jaundiced patients, and it increased slightly after drainage. Serum type III collagen propeptide levels were elevated in patients with biliary obstruction and dropped after drainage. The elevated levels may be related to the increased synthesis due to fibrosis.

As a conclusion, diabetes mellitus impairs epidermal wound healing, while obstructive jaundice does not.

Keywords: biliary drainage, collagen, inflammation, occludin, wound model, ZO-1
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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Acronym</th>
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<tr>
<td>Aminoterminal propeptide of type I procollagen</td>
<td>PINP</td>
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<tr>
<td>Aminoterminal propeptide of type III procollagen</td>
<td>PIINP</td>
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<tr>
<td>Basement membrane zone</td>
<td>BMZ</td>
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<tr>
<td>Blood flow</td>
<td>BF</td>
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<tr>
<td>Cytokeratin</td>
<td>K</td>
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<tr>
<td>Epidermal growth factor</td>
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<tr>
<td>Keratinocyte growth factor</td>
<td>KGF</td>
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<td>Insulin-dependent diabetes mellitus</td>
<td>IDDM</td>
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<tr>
<td>Interferon ?</td>
<td>IFN ?</td>
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<tr>
<td>Interleukin</td>
<td>IL</td>
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<td>Matrix metalloproteinases</td>
<td>MMP</td>
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<td>Non-immunologic immediate contact reaction</td>
<td>NIICR</td>
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<tr>
<td>Non-insulin-dependent diabetes mellitus</td>
<td>NIDDM</td>
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<tr>
<td>Nitric oxide</td>
<td>NO</td>
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<tr>
<td>Transforming growth factor a</td>
<td>TGF α</td>
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<td>Transforming growth factor β</td>
<td>TGF β</td>
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<tr>
<td>Tumor necrosis factor a</td>
<td>TNF α</td>
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<td>Vascular endothelial growth factor</td>
<td>VEGF</td>
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<td>Water evaporation</td>
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List of original publications

This book is based on the following articles, which are referred to in the text by their Roman numerals.


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1 Introduction

The mechanisms of wound healing are elemental for life, since not only the healing of visible wounds, but also the curing of small, principally epithelial defects takes place continuously in a human being (Jacinto et al. 2001, Martin 1997). There is a reason to continue wound healing research, because several basic phenomena of cell motility and inflammation remain to be elucidated, and because non-healing wounds pose a significant burden to patients (Jeffcoate & Harding 2003, Falanga 1993).

The majority of studies have been done using tissue cultures or experimental animals. Recently, further insight into regulatory proteins and growth factors has been gained by using transgenic or knockout gene animals. Cell cultures have also yielded new information about the regulation of cell motility (Coulombe 2003, Singer & Clark 1999). There are also wound healing models that have been used in humans. These are important, since the application of information from cell culture and animal experiments to humans must be done with certain diffidence. Different models describe different parts of the process of healing. The cell stick device gives information about the cellularity and protein synthesis of full-thickness wounds, while wound chamber models illustrate the contents of wound fluid (Fahey et al. 1991, Viljanto 1969, Viljanto 1976).

The suction blister wound model gives information about the re-epithelization and inflammation of wounds. The prolonged vacuum on the skin leads to separation of the basement membrane at the level of lamina lucida, and when the blister roof is removed, healing takes place on the partially intact basement membrane. The healing process can be followed up by biopsies or by non-invasively examining water evaporation and blood flow on the wound base. The suction blister model was developed in the 1960s, and it has been used since in studies on the cellularity and collagen synthesis of the skin in different diseases and after surgical procedures and in wound healing studies (Kiistala 1969, Oikarinen et al. 1992, Svedman et al. 1991a, Svedman et al. 1991b).

Cytokeratins (CK) are intermediate filaments of the epithelial cytoskeleton. They are part of the cellular scaffolding. Cytokeratins reflect cellular maturity: immature cells express lower molecular weight proteins. It is known that, in diseases where cell kinetics is altered, such as inflammatory disorders or cancer, the cytokeratins are immature. CKs are linked to desmosomes and hemidesmosomes, which provide firm cell-cell and cell-basement membrane contacts. It is thus likely that an alteration in cytokeratin expression
enables cell movement. Earlier studies have revealed that cytokeratin expression is altered during wound healing (Fuchs & Cleveland 1998).

Tight junctions form a barrier between cells that regulates the passage of water and solutes. Tight junctions are present in all epithelia, and their function has been examined especially in the intestinal barrier. Traditionally, the epidermal diffusion barrier was considered to consist of lipids. In the recent years, the significance of tight junctions in the epidermal barrier has been highlighted further. Tight junctions may have a role in the conditions where the lipid barrier is absent or abnormal, e.g. in fetal skin and in skin diseases. Cellular junctions also accomplish paracellular communication. Since barrier restoration and cellular communication are essential to wound healing, the expression of tight junctions on healing is interesting (Stevenson & Keon 1998).

Diabetes mellitus is known to cause deficits in wound healing. This is most obvious in foot ulcers, where neuropathy, ischemia and liability to infection are the main impairments. The healing deficit in patients with diabetes is multifactorial. Impairments are seen in wound leukocyte functions, growth factor secretion and cell proliferation (Greenhalgh 2003b). The suction blister model was used to reveal whether there is impairment in wound re-epithelization or in the inflammatory response in patients with diabetes.

Obstructive jaundice is considered to have adverse effects on tissue healing and postoperative recovery. These deficits are linked to the inflammatory state induced by jaundice. Biliary obstruction has been found to decrease skin collagen synthesis. Obstructive jaundice is commonly treated by biliary drainage before surgery to avoid complications (Kimmings et al. 2000, Sewnath et al. 2002). In the present work, the effect of biliary drainage on experimental wound healing and collagen synthesis was investigated.
2 Review of literature

2.1 Skin

The skin is divided into a superficial epithelial layer mainly consisting of keratinocytes called epidermis and a deeper connective tissue layer called dermis. The epidermis and the dermis are separated by the basement membrane (Lever & Schaumburg-Lever 1990).

2.1.1 Epidermis

The epidermis consists of stratified squamous epithelium. The predominant cells are keratinocytes. The other cells are melanocytes, Langerhans cells and Merkel cells. The epidermis is divided into different layers: the basal, squamous and granular layers and the uppermost stratum corneum. Basal cells form a single layer resting on the basement membrane. Squamous cells form a mosaic pattern of cells four to five layers thick, and the stratum of granular cells is one to ten cell layers thick, depending on the thickness of the uppermost stratum corneum (Fig.2). There is continuous turnover of superficial keratinised cells, which are lost and replaced by cells arising from the basal layer. The structural organization of the epidermis reflects the life of keratinocytes: cells proliferate, differentiate and migrate outwards, forming a dynamic and continuously changing structure (Lever & Schaumburg-Lever 1990).

The epidermis has numerous functions: it protects the body against environmental microbes and chemicals. It also has a role in immunology, and it absorbs ultraviolet irradiation and performs sensory functions (Eady et al. 1998).
2.1.2 Basement membrane

Between the epidermis and the dermis lies the basement membrane zone (BMZ), which connects the epidermis to the dermis and restricts the passage of molecules between them. BMZ is composed of hemidesmosome, lamina lucida, lamina densa and sub-lamina densa. The intermediate filaments in basal keratinocytes are attached to the intracellular plate of hemidesmosome. The main protein components of hemidesmosome are bullous pemphigoid antigens 1, type XVII collagen and α6β4 integrin (Ghohestani et al. 2001, Franzke et al. 2003). The anchoring filaments connect hemidesmosome to lamina lucida. The main constituent of anchoring filaments is laminin-5. The basement membrane is attached to the dermis by anchoring fibrils. The anchoring fibrils form loops in the upper dermis and are attached to type IV collagen and laminin-5 in the basement membrane. When mechanical forces cause skin blistering, as in the suction blister model, the detachment takes place in the lamina lucida layer (Oikarinen et al. 1982, Uitto et al. 1996, Willsteed et al. 1991) (Fig. 1).

![Diagram of the basement membrane](image)

**Fig. 1.** The cutaneous basement membrane zone. Basal keratinocytes lie on the dermis. The two compartments are separated by the basement membrane. The morphological structures are named on the left side and their protein components on the right side of the figure (modified from Uitto et al. 1996).
2.2 Cytokeratins

Cytokeratins, or keratins, are a group of intermediate filaments that form part of the intracellular cytoskeleton system. The other components are microtubules, actin microfilaments, vimentin and desmin (Chou 1997, Fuchs & Cleveland 1998, Geiger & Karsenti 1997). Cytokeratins are only present in epithelia. The diversity of cytokeratins is most pronounced in the skin, where there are over 20 distinct keratins. Cytokeratins are divided into two types based on their amino acid sequence: type I (acidic) keratins and type II (neutral/basic keratins). Type I keratins include 11 keratin polypeptides, named K10 to K20 with molecular weights ranging from 40 to 56.5 kDa. Type II keratins include nine polypeptides, K1 to K9, which have molecular weights of 52-67 kDa. In normal human epidermis, keratins are expressed in specific pairs consisting of members of both families (type I and type II). The largest molecules in each group, namely K1 and K10, make up a pair, which is expressed in the suprabasal layer of normal epidermis, while the intermediate-sized molecules, K5 and K14, appear in the basal layer of epidermis (Roop 1995, Smack et al. 1994) (Fig. 2).

The cytoskeletal network changes in diseases with increased cell turnover. During wound healing, keratinocytes proliferate and migrate, and they express different cytokeratins compared to their resting condition. For example, the K1 and K10 in the suprabasal layer are replaced by K6, K14 and K16. The alterations in keratin expression probably enable cell migration (Coulombe 2003).

2.3 Tight junctions

Cell junctions were originally classified according to their shape and the nature of the cell contact. A spot-like junction (e.g. desmosome) is called macula and a belt-like one zona. If the cells appear to be in contact or fused, the junction is said to be occluding or tight (Stevenson & Keon 1998). If there is a space of appr. 20 nm filled with dense material associated with cytoplasmic surfaces, we speak about an adhering or adherens junction. Tight junctions are located apically in epithelial cells. Spot desmosomes are located at various levels of cells, and there is a 20-30nm space filled with filamentous material at the junction. Adherens junctions are present in all layers of epidermal cells in close apposition to gap junctions. At gap junctions, there is a narrow space between cells, and the gap between cells basically consists of a hexagonal arrangement of connexons. Adherens and tight junctions are connected to actin microfilaments, whereas desmosomes and are connected to the intermediate filaments of the cytoskeleton. The main task of a tight junction is to form a barrier, whereas adherens and desmosome junctions adhere cells and probably also participate in cell dynamics (Garrod et al. 1996). Gap junctions are chiefly used as paths for communication (Evans & Martin 2002).

Recently, cellular junctions have been associated with cellular signalling. There are various kinds of interplay between cell junctions (particularly adherens junctions) and microtubules, actin and intermediate filaments. The cell-cell adhesion via cadherin receptor (component of adherens junctions) has been shown to activate small Rho
ATPases, which in turn regulate the other parts of the junction assembly and thus maintain tissue integrity and the epithelial phenotype (Tsukita et al. 1999).

Tight junctions form a paracellular permeability barrier that regulates the movement of water, solutes and immune cells in simple epithelia (Anderson et al. 1995). Additionally, tight junctions divide the plasma membrane into the apical and basolateral membrane domains. The tight junction forms a belt encircling the apical portion of the cell (Stevenson & Keon 1998). For example, in the intestinal epithelium, tight junctions (zonula occludens) form the most apical component of the lateral junction complex (Berkes et al. 2003). Several structural components of tight junctions have been characterized during recent years (Tsukita & Furuse 1999, Tsukita & Furuse 2000).

Occludin is a transmembrane protein that only occurs at tight junctions. Occludin has four hydrophobic transmembrane helices, and both the NH$_2$ and the COOH terminals point towards the cytoplasmic side of the plasma membrane. ZO-1 (zonula occludens-1) is a protein found in the cytoplasmic plate of tight junctions, but also in adherens type junctions in cells that lack tight junctions, such as fibroblasts and cardiac myocytes (Tsukita et al. 1999). ZO-1 is believed to contribute to the proper organization of proteins within the tight junction plate (Tsukita S & Furuse 1999). In addition, the family of claudins as well as the cytoplasmic proteins ZO-2, ZO-3, cingulin, symplekin and 7H6 have been characterized as components of tight junctions (Tsukita & Furuse 2000).

Early studies using freeze-fracture scanning electron microscopy on human and mouse stratified epithelia revealed that tight-junction elements were either fragmentary or absent (Elias et al. 1977). The epidermal diffusion barrier has thus been considered not to consist of tight junctions, but of epidermal lipids, especially the lipid bilayers present in the cornified layer (Wertz 2000). However, the characterization of the structural components of tight junctions has provided new means to study the tight junctions, and the tight junction components occludin and ZO-1 have been found in adult human epidermis (Morita et al. 1998, Pummi et al. 2001). The study on claudin-1 knockout mice suggests that tight junctions function as components of the diffusion barrier (Furuse et al. 2002).

On wound healing, the epidermal barrier is incomplete and epidermal keratinocytes undergo proliferation, migration and differentiation (Martin 1997, Singer & Clark 1999). Rearrangement of tight junctions in the gut restores the mucosal barrier in small mucosal defects (Berkes et al. 2003). Tight junctions may thus play a role in the restoration of the epidermal barrier during wound healing. In the present work, the tight junction components ZO-1 and occludin were studied during re-epithelization of the skin.
Fig. 2. Layers and cytokeratins (K1 etc.) of epidermis. Modified from Roop 1995.

2.4 Collagen

Collagen is the major constituent of the extracellular matrix together with proteoglycans. The extracellular matrix binds cells together, giving structure to tissues. Collagens are large fibrillar proteins, which provide tensile strength to tissues. The collagen protein has a basic sequence glycine – X-Y, where X and Y are other amino acids. The collagen molecule consists of three polypeptide β-chains. The typical collagen is arranged in a triple-helix form. The triple-helix collagens I-III are then packed into fibrils, which in turn make up collagen fibres (Myllyharju & Kivirikko 2004, Prockop & Kivirikko 1995).

By now, over 20 collagen types have been discovered. The most abundant collagen in the human body is type I collagen. It is present in bone and in soft tissues. It is also the main constituent of skin. Type II collagen is present in bone and cartilage. Type III collagen occurs together with type I collagen in soft tissues. In the provisional matrix of wound healing, which is called granulation tissue, type III is the most abundant collagen.
Basement membrane contains type IV collagen. Type VII collagen is found in the anchoring fibrils of skin (Borradori & Sonnenberg 1999, Uitto et al. 1996).

2.4.1 Collagen synthesis

Collagen synthesis proceeds via an intracellular precursor molecule pathway. Precursor collagen undergoes posttranslational modifications before becoming part of a mature collagen fibre. The earliest collagen, preprocollagen, contains a signal sequence that is cleaved after synthesis in the endoplasmic reticulum. After that, the prolyl and lysyl residues are hydroxylated. Formation of interchain S-S bonds assembles collagen as a triple helix. In the following intracellular processing, the triple-helix procollagen is extracted via the Golgi apparatus. In the extracellular space, procollagen aminoproteases and carboxyproteases cleave amino- and carboxyterminal propeptides. Mature collagen molecules assemble into collagen fibres. The final tensile strength of collagen is achieved after the formation of covalent crosslinks between fibres (Myllyharju & Kivirikko 2004, Prockop & Kivirikko 1995).

Collagens occur and are synthesized in connective tissues of all types. In skin, the synthesis takes places in fibroblasts, in bone in osteoblasts, in cartilage in chondroblasts and in the alimentary tract in smooth muscle cells (Myllyharju & Kivirikko 2004).

2.4.1.1 Measuring collagen metabolism

The synthesis of types I - III collagens is measured by assaying collagen propeptides from extracellular fluid (mostly serum). Collagens I - III are synthesised as larger precursor proteins, which contain extra domains at both ends of the molecule. The protein is called procollagen, and it contains the proper collagen molecule and aminoterminal and carboxyterminal propeptides. Propeptides are formed in a stoichiometric ratio to the number of collagen molecules formed (Risteli & Risteli 1995, Risteli et al. 1995). The concentration of collagen propeptides in serum depends on several factors. Propeptides of type I and III collagens are exchanged to serum in a 1:1 ratio. In the case of IV and VI collagens, the antigens are liberated into serum through a process different from straightforward precursor-product conversion. The propeptides may be extracted during synthesis or released upon breakdown, which means that the level of procollagen may mirror both degradation and synthesis. Collagens are synthesised in liver, in other soft tissues and in bone. After cleavage, procollagens pass into blood directly or via lymph. From blood, they are eliminated via certain receptors on hepatic vessel endothelial cells. Procollagens are too large to be cleared by the kidneys. An elevated serum level of collagen propeptides may result from increased synthesis or decreased uptake by liver cells (Risteli & Risteli 1995).

Collagen degradation assays are based on the cross-linked telopeptide parts of collagen molecules. Assays for telopeptides of type I and III collagens have been
developed (Kauppila et al. 1999, Risteli et al. 1993). Telopeptides are cleared from serum by the kidneys, and glomerular filtration rate hence affects its levels (Risteli & Risteli 1995).

2.4.1.2 Suction blisters in measuring collagen synthesis in skin

There are only a few tissues where the local production of collagen propeptides can be measured, even though that would often be the most interesting matter. Local production can be measured from skin using the suction blister method (Oikarinen et al. 1992).

Suction blister fluid represents interstitial fluid (Herfst & van Rees 1978). Most polypeptides are produced locally. The fraction of proteins derived from serum is related to molecular weight in such a way that the higher the molecular weight is, the lower is the serum fraction. Proteins enter the blister by diffusion (Kiistala 1976, Vermeer et al. 1979).

The aminoterminal propeptides of type I and III collagens (PINP and PIIINP) can be measured from suction blister fluid. The method is a commercially available enzyme immunoassay (Orion Diagnostica, Finland). The method has been used to study baseline skin collagen synthesis and the effects of glucocorticoid treatments and surgical procedures on skin collagen synthesis (Oikarinen et al. 1992, Ihlberg et al. 1993).

2.5 Wound healing

Wound healing is essential to the human body. The wounded body is disposed to threats, such as bleeding, microbes, and dehydration. The healing process is principally similar in distinct connective tissues and epithelia (Heath 1996, Jacinto et al. 2001). The healing of the wound requires orchestrated collaboration between different tissues and cell lineages (Clark 1996). Most skin lesions heal without problems within one or two weeks. Complications in wound healing are most often associated with systemic diseases, such as diabetes, cancer or malnourishment, or with local conditions, such as venous hypertension, severe trauma and bacterial contamination (Jeffcoate & Harding 2003, Falanga 1993). However, even if the wound heals without complications, the end product is neither aesthetically, nor functionally perfect. In the scar, the collagen matrix is poorly reconstituted and contains dense parallel bundles instead of mechanically efficient basket-weave meshwork (Martin 1997). Wound healing can be divided in different phases starting from inflammation and ending to matrix remodelling (Fig.3).
2.5.1 Fibrin clot, provisional matrix and inflammation

Tissue injury causes extravasation of blood, and the coagulation cascade establishes hemostasis. Immediately after tissue injury, blood flow is slowed down by the interplay of vasoconstricting substances, such as serotonin, thromboxane A2 and epinephrine. Blood clotting is initiated by activation of factor XII (Hageman factor) in the intrinsic coagulation pathway and by tissue activation of factor VII in the classical pathway. The essential prerequisite for coagulation is the availability of surfaces that allows the activation of specific coagulation proenzymes. If not enough surfaces are available, the process is blocked by several serum inhibitors (English 1995).

Platelets regulate bleeding first by forming a platelet plug in the severed blood vessel and then by catalysing thrombin production, which facilitates early events in clot formation. Thrombin turns fibrinogen to fibrin, which forms a mesh-like network in the wound. The fibrin network is the most abundant constituent in the blood clot. In addition, the clot also contains other extracellular matrix constituents, such as fibronectin,
vitronectin and thrombospondin. Red cells, leukocytes and platelets are trapped from the bloodstream into the clot (Yamada & Clark 1996).

Fibronectin is a multifunctional protein, which interacts with a wide range of cell types and the extracellular matrix. Fibronectin is present in blood in notable concentrations, and the clot is therefore rich in fibronectin. Fibronectin is first deposited in conjunction with fibrin, but it is later located around wound cells, especially fibroblasts. Fibronectin supports keratinocytes, fibroblasts and endothelial cell adhesion and migration. It also serves as a template for collagen deposition, and it can control fibroblast protein synthesis. Hyaluronan is a large glycosaminoglycan, which is a very hydrophilic molecule and hence a major space-occupying constituent of the extracellular matrix. Hyaluronan is considered to promote cell movement in the early provisional matrix. It can facilitate movement by lubricating cell-cell or cell-matrix surfaces or via cell receptors for hyaluronan (Yamada & Clark 1996).

The clot protects the wound from dehydration and microbes and serves as a provisional matrix during healing (Clark 1996). The clot contains healing-promoting cytokines, namely platelet-derived growth factor (PDGF), transforming growth factor α (TGFα) and transforming growth factor β (TGFβ), thus controlling the early steps of healing (Werner & Grose 2003).

PDGF, proinflammatory cytokines (interleukin-1 and -6 (IL-1 and -6), tumor necrosis factor α (TNFα) and interleukin-8 (IL-8)) and other chemokines guide leukocyte infiltration to the injury scene (Werner & Grose 2003). Chemoattractants are secreted by platelets, endothelial cells, keratinocytes and fibroblasts in the wound area. Proinflammatory cytokines, particularly TNFα and IL-1, increase the expression of leukocyte adhesion molecules in capillary endothelium (Singer & Clark 1999). The adhesion molecules expressed are the intercellular adhesion molecule, the endothelial-cell adhesion molecule and the leukocyte-cell adhesion molecule. They trap leukocytes from the bloodstream, thereby initiating leukocyte infiltration (Clark 2003). From the capillaries, leukocytes migrate towards the increasing chemotactic gradient of various substances (e.g. kallicrein, fibrinopeptides and cytokines) to the wound scene (Singer & Clark 1999).

In the early stages of wound healing, the recruited neutrophils are the predominant leukocytes in the wound. The primary function of neutrophils is microbial clearance of the wound. Neutrophils secrete proteases (matrix metalloproteinases and plasminogen activators) that degrade the provisional matrix, thus enabling arrival of the more mature wound components (Clark 1996). Neutrophils also have some creative functions: they support granulation tissue formation by secreting vascular endothelial growth factor (VEGF) and PDGF. Neutrophils secrete chemotactic factors, which attract macrophages, the most significant leukocyte in wound healing (Werner & Grose 2003). Neutrophil predominance is characteristic of the early inflammatory phase of wound healing, and by the time macrophages become predominant, healing has proceeded to the late inflammatory phase (Clark 1996).

Monocytes start intrusion into the wound on the first day after wounding, and they replace neutrophils as the predominant leukocyte type on the third day. The emigration of monocytes from the vasculature and their migration to the wound retain an appropriate inflammatory infiltrate. Macrophages are attracted to the wound site by cytokines and growth factors (TGF-β, IL-1 and TNFα) and by chemokines (chemotactic cytokines).
Chemokines are subdivided into the α and β groups. The α group generally acts on neutrophils, whereas the β group attracts macrophages and includes a factor called macrophage chemoattractant protein – 1 (MCP-1). Monocytes undergo metamorphosis into inflammatory macrophages in the wound. Monocytes mature into macrophages through activation by PDGF (secreted from platelets and keratinocytes) and IL-2, TNFα and IFN-γ (released by T lymphocytes). The binding of monocytes to fibronectin surface receptors also promotes their transformation to macrophages. Other inflammatory factors, e.g. leukotrienes, complement 5α, and bacterial products such as lipopolysaccharides also support the formation of leukocyte infiltrate (Martin 1997, Riches 1996, Singer & Clark 1999).

Macrophages orchestrate tissue repair. Macrophages are the most important leukocytes of wound healing that secrete over 100 different substances affecting the healing process. Wound healing in macrophage-depleted experimental animals is severely disturbed, while a lack of neutrophils mainly causes an increased incidence of wound infections. Macrophages secrete colony-stimulating factors, which maintain macrophage and monocyte infiltration, and PDGF, which is a mitogen for fibroblasts, TNFα and IL-1, which further activate macrophages. Macrophages also secrete TGFα and β and insulin-like growth factor (IGF), which affect the proliferation and migration of keratinocytes and protein synthesis in fibroblasts (Martin 1997, Riches 1996) (Fig.5). Macrophages are also the main source of nitric oxide in healing wound. Nitric oxide is a free radical, which acts in molecular, cellular and physiologic level regulating inflammation, angiogenesis, cell proliferation and protein synthesis in healing wound. Its role in impaired healing is under intensive research (Rizk et al. 2004).

Macrophages contribute to matrix degradation. The damaged connective tissue components, such as collagen, elastin and proteoglycans, must be removed from the wound, and the provisional matrix must make room for new epithelium and connective tissue. Macrophages secrete matrix metalloproteinases, which degrade collagen and elastin, and plasminogen activators, which cleave the fibrin clot from the way of keratinocyte migration (Riches 1996, Madlener et al. 1998).

Other inflammatory cells also play a role in wound healing. Lymphocytes release some inflammatory cytokines. However, they play a minor role compared to macrophages and neutrophils (Werner & Grose 2003).
Fig. 4. A cutaneous wound three days after injury. The arrow starts from the source of the growth factor and ends at the target cell. VEGF refers to vascular endothelial growth factor, TGFα and TGFβ to transforming growth factors, FGF to fibroblast growth factor, PDGF to platelet-derived growth factor, KGF to keratinocyte growth factor and IGF to insulin-like growth factor (modified from Singer & Clark 1999).

2.5.2 Re-epithelization

The principal function of any epithelium is to provide a self-sealing dynamic barrier against the environment. Basically the same dynamics prevail in the skin and in other epithelia, including the gut mucosa (Heath 1996). Directed cell migration and proliferation take place from the first moments of life onwards until the moment when the neural tube closes to repair the last skin scratches in a dying human (Coulombe 2003, Woodley 1996).

Re-epithelization starts within four hours after injury and lasts for a variable time alongside the other healing events, until the epithelial defect has been covered. Re-epithelization starts from the wound edge and from the dermal appendages, such as hair follicles. Epidermal cells undergo marked alterations that facilitate cell motility (Dennis et al. 1995, Jacinto et al. 2001, Martin 1997). The migrating and proliferating cells exhibit different patterns of cytokeratins (Coulombe 2003). The most intercellular
desmosomal and hemidesmosomal junctions between the basal lamina and basal keratinocytes are dissolved. Cytoskeletal and junctional alterations allow lateral motion of epidermal cells. Epidermal cells interact with their neighbouring cells and matrix via integrin receptors. The epidermal cells in wound healing express primarily the integrins that attach to the provisional matrix, namely α5β6 and αvβ1 fibronectin/tenascin receptors and αvβ5 vitronectin receptor (Cavani et al. 1993, Haapasalmi et al. 1996). Laminin-5, which is a component of anchoring filaments in the basement membrane, is proposed to promote keratinocyte migration. Laminin-5 is expressed in migrating keratinocytes and connected to α3β1 and α6β4 integrins in keratinocytes, thus establishing the contact between wound keratinocytes and the surrounding matrix (Kainulainen et al. 1998).

In an adult skin wound keratinocytes move by lamellodpodial crawling. The contraction of actinomyosin filaments creates the required mechanical force (Vasioukhin et al. 2000). The filaments are attached to the adhesional complex, so that the junction grasps and holds on while the filament contracts (Welch et al. 1997). In the embryo, the mechanisms differ, and epithelial defects are sealed by the contractile purse string mechanism (Brock et al. 1996). Actinomyosin cables assemble at the leading edge of the healing epithelium. Adherens junctions connect the cables, forming a line at the edge of the wound and the contraction of the line closes the defect. In an adult wound, the purse string mechanism is considered to have only a supportive role affecting the evenness of the advancing epithelial edge. It is still possible that considerable overlap exists, as, for example, small mucosal defects in gut are closed by this mechanism (Bement et al. 1993, Jacinto et al. 2001).

The contractile forces from extracellular signals to intracellular events are mediated by Rho guanosine triphosphatase (GTPase) signal proteins. The most widely studied signal protein in epithelization is Rac, which mediates lamellodpodial contraction in a tissue-culture epithelium, Rho, which operates in embryonal wound healing and makes the migrating epidermal sheet even, and Cdc42, which directs the lamellodpodial contraction (Fenteany et al. 2000, Nobes & Hall 1999, Tapon & Hall 1997).

The initiative signal for epidermal closure is presumed to be the exposure of keratinocytes to growth factors released by de-granulating platelets and mechanical factors related to trauma. Keratinocytes are stretched and damaged at the time of wounding, which makes keratinocytes leaky for small ions, such as Ca²⁺, that might act as a transcriptional factor for cells. Also, the release of contact inhibition may activate the wound edge keratinocytes (Woolley & Martin 2000). When activated, the wound area cells secrete many cytokines, which are mitogenic and motion-promoting for keratinocytes. The most essential growth factors for re-epithelization are epidermal growth factor (EGF), transforming growth factor α (TGF α) and keratinocyte growth factor (KGF) (Stoscheck et al. 1992, Werner et al. 1992, Werner & Grose 2003).

Once the inhibition is released, keratinocytes start their migration to cover the wound. Some hours after the start of migration, the epidermal cells just behind the wound margin undergo a proliferative burst that provides an extra pool of cells to replace the cells lost upon injury. Continuous proliferation of keratinocytes takes place near the original wound edge. There is some controversy about the leading cells in the migrating sheet. Studies using virally tagged cells in an organotypic model of wound healing have shown that the hypothesis of a coherent epidermal sheet being dragged forward with the
migrating edge is not accurate, but that there is a lot of interchange between the first few rows of migrating epidermal cells. The mobile integrin profile extends more than 10 cell rows back from the wound edge (Garlick & Taichman 1994). It has also been shown that blocking of cell motility in the first row of migrating cells does not prevent repair (Coulombe 2003, Hertle et al. 1992).

Migrating epidermal cells must make their way through the provisional matrix and the chaotic connective tissue of the wound base to reconstitute the barrier. The chief fibrinolytic enzyme cutting the route through the fibrin clot is plasmin. Plasmin is derived from the plasminogen of the clot. The plasminogen is activated by plasminogen activator, which is secreted from the activated keratinocytes of the wound edge (Grondahl-Hansen et al. 1988, Romer et al 1994). This is considered essential for healing because, if the plasminogen activator gene is knocked out in mice, migration is blocked (Romer et al. 1996). Keratinocytes are also a resource for matrix metalloproteinases (MMP) in acute wound healing (Ravanti & Kähäri 2000). MMP-9 (gelatinase type B) and MMP-2 (gelatinase A) cleave type IV collagen in the basal lamina and type VII collagen in the anchoring fibrils, and they thus liberate keratinocytes from their bonds to the basal lamina (Salo et al. 1994). MMP-1 (interstitial collagenase) is required for the migration of keratinocytes, and it cuts the way through type I and III collagens (Saarialho-Kere et al. 1995). Stromelysins-1 and –2 (MMP-3 and –10) have wider substrate specificity, and they can also degrade fibronectin, laminin and glycosaminoglycans (Saarialho-Kere et al. 1994). MMP-19 has been found in fibroblasts and capillary endothelial cells of dermal wounds, where it has a possible role in angiogenesis (Hieta et al. 2003). MMP-28 has been recently found in keratinocytes distal to the wound edge. The in vivo substrates of MMP-28 are not yet known, but it is possibly needed in the remodelling of the basement membrane or to degrade the adhesive proteins between keratinocytes (Saarialho-Kere et al. 2002) (Fig. 5).

The stop signal for epithelization is not exactly known, but there are some postulations. The phenomenon called “contact inhibition” (Abercrombie et al. 1954) is employed to stop epithelial migration when the epithelial sheets meet. Studies on dorsal closure in Drosophila and epidermal cell cultures have demonstrated the rapid formation of adherens junctions when two epidermal sheets meet (Williams-Masson et al. 1997). It is possible that the formation of intercellular junctions has a role in stopping migration (Adams et al. 1998). Once the wound is covered by a monolayer of keratinocytes, the other keratinocyte layers and the basement membrane are established from the wound margins to the centre (Leivo et al. 2000).
Fig. 5. A cutaneous wound five days after injury. Granulation tissue with early neovascularization is seen. The proteinases needed for cell movement are shown. The arrow, which points to the abbreviated name, starts from the source of the proteinase. The second arrow shows the target area. MMP refers to matrix metalloproteinase, t-PA to tissue-type plasminogen activator and u-PA to urokinase-type plasminogen activator (modified from Singer & Clark 1999).

2.5.3 Granulation tissue and fibroplasia

The new stroma, called granulation tissue, starts to form approximately four days after injury. The name refers to its granular appearance derived from numerous capillaries. The fibrin clot forms the first provisional matrix that creates a conduit for granular tissue formation. The fibronectin present in the clot provides contact guidance for cells, while hyaluronic acid provides low impedance for cell migration (Clark et al. 1982). The activated platelets in the clot release PDGF, which is mitogenic and chemotactic for fibroblasts. The clot acts as a reservoir of cytokines. Monocytes, which are activated into macrophages in the wound area, are an important source of several cytokines, including those chemotactic and mitogenic for fibroblasts. By secreting FGF, TFGβ, VEGF and TGFα, macrophages orchestrate the formation of granulation tissue (Singer & Clark 1999).

PDGF, TFGβ and extracellular matrix and its fragmentation components (e.g. fibrin, fibronectin, vitronectin and their parts) stimulate the fibroblasts around the wound to proliferate and to invade the wound (McClain et al. 1996). The new integrin expression on the fibroblasts facilitates cell migration (Xu & Clark 1996). The fibroblasts move as keratinocytes by lamellipodial crawling towards the chemotactic stimulus. The new
adhesions are formed in extrusion while the opposite pole is released. The contraction of intracellular actinomyein filaments moves the cell. Fibroblasts get their direction from the chemotoatic gradient, from the free edge effect and from extracellular matrix fibrils (McCarthy et al. 1996).

Keratinocytes have been known since the early 90’s to produce cytokines, which have a paracrine effect on the nearby epidermal cells. Lately, keratinocytes in the injured area have also been found to secrete cytokines and chemokines, which have no effect on the epithelial but only on the mesenchymal cells, such as fibroblasts. For example, the PDGF secreted by keratinocytes promotes fibroblast proliferation and migration, and the vascular endothelial growth factor (VEGF) stimulates angiogenesis and hyperpermeability directly but also indirectly via PDGF released from fibroblasts (Ansel et al. 1993, Arkonac et al. 1998, Brown et al. 1992). Lately, keratinocytes have been found to produce interferon gamma inducible protein, which abrogates fibroblasts’ responsiveness to growth factors (Satish et al. 2003).

Movement of cells into the fibrin clot or the collagenous matrix necessitates proteolysis. Fibroblasts secrete plasminogen activators and matrix metalloproteinases (interstitial collagenases, gelatinases and stromelysins) (Rechardt et al. 2000, Romer et al. 1994, Vaalamo et al. 1997). These enzymes lay open the path for fibroblast into the clot. Fibronectin provides a conduit for fibroblast migration into the fibrin clot (Greiling & Clark 1997, Mignatti et al. 1996).

Once in the clot, fibroblasts switch their major function from proliferation and migration to protein synthesis. TFGß is most likely to be responsible for this change (Clark et al. 1995). Fibroblasts start to synthesize collagens (predominately type I and III) and elastins. The provisional extracellular matrix is then replaced by collagenous matrix. The PDGF isoform AB has been shown to increase type I and type III collagen gene expression, while PDGF-BB has been mitogenic to fibroblasts in wound healing (Lepistö et al. 1996). When an adequate amount of collagen has been produced, the synthesis is down-regulated and ceases. The mechanism is not entirely known, but gamma interferon may be one factor (Laato et al. 2001). It is also known that collagenous matrix decreases collagen synthesis in fibroblasts, while fibrin matrix increases it (Xu et Clark 1996). Finally, fibroblasts disappear via apoptosis, and a scar, which is relatively acellular, develops (Desmouliere et al. 1995).

2.5.4 Neovascularization

New blood vessels are produced because granulation tissue needs oxygen and nutrients. Angiogenesis is a complex process, which includes interactions with the extracellular matrix and endothelial cells and migratory and mitogenic stimulation of endothelial cells (Madri et al. 1996, Martin 1997, Singer & Clark 1999, Tonnesen et al. 2000). Shortly after tissue injury, the wound scene is devoid of oxygen, which is a strong angiogenic and wound healing promoting stimulus. Aim of the neovascularization is to provide adequate tissue oxygenation for reparative phases of healing. In contrast to beneficial acute hypoxia, chronic hypoxia decreases all processes in healing (Tandara et al. 2004).
The initiation of angiogenesis is attributed to FGF, TFGß and VEGF released from cells of the injured area (Cao et al. 1996, Frank et al. 1995). The main sources of cytokines are activated macrophages, keratinocytes and endothelial cells. There are also numerous other angiogenous factors, including angiopoietins, angiogenins, angiotropins, low oxygen tension and elevated lactic acid level (Risau 1997). Nitric oxide is an important mediator of inflammation and angiogenesis, and decreased levels of it have been associated to impaired healing in diabetes and in malnutrition (Rizk et al. 2004).

Angiogenic factors stimulate endothelial cells to release plasminogen activators and matrix metalloproteinases, which degrade the basement membrane beneath endothelial cells (Fisher et al. 1994). Endothelial cells move towards the chemotactic gradient through the basement membrane and form capillary sprouts. The endothelial cells are surrounded by fibronectin that acts as a conduit sheet for endothelial cells. New capillaries grow, and one sprout finally connects with another, forming a capillary loop where blood flow is initiated. When the granulation tissue is replaced by collagenous matrix, and ultimately, by a scar, the need for oxygen diminishes. Angiogenesis ceases, and many of the newly formed capillaries disintegrate as a result of apoptosis. The process is relatively slow, and the time perspective for the whitening of scars is years rather than months (Ilan et al. 1998, Iruela-Arispe et al. 1997, Pintucci et al. 1996).

2.5.5 Wound contraction and extracellular matrix remodelling

After the accumulation of suitable extracellular matrix components, predominantly fibronectin and type I and III collagen, the wound undergoes contraction. This is clinically important, especially for the closure of a wound with a large tissue defect. Wound contraction involves complex interactions between different cells in the wound area and the extracellular matrix. The key phenomenon in the contraction is phenotypical transformation of fibroblasts into myofibroblasts. Myofibroblasts contain large bundles of actin microfilaments, which give them an ability to contract (Desmouliere et al. 1996, Welch et al. 1990). Differentiation starts on the second week of healing and, as a result, myofibroblasts will be the most abundant cell type of granulation tissue. Myofibroblast differentiation is triggered by certain isoforms of PDGF. PDGF isoforms capable of this are found in platelets and in activated macrophages of the wound. The macrophages of a one-week-old wound secrete an efficient PDGF isoform, which is possibly the essential one (Clark et al. 1989). There is also evidence that TGFß participates in contraction (Montesano & Orci 1988).

Myofibroblasts are organized on the lines of wound contraction. The condensation of the collagen bundle is executed by myofibroblasts, which collect bundles using extension and retraction of pseudopodia attached to collagen fibres. Actinomyosin filaments generate the contractile force. Transmission of traction forces is mediated by integrin attachments between collagen and fibroblasts, by covalent cross-links between individual collagen bundles and by adherens junctions between individual cells (Desmouliere & Gabbiani 1996, Schiro 1991 et al., Woodley et al. 1991).
The remodelling of a wound may take years after wounding. Collagen synthesis and catabolism continue throughout the healing process, and collagen bundles become more ordered over time, and the proportions of type I and III collagens approach the values recorded in normal skin (Singer & Clark 1999). Collagen metabolism is controlled by matrix metalloproteinases, inhibitors of metalloproteinases and the level of collagen synthesis. This requires interactions between cells and the extracellular matrix and is mediated by a complex mixture of cytokines and cytokine receptors (Madlener et al. 1998, Ravanti & Kähäri 2000).

The scar gains tensile strength reasonably slowly: after three weeks, the wound has gained 20% of its final tensile strength. The formation of larger collagen bundles and new cross-links increases tensile strength for months, but the outcome remains incomplete: the final tensile strength is 70% of that of normal skin (Levenson et al. 1965).

2.5.6 Fetal wound healing

Since the scarless repair of fetal wounds was reported two years ago, there have been intensive research efforts to solve the mechanism of fetal healing (Rowlatt 1979). Also, since then, there has been a renewed hope that it would be possible also to attain scarless healing of adult wounds.

The mechanisms of fetal wound repair differ from those of adult healing: re-epithelization, inflammation, connective tissue synthesis and tissue remodelling are different. Initially, it was hypothesized that the conditions (the fetus is bathed in steril, growth factor-rich fluid) in utero contributed to optimal repair. Later studies have revealed, however, that intrauterine conditions are not necessary for scarless repair, but that fetal tissue has this intrinsic property (Armstrong & Ferguson 1995, Lorenz et al. 1992).

Wounded fetal epidermis re-epithelialized by the contractile purse-string mechanism in contrast to the adult lamellipodial crawling of cells. The cells at the leading edge are connected via adherens junctions, which connect to the actonomycin cables in the cells. The defect is closed when the cables around the defect contract (Jacinto et al. 2001). The purse-string mechanism is not fully restricted to embryonic tissues: corneal and gut mucosal wounds close, at least partially, by the same mechanism (Heath 1996, Danjo & Gipson 1998).

The collagen fibers in healing fetal dermis exhibit fine reticular patterns similar to the surrounding dermis. Fetal fibroblasts synthesize more total collagen and a larger proportion of type III collagen from total collagen than adult ones (Bullard et al. 2003). Fetal fibroblasts secrete an increased amount of hyaluronic acid (a lubricating and space-occupying constituent of the provisional matrix) and have more hyaluronic receptors (Chen et al. 1989). In addition to synthesis, degradation of the matrix is also needed for wound repair. The gene expression of matrix metalloproteinases (which degrade collagens) has been found to be increased in scarless fetal wounds (Lorenz et al. 2001). In the recent study, fetal skin fibroblasts expressed collagenase-3 (MMP-13) in contrast to adult fibroblasts. Collagenase-3 is a proteinase with a wide substrate specificity and
different expression may contribute to the scarless repair in fetal skin (Ravanti et al. 2001).

Growth factors govern wound healing. A number of differences have been found between the processes of fetal and adult wound healing. The expression of TGFß isoforms is different in scarless and scarring wound healing. TGFß1 and TGFß2, which are scar-promoting cytokines, have been found to have diminished activity in scarless wound repair, while TGFß3, which may reduce scar formation, has increased activity in scarless repair (Soo et al. 2003).

### 2.6 Suction blister method in studying wound healing

The suction blister method was developed to separate the epidermis from the dermis by U.Kiistala 1968 (Kiistala 1968). Over the decades, new applications of this method have enabled assessment of collagen synthesis in human skin *in vivo*, determination of pharmacological agents and cytokines in skin and measurement of the healing rate of the suction blister wound (Ihlberg et al. 1993, Oikarinen et al. 1992, Svedman et al. 1991a, Svedman et al. 1991b).

To produce blisters, a suction cup with holes is placed on the skin. After that, 150-250 mmHg negative pressure inside the cup is created by a pump. Hole size can vary from 3 to 10 mm. The total diameter of the cup can vary from 20 to 50 mm. The suction results in the separation of the epidermis from the dermis at the level of lamina lucida in the basement membrane. The basement membrane forms the blister floor and the epidermis forms the blister roof. The blister cavity is filled with tissue fluid. The blister fluid represents interstitial fluid, although a small portion of proteins is diffused from serum (Kiistala 1968, Vermeer et al. 1979).

The suction blister method can be used to study two main topics of wound healing: re-epithelization and inflammatory response. It has the advantage of being a standard and non-invasive technique (Eaglestein & Mertz 1978, Levy et al. 1995, Svedman et al. 1991a, Svedman et al. 1991b).

The level of separation of the epidermis from the dermis occurs above the layer of type IV collagen, since all of this collagen remains in the blister floor after blistering. In contrast, some laminins, such as laminin 5, can be found variably in the blister floor and in the basal cells of the detached epidermis, indicating that the separation occurs below the basal cells above the lamina densa layer of BMZ (Oikarinen et al. 1982, Kainulainen et al. 1998, Leivo et al. 2000).

Epidermal cell proliferation begins at the edge of the wound and at the dermal appendages. A few hours after the onset of proliferation, cells start to migrate on the partially intact basement membrane (Fig. 6). Simultaneously, the synthesis of basement membrane components occurs (Leivo et al. 2000). The changes in the cytoskeleton and the cell-to-cell and cell-to-matrix connections enable this migration. The process is controlled via cytokines originating from epidermal cells, platelets, fibroblasts and wound leukocytes (Jacinto et al. 2001, Martin 1997).
Epithelization may be followed up by taking skin samples from the healing blisters and by studying the samples with appropriate techniques. Skin samples can be taken at any phase of healing until the healing is complete. The whole healing blister is biopsied under local anaesthesia (Leivo et al. 2000, Oikarinen et al. 1982).

Re-epithelialization can be followed up non-invasively by measuring water evaporation from the blister area. Epidermis, as any other epithelium, forms a barrier towards the environment, which limits water passage. The epidermal barrier function can be measured by studying water evaporation on the skin (Oestman et al 1993). Initially, after blister induction (when there is no epidermis), the rate of water evaporation from the blister wound is 15- to 20-fold compared to basic evaporation from intact skin. When epidermal healing proceeds, water evaporation decreases. This enables non-invasive follow-up of wound healing by measuring the decrease in water evaporation as a function of time, i.e. the restoration of epidermal barrier function (Levy et al. 1995, Nilsson 1997, Svedman et al. 1991a, Svedman et al. 1991b).

Fig. 6. The healing of a suction blister wound. Keratinocytes proliferate at the edge of the wound and then migrate as a sheet to cover the wound bed. Normal epidermis, the hyperproliferative zone of keratinocytes and the migrating epidermal sheet can be distinguished.
Inflammation is an essential part of the healing process. The features of inflammation are increased blood flow in the affected area, leukocyte recruitment and initiation of regenerative processes. Inflammation is controlled via neuropeptides (e.g. substance p and calcitonin gene-related peptide), prostaglandins, histamine and various cytokines (Clark 1996, Schaffer et al. 1998). The increased blood flow in the blister wound is due to vasodilatation. The local inflammation is difficult to measure solely based on levels of secreted substances, since the phenomenon itself is complex, and substances have overlapping functions. In the suction blister model, the level of blood flow and, hence, the level of inflammation can be measured using a laser-Doppler flowmeter (Periflux Pfl, Perimed KB, Stockholm, Sweden) (Oberg et al. 1984).

2.7 Wound healing in diabetes mellitus

Patients with diabetes mellitus have an increased risk of foot ulcers in complicated disease. The healing disorder is a feature of both adult onset and juvenile diabetes. In the literature, both types have mostly been discussed together, because the main features of the deficits are similar. Both types of diabetic experimental animals have shown impaired wound healing (Falanga 1993, Greenhalgh 2003a, Greenhalgh 2003b, Silhi et al. 1998).

The wound healing disorder in diabetes mellitus is multifactorial. The most important predisposing factors for faulty healing are abnormal inflammatory pathways, peripheral neuropathy and vascular disease (LoGerfo et al. 1984, Pecoraro et al. 1991). This is most obvious in the foot (Jeffcoate & Harding 2003). Many functional abnormalities, such as defects in granulocyte functions and chemotaxis, reduced collagen synthesis, pathology in the regulation of inflammation, microvascular dysfunction, reduced capillary neovascularization and impaired keratinocyte functions have been found in patients with diabetes (Fahey et al. 1991, Schaffer et al. 1997, Witte et al. 2002). It appears that insulin administration and good metabolic balance improve functional abnormalities. However, 15% of diabetic patients suffer from non-healing wounds during their lifetime (Sandeman & Shearmann 1999).

The effect of diabetes on surgical wound healing is not as clear as the risk for chronic foot ulcer. One study showed that surgical patients with diabetes had a higher rate of wound complications than patients without diabetes (Casey et al. 1983). In another study, diabetic patients did not have a higher complication rate when they had uncomplicated disease and no other impairing conditions such as obesity (Hjortrup et al. 1983).

2.7.1 Keratinocytes in the wound healing of diabetic patients

Diabetes affects directly keratinocytes, which have been found to secrete diminished amounts of growth factors in diabetic mice and in cell culture (Frank et al. 1995, Mellin et al. 1995, Werner et al. 1994). There has been also diminished activity of keratinocyte-derived inducible nitric oxide in the skin of diabetic mice (Stallmeyer et al. 2002). It has
further been found that glucose impairs keratinocyte proliferation in cell culture (Spravchikov et al. 2001) In humans, it has been found that insulin-like growth factor is absent in basal keratinocytes (Blakytny et al. 2000).

2.7.2 Microvascular dysfunction

Up till the 1980s, it was thought that the diabetic foot ulcers were caused by cutaneous diabetic microangiopathy in analogy to microangiopathy in the retina and the kidneys (Shimomura & Spiro 1987). This was postulated in a retrospective study in 1959, where amputation specimens were examined (Goldenberg et al. 1959). The result was contradicted in several studies where no differences in microvascular occlusive disease were found between patients with diabetes and atherosclerosis, and where there were no differences in the skin oxygen gradient between diabetic and vascular patients (Conrad et al. 1967). On the contrary, diabetic patients appear to have foot ulcers in the presence of high transcutaneous oxygen tension, suggesting that the etiology of their ulcers is other than ischemia (Wyss et al. 1984).

However, even though there is evidence that diabetic ulcers are not principally caused by ischemia, diabetic patients have ischemic ulcers. Diabetic patients are more prone to atherosclerosis than patients without diabetes. Typically, the tibial and peroneal arteries are affected. Pedal vessels are usually spared, which makes bypass surgery possible (Akbari & LoGerfo 1999, Strandness et al. 1964).

Even when there are no occlusive lesions in microvessels, microvascular disease may have a significant role in diabetic foot ulcer. Autonomic neuropathy leads to dilatation of the shunt vessels before the capillaries, thus directing the flow away from oxygenation and nutrition (Arora et al. 1998, Rendell & Bamisedun 1992). This is the reason why the feet of diabetic patients are usually warm (Flynn et al. 1988, Netten et al. 1996). There is thus increased capillary pressure and flow at the early stage of the disease (Candido & Allen 2002, Lefrandt et al. 2003, Tooke & Brash 1996). Later, this phenomenon is thought to lead to vascular sclerosis and to reduce hyperaemic responses. It has been found that the microvascular responses to heating and trauma in the feet of insulin-dependent diabetes mellitus (IDDM) patients are reduced, which is postulated to be partly due to sensory and autonomic neuropathy (Rayman et al. 1986).

There is also evidence that diabetes has a negative impact on hemorrheology (LeDevehat et al. 2001). Patients with diabetes have increased blood viscosity as a result of stiffened red blood cells (Linderkamp et al. 1999 McMillan et al. 1981). Red cells have to undergo deformation in shape in order to pass through capillaries, and their stiffness increases flow resistance (Simpson 1985). The mechanism for that seems to be non-enzymatic glycosylation of the red cell protein spectrin. This process is induced by hyperglycemia and can be reduced by good glycemic control (Resmi et al. 2001, Schwartz et al. 1991).
2.7.3 The diabetic foot

Studies on the aetiology of diabetic foot ulceration have shown that 40% of the ulcerations are neuropathic, 40% neuro-ischemic and only 10% solely ischemic, which means that neuropathy is the most important factor predisposing to foot ulceration (Jeffcoate & Harding 2003 Sandeman & Shearman 1999).

This damage affects all nerve types and mostly starts from sensory nerves. Sensory neuropathy makes the foot vulnerable because small scratches are not noticed. Sensory dysfunction disturbs foot proprioceptors and causes prolonged pressure effects on the foot. The loss of innervation of sweat glands leads to dry skin, which is vulnerable. Motor neuropathy leads to weakness in the intrinsic foot muscles. Unopposed action of the proximal flexors leads to flexion of the metatarsal phalangeal joints and typical dislocation of the foot, in which the metatarsal head is prominent and vulnerable. Autonomic nerve dysfunction leads to alteration in the distribution of skin microcirculation (Vinik et al. 2001). The arteriovenous shunts before the nutritive capillaries are enlarged as a result of neuropathy, and the flow is directed through them instead of the nutritive capillaries (Akbari & LoGerfo 1999 LoGerfo & Coffman 1984 Strandness et al. 1964). The pathophysiology of the microvascular anatomic alteration is still only partially understood. Still, it is not likely that pathologic vascular changes cause skin ischemia, but that the vascular disorder is principally functional. The classical diabetic foot complication is Charcot foot, which is characterised by collapse of joints and bones. Autonomic vascular neuropathy is a possible cause of Charcot foot because arteriolar-venular shunting is considered to lead to osteopenia. Motor and sensor neuropathies contribute to this syndrome (Jeffcoate et al. 2000, Rajbhandari et al. 2002).

The mechanisms leading to neuropathy in diabetes are not fully understood. Several theories exist. The diabetic nerve damage may result from metabolic abnormalities, such as accumulation of sorbitol in the perineural space leading to neural edema, and from disease in endoneural microvessels. Sorbitol is derived from glucose via the aldose reductase pathway. There is also demyelination of Schwann cells in diabetic patients. This may be the reason for the reduced conduct velocity in their nerves. However, these lesions are not found in the early stages of nerve damage. The Swann cell damage is possibly mediated by increased blood glucose. It is further possible that the damage is related to abnormalities in the metabolism of myo-inositol. Myo-inositol is a sugar molecule which is bound to the plasma membranes of neural cells. In diabetic patients, decreased levels of myo-inositol have been found (Dejgaard 1998, Greene et al. 1999, Vinik et al. 2000).

2.7.4 Inflammation in diabetes

There are several unfavourable alterations in inflammatory processes in diabetes mellitus (Olerud et al. 1995, Wetzler et al. 2000). Since inflammation is a core process in wound healing, defects in the inflammation process are probably important in the healing disorder. Both neutrofil and macrophage functions are abnormal in diabetes (Goren et al.
Adherence to the blood vessel wall, diapedesis, chemotaxis and phagocytosis are all impaired. Leukocytes in patients with diabetes also show impaired deformability due to changes in cell membranes, and leukocytes may be trapped in microvessels causing ischemia. The defects in neutrophil function may cause liability to wound infection (Bagdade et al. 1974). Incomplete macrophage function may disturb matrix remodelling, angiogenesis and epithelization (Loots et al. 1998).

There are a few observations on reduced vascular reactions in patients with diabetes (Bassirat & Khalil 2000, Wilson et al. 1992). The microvascular responses to heating and trauma in the feet of IDDM patients have been reduced (Rayman et al. 1986, Shore et al. 1991, Walmsley et al. 1989). In non-insulin-dependent diabetes mellitus (NIDDM), the microvascular responses to acetylcholine and sodium nitroprusside were found to be reduced in the forearm skin, which suggests pathology in NO-mediated vascular dilatation (Morris et al. 1995).

The healing process is regulated by growth factors. Wounds in diabetic experimental animals have lower levels of PDGF, insulin-like growth factor (IGF), nerve growth factor (NGF) and keratinocyte growth factor (KGF) than wounds in normal animals (Brown et al. 1997, Frank et al. 1995, Matsuda et al. 1998, Werner et al. 1994). It has also been found that topical application of PDGF accelerates, albeit slightly, wound healing in experimental diabetes and in patients with diabetes (Greenhalg et al. 1990, Steed 1995). PDGF has been licensed for clinical use (Wieman et al. 1998). Also, reduced levels of growth factors have been found in the wounds of diabetic patients (Blakytny et al. 2000).

2.7.5 Infection in diabetes

Patients with diabetes have an increased risk for infections. Necrotizing fascitis, wound infection, pneumonia and pyelonephritis and septicemia occur more often in patients with diabetes than in healthy individuals. Diabetic patients are particularly prone to Staphylococcal infections. Infections in diabetic patients are more severe than those in non-diabetic subjects (Pozzilli & Leslie 1994). Infection also often causes a loss of metabolic control, and it has been estimated that some 75% of patients with ketoacidosis have had infection as a trigger (Foster & McGarry 1983, Lipsky 1994).

Diabetes increases in several ways the risk for infection. Diabetes impairs the immune defence, disturbing neutrophil and macrophage functions (Bagdade et al. 1974, Goren et al. 2003, Loots et al. 1998, Wetzler et al. 2000). Elevated blood glucose concentration favours the growth of gram-positive organisms. Ischemia due to vascular disease impairs healing processes and immune functions (Olerud et al. 1995). Finally, sensor and motor neuropathy makes the patient prone to small scratches, where infection may develop (Falanga 1993).
2.7.6 Connective tissue and basement membrane in diabetes

There are various alterations in connective tissue in diabetes (Perez & Kohn 1994). In the scope of wound healing, those related to collagen synthesis are of special interest. Diabetic experimental animals have been found to have reduced tissue strength and collagen accumulation in the wound (Greenhalg 2003b, Schaffer et al. 1997, Witte et al. 2002).

The basement membrane separates the epithelial surfaces from the connective tissue matrix. The basement membrane is composed of type IV collagen, laminin, entactin and heparan sulfate proteoglycans (Uitto et al. 1996). The hallmark of the diabetic microvascular disease is thickening of the basement membrane (Tsilibary 2003). Similar alterations, e.g. accumulation of glycosylation end products and increased amounts of collagen type I, have been found in both the skin and the glomerular basement membrane (Pijl et al. 1998). In the early investigations, thickening of the basement membranes was thought to lead to impaired tissue oxygenation, and the myth of “diabetic small vessel disease”, which caused chronic ulcers in the presence of palpable pedal pulses was established. This led to nihilism in the treatment of diabetic foot ischemia (Falanga 1993). The thick diabetic basement membrane has been shown to actually increase permeability (Shimomura & Spiro 1987). The significance of basement membrane alterations for wound healing in patients with diabetes is not clear.

2.8 Obstructive jaundice

Obstruction of bile flow from the liver causes elevation of the serum bilirubin level. Bilirubin is synthesized in the reticuloendothelial system from biliverdin, which is formed from the heme molecule of dissolute red cells, myoglobin or cytochromes. In hepatocytes, bilirubin is conjugated and then secreted into bile. Bile flows via the biliary ducts into the bowel. From the bowel, part of it passes out from the system in stools and part is recovered from the intestine. The latter process is called enterohepatic circulation. If the passage of bile is disturbed, the level of conjugated bilirubin rises. The obstruction may occur at any level from the bile canaliculus to the sphincter of Odd. The level of conjugated bilirubin also rises in non-obstructive jaundice, such as hepatocellular necrosis and hepatic cirrhosis. Increased unconjugated bilirubin may result from hemolysis or impaired conjugation of bilirubin in Gilbert’s syndrome or some other disease (Fisher et al. 2002, Tso 1995).

Bile contains bile salts, bile pigments, cholesterol, phospholipids and proteins. Bile has numerous important functions. Bile salts facilitate the absorption of lipids in the intestine. Bile flow maintains the enteral barrier function (Tso 1995).

There are several benign and malignant diseases that cause obstructive jaundice. The most frequent benign diseases are common duct stones, chronic pancreatitis in the head of the pancreas, sclerosing cholangitis and postoperative strictures. The most frequent malignant diseases are adenocarcinomas of the pancreas and the periampullary area and cholangiocarcinomas (Yeo et al. 2002, Dalton & Gadacz 2002).
Because biliary obstruction compromises hepatocyte function, these patients are at risk for hepatic failure. The likelihood of hepatic failure increases when the obstruction has lasted for 1-2 months if the jaundice is deep, or if the patient has previous liver disease. Some patients will have permanent hepatic insufficiency after a reasonably short period of obstruction (Fisher et al. 2002).

Biliary obstruction causes hepatic fibrosis. Changes have been observed as early as one day after complete obstruction (Desmouliere et al. 1997). However, it usually takes months for irreversible fibrosis to develop. In cholestasis, fibroblasts within the portal tracts proliferate and differentiate into myofibroblasts and synthesize increased amounts of extracellular matrix proteins (Pasha & Lindor 1996).

Biliary obstruction has systemic adverse effects on the patient. Most of them are considered to result from endotoxemia (Padillo et al. 2002). Endotoxemia is caused by two mechanisms. In the first place, the lack of bile in the intestine disturbs the mucosal barrier function and increases the translocation of endotoxins from the lumen to the body. It is further possible that bacterial translocation causes inflammation in the bowel wall, which increases systemic endotoxins and inflammatory mediators. Secondly, biliary obstruction causes reduction in the liver reticuloendothelial cell function, leading to diminished clearance of endotoxin by Kupffer cells. Endotoxemia (which is mediated via lipopolysaccharides) may cause a systemic inflammatory response (in which the chief mediator is tumor necrosis factor alpha) with adverse effects on immune, renal, haemostatic and reparative functions (Bemelmans et al. 1992, Bemelmans et al. 1996, Kimmings et al. 2000).

2.8.1 Wound healing in obstructive jaundice

The evidence relating to the effect of biliary obstruction on wound healing remains controversial. Jaundice has been associated with an increased incidence of postoperative hernias and wound dehiscence (Armstrong et al. 1984). There have also been observations to show that jaundice has no effect on wound healing in rats, and that conjugated bilirubin has no effect on tissue culture fibroblasts (Greaney et al. 1979, Taube et al. 1988). Prolylhydroxylase activity assay has shown skin collagen synthesis to be decreased in jaundiced patients compared to controls (Grande L et al. 1990). Ligation of the bile duct caused a decrease in wound breaking strength and anastomotic bursting pressure in experimental animals (Cömert M et al. 2000).

2.8.2 Effect of preoperative biliary drainage on healing

The effects of treatment of jaundice on tissue healing are clinically interesting because surgical procedures are often required in the management of jaundiced patient. Surgery of jaundiced patients is considered to involve a risk of septic complications. These are linked with the proinflammatory state resulting from endotoxemia in patients with
jaundice. It has also been proposed that preoperative biliary drainage improves hepatic function and recovery from operations (Kimmings et al. 2000, Pain et al. 1985). Biliary drainage has also been shown to reduce the levels of inflammatory mediators. Later investigations have yielded conflicting results on the effect of biliary drainage on patients’ recovery from surgery (Marcus et al. 1998, Pisters et al. 2001, Povoski et al. 1999).

The recent meta-analysis by Sewnath et al focused on the effect of preoperative biliary drainage on recovery from operations (Sewnath et al. 2002). There was an increased complication rate in patients with preoperative biliary drainage but no differences in mortality. The increased complication rate was explained by complications of the drainage procedure, and it was estimated that if the complication rate of biliary drainage (27 %) could be decreased, biliary drainage would result in fewer complications than the non-drainage practice. The problem of this meta-analysis was the small number of randomised controlled trials. There has been progression in the treatment of these patients over the last few decades, and the studies dating back to the 1970s and 1980s cannot be directly compared to more recent studies. For example, the complication rate of biliary stenting in meta-analysis may be higher than the current rate. As a result of the diversity of knowledge, there are various ways to treat patients with malignant obstructive jaundice. Pisters et al suggest that patients in a good clinical condition should be operated when jaundiced, but that if the operation is delayed, endoscopic stenting before surgery is a preferred option (Pisters et al. 2003).
3 Aims of the study

1. To describe the suction blister model of wound healing.
2. To find out whether there are changes in cytokeratins on re-epithelization.
3. To study the expression of tight junctions on re-epithelization.
4. To study the healing of suction blister wounds in patients with diabetes.
5. To compare microcirculatory responses between patients with diabetes and healthy controls.
6. To find out whether obstructive jaundice and its resolution have an effect on the healing of suction blister wounds.
7. To examine whether the drainage of biliary obstruction has any effect on collagen synthesis and serum collagen propeptide levels.
4 Patients and methods

4.1 Suction blister method (I)

Suction blisters were induced on healthy-looking abdominal skin. To produce blisters, a cup with 250 mmHg negative pressure was applied on the skin for 30 min. After 30 min, the pressure was decreased to 150mmHg for another 15-30 min. The suction area was slightly heated during the induction. Commercially available disposable suction blister devices (Dermovac blistering device, Ventipress, Lappeenranta, Finland) were used. The device contained five holes 6 mm in diameter.

The restoration of the barrier function was followed up by measuring water evaporation from the wound. The measurements were done after induction and during the first week of healing. Water evaporation was measured using Evaporimeter EP1 (Servomed, Stockholm, Sweden). This instrument records air humidity in an open cylinder probe with a 12mm diameter. The probe was placed on the wound, measurements were made from the five wounds induced, and the mean value of these was calculated (Nilsson 1997, Svedman et al 1991a, Svedman et al 1991b).
Blood flow (BF) in the wound area was measured using a laser-Doppler flowmeter (Periflux PF1, Perimed KB, Stockholm, Sweden). The function of the flowmeter is based on the Doppler-shift of the laser light when it is backscattered from moving red cells (Oberg et al. 1984). The equipment has a multifibre probe 5 mm in diameter. The laser beam penetrates about 1 mm into the tissue. The output signal is relative and shows the amount and speed of the blood cells moving in the measured area. BF was measured from the five wounds created, and the mean value was calculated. The probe was in contact with the wound surface, but did not damage it.

Suction blister wound model is described in detail in the original article I.
4.2 Examination of cytokeratins in regenerating epidermis (II)

Seventeen blisters were induced on the fifteen healthy adult volunteers. The blisters were induced on abdominal skin. The healing blister wounds were biopsied at different phases of healing: one biopsy specimen was taken on the first day, fifteen on the fourth or fifth day, and one on the ninth day. Half of each blister specimen was snap-frozen in liquid nitrogen, and the other half was fixed in formalin.

The monoclonal antibodies against the cytokeratins K4, K7, K8, K10, K13, K14, K18, K19 and K20 were used. The monoclonal antibodies keratin-903 (against K1, K5, K10, K14), MAK-6 (against K8, K14, K15, K16, K18, K19) and AE1/AE3 (against K10, K14, K15, K16 and K19) were used as well. The immunohistochemical staining was performed using the avidin-biotin technique with an ABC-HRP kit (Dako A/S, Glostrup, Denmark) according to the manufacturer’s instructions. The paraffin sections were pretreated with pepsin digestion. When using the keratin-903 antibody, the sections were additionally pretreated in a microwave oven. The frozen sections were fixed in acetone.
4.3 Examination of tight junctions in regenerating epidermis (III)

Suction blisters were induced on eight healthy medical students. The blister roofs were removed after blister induction, and the wounds were left bare to heal. The healing process was observed by measuring WE in the blister area. Four biopsies were taken on the fourth day and four on the sixth day of healing.

The following antibodies were used: mouse monoclonal antibodies to human ZO-1 and occludin, polyclonal rabbit antibodies to human ZO-1 and occludin, all from Zymed Laboratories Inc., and mouse monoclonal antibody to human involucrin (Ab-1, MS-126-P0, NeoMarkers, Fremont, CA).

To reveal the tight junction antigens from formalin-fixed and paraffin-embedded sections, immunohistochemical staining was made using the avidin-biotin method and the Histostain-Plus Kit (Zymed Laboratories Inc., San Francisco, CA) according to the protocol supplied by the manufacturer.

Frozen sections of suction blisters were cut on slides for indirect immunofluorescence labelling. The primary antibodies were diluted in 1% BSA-PBS and incubated. Following washes in PBS, the slides were incubated with secondary antibodies. After the incubation, the samples were mounted with Glycergel (Dako, Glostrup, Denmark).

Confocal laser scanning microscopy was carried out using a Leica TCS SP Spectral confocal laser scanning microscope equipped with an air-cooled Argon-Krypton ion-laser system (Leica Microsystems Heidelberg GmbH, Heidelberg, Germany) and Leica TCS NT software (Version 1.6.551 Heidelberg, Germany).

4.4 Patients and methods in studying diabetes (IV, V)

Seventeen patients with type I diabetes (insulin-dependent) participated in the study. The diabetic patients were otherwise healthy, and all were male. Their average age was 35 years (SD: 7.0) and age range 22-45 years. The mean duration of diabetes was 16 years, and the range of duration from 2 to 35 years. Diabetic microangiopathy was evaluated by measuring 12h overnight urinary albumin excretion. An ophthalmologist assessed retinopathy. Patient characteristics are presented in the table (IV table1).

Eleven healthy Caucasian males served as control subjects. Their mean age was 29±8.2 years and age range 22-45 years.

Suction blisters were induced on abdominal skin outside the insulin injection area. The blister roofs were removed after blister induction, and the wounds were left open to heal. The healing was followed by measuring water evaporation and blood flow as described previously.

The examinations were made immediately after blistering, on the second day, on the fourth day and on the eighth day.
4.4.1 Microcirculatory response to irritants in diabetic patients (V)

The microcirculatory responses of diabetic skin were studied using benzoic acid and methyl nicotinate. These substances cause a hyperaemic non-immunologic contact reaction. Prostaglandins and nitric oxide are considered to mediate the reaction (Downard et al 1995, Lahti 1997, Morrow et al 1989).

The patients were divided into two groups according to their disease duration (less or longer than 10 years), because diabetic vascular complications are dependent on the duration of the disease (Akbari & LoGerfo 1999).

The test substances were benzoic acid (BA) and methyl nicotinate (MN) in a mixture of 2-propyl alcohol and 1,2-propylene glycol (75%/25%) as a vehicle. They were applied openly on 1x1 cm areas of the lateral aspects of the upper arms as a dilution series. The BA concentrations were 250, 125, 62, 31, 15 mM and the MN concentrations 25, 5, 1, 0.2 mM. The measurements were done at 20 minutes and at 40 minutes after application. Blood flow was measured using a laser-Doppler flowmeter. The subjects had been asked to refrain from non-steroidal anti-inflammatory drugs for 1 week and to avoid UV radiation for 1 month before the study. The tests and measurements were made in a supine position (Lahti 1997).

4.5 Patients and methods of studying the effects of jaundice and its resolution on wound healing (VI)

Twenty-four patients with clinical jaundice were enrolled in the study group and seventeen anicteric patients with corresponding diseases in the control group to find out whether jaundice itself cause alterations in experimental wound healing or collagen synthesis. The second set of blisters were induced on thirteen patients in the jaundiced group and fourteen control patients, to find out whether the parameters are changed by biliary drainage or as a function of time.

The second experiment included patients who were compliant with prolonged investigations (i.e. lived relatively close to the hospital) because blister induction and follow-up require time and two hospital visits. On the basis of a review of patient charts, there were no marked differences in disease progression between the groups who were studied once or twice.

The healing process was observed by measuring water evaporation and blood flow in the blister area as described above. The first measurements were done immediately after blister induction and the follow-up measurements on the fourth day of healing. The first set of suction blisters were induced when the patient was jaundiced and the second set when the jaundice had been resolved after a drainage procedure. The same study protocol was applied to the control patients.

The study group included 17 patients with pancreatic adenocarcinoma, 5 patients with cholangiocarcinoma, 1 patient with neoplastic pancreatic cyst and 1 patient with periampullary carcinoma. In the control group, 7 patients had pancreatic adenocarcinoma,
4 cholangiocarcinoma, 2 periampullary carcinoma, 2 neoplastic pancreatic cysts, 1 ampuillary polyp with grave dysplasia and 1 pancreatic endocrine carcinoma. Patients with hepatic diseases, other neoplasms, metatstatic disease, corticosteroid medication, or sepsis were excluded. There was one patient with adult onset diabetes mellitus in the study group and two in the control group.

The mean age of the jaundiced patients was 68 years (range 48-89 yrs) and that of the control patients 65 years (range 40-81 yrs). There were 16 male and 7 female patients with jaundice and 8 male and 9 female control patients. Nutritional state of the patients and control persons is presented in the table 1, original article VI.

The patient charts were reviewed when analyzing the results, and one patient was excluded because of metastatic disease and one because of parenchymal liver disease. All of the studied patients are included in the analysis.

The biliary drainage was done by endoscopic biliary stenting or percutaneous transhepatic cannulation and stenting with drainage to the bowel (combined internal and external percutaneous transhepatic cannulation). The drainage was done by endoscopic biliary stenting in 10 cases and by percutaneous transhepatic cannulation in 3 cases. Biliary drainage failed in one case, and this patient was excluded from the analyses of treated jaundice.

The duration of jaundice ranged from 7 to 40 days, and the mean was 18 days. The mean bilirubin level was 225 µmol/L (range 92-800 µmol/L) before drainage and 25 µmol/L (range 4-32 µmol/L) (normal reference interval 2-20 µmol/L) after drainage. Suction blisters were induced on healthy-looking abdominal skin as described earlier.

The median time between the first blisters on the jaundiced patients and the second blisters induced when patients were anicteric was 17 days (mean 29). The biliary drainage was done within four days from the first blister induction. The number of patients at each time point is shown in the figures.

The blister fluids were used for assays of the aminoterminal propeptide of type I procollagen (PINP) and the aminoterminal propeptide of type III procollagen (PIIINP). (method described above) The serum bilirubin, albumin, hemoglobin, alanine amino transferase, thrombotest and creatinine levels of each patient were measured.
Table 1. Summary of patients and methods in the present study.

<table>
<thead>
<tr>
<th>Study</th>
<th>Studied subjects</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratins in regenerating human epidermis (II)</td>
<td>15 healthy adults</td>
<td>Suction blisters, biopsy, immunohistochemistry</td>
</tr>
<tr>
<td>Tight junction proteins in regenerating human epidermis (III)</td>
<td>8 healthy adults</td>
<td>Suction blisters, assessment of evaporation and blood flow on the blister base, biopsy, immunohistochemistry</td>
</tr>
<tr>
<td>Restoration of barrier function in patients with diabetes (IV)</td>
<td>17 patients with juvenile diabetes and 11 healthy controls</td>
<td>Suction blisters, evaporation and blood flow in the blister</td>
</tr>
<tr>
<td>Microvascular reactivity in patients with diabetes (V)</td>
<td>17 patients with juvenile diabetes and 11 healthy controls</td>
<td>Non-immunologic contact reaction, blood flow in the skin</td>
</tr>
<tr>
<td>Effect of jaundice and its resolution on wound re-epithelization and skin collagen synthesis and serum collagen propeptide levels (VI)</td>
<td>24 patients with neoplastic pancreaticobiliary obstruction and 17 patients with corresponding disease without obstruction</td>
<td>Suction blisters, assessment of evaporation and blood flow on the blister base, assessment of collagen propeptides in blister fluid and in serum</td>
</tr>
</tbody>
</table>

4.6 Statistical methods

In the analysis of diabetic patients, the median, percentiles and range in each group were determined to create a box plot presentation of the results. The t-test for independent samples was used at the individual time points in analysing the differences between the means of the healing parameters. The standard deviations are given after the mean values. When assessing the microcirculatory response to irritants, the results were studied using analyses of variances with the least significant difference test at the 0.05 level.

When analyzing jaundiced patients, the paired samples t-test was used when the two time points of an individual patient were compared (e.g. jaundiced patient before and after biliary drainage). The results were presented graphically as line series. The Mann-Whitney U-test was used to compare the patients with jaundice and the control patients, and the results are presented graphically by using a box plot to represent each group’s median, percentiles and range. Analyses were done using the SPSS program version 11.5.

The normality of the distributions was confirmed graphically by using a normal plot.

4.7 Ethical considerations

The studies were approved by the Ethical Committee of the Medical Faculty of the University of Oulu. All patients and control subjects gave their informed consent before participating in the trials.
5 Results

5.1 Expression of cytokeratins in regenerating human epidermis (II)

The healing blister wounds showed no signs of epithelization on the second day. Partial epithelization was seen on the fourth and fifth days and complete epithelization on the eighth day.

No small- and intermediate-size keratins K4, K7 and K8 were present in either the epithelium surrounding the wound or the regenerative epidermis. The large molecular size keratin K10 was present in the suprabasal layer of the surrounding epidermis but not in the regenerating epidermis (II, Fig 1). Keratin 13 was present in all layers of the surrounding and healing epithelium. Keratin 14 was present in the basal but not in the suprabasal layer of the surrounding epidermis and in both layers of regenerative epidermis (II, Fig 2). Low molecular size K18 was not present in the surrounding epidermis, but it was expressed in both layers of the regenerating epidermis. The small keratins K19 and K20 were not present in the surrounding epidermis, nor in the regenerating epidermis (II, Table 2).

Keratin-903, which is an antibody against 1,5,10,14, e.g. large and medium size keratins, caused staining in all layers of both the surrounding and the regenerating epidermis indicating the presence of one or several of those keratins (II, Table 2).

MAK-6 (antibody against 8, 14, 15, 16, 19, e.g. medium and small size keratins) caused staining in the basal layer of the surrounding epidermis and in both layers of the regenerating epidermis suggesting the presence of corresponding keratins (II, Table 2).

AE1/AE3 (antibody against 10, 14, 15, 16, 19, e.g. large, medium and small size keratins) detected its antigens in the basal layer of the surrounding epidermis and in both layers of the regenerating epidermis (II, Table 2).
5.2 Tight junction components in regenerating human epidermis (III)

On the fourth day, the WE values from blister wounds were 9, 25, 18 and 29 g/m²h higher than the normal skin values, and on the sixth day, the WE values were 7, 12, 12 and 13 g/m²h above the baseline, indicating that restoration of the barrier had proceeded but was not yet complete.

Histological analysis showed re-epithelization that covered the wound partially in all samples.

The epidermis in the samples had three distinct areas: the surrounding normal-looking epidermis, the hyperproliferative zone at the wound edge and the regenerating epidermis.

In the normal epidermis, ZO-1 and occludin were present in the granular layer, as mentioned earlier. In the hyperproliferative zone, the expression of ZO-1 and occludin was spread to the spinous layer of keratinocytes. At the leading edge, ZO-1 and occludin were localized in the uppermost layer of keratinocytes (III, Fig.1).

5.3 Blister wound healing in patients with diabetes (IV)

5.3.1 Restoration of epidermal barrier function

Water evaporation from the wound was at a higher level in the patients with diabetes than in the control subjects on the wounding day (116±11 g/m²h vs. 95±13 g/m²h, p < 0.001), on the second day (90±21 g/m²h vs. 60±24 g/m²h, p= 0.002) and on the fourth day of healing (40±17 g/m²h vs. 14±8 g/m²h p< 0.001) (Fig.9). The difference was most prominent on the fourth day, when the WE of diabetic men was 37 % and that of control men 16 % of the value recorded after blister induction (p<0.001). On the eighth -day, water evaporation in both study groups was close to that in normal skin (IV, Fig. 1).
Fig. 9. Water evaporation on the fourth day after wounding. Box plots show range (vertical line), median (horizontal line) and values between the 25th and 75th percentiles (box height). Circles indicate the outlying variables (IV, Fig. 1).

5.3.2 Inflammation in diabetic patients

Initially after blister induction, blood flow was at a lower level in the patients with diabetes than in the controls (93±20 vs.112±18, p=0.02) (Fig.10). On the second and fourth days, there were no differences between the patients and controls. On the eighth day, the flow in the diabetic patients tended to be at a higher level (52±24 vs.78±32, p=0.05) (Fig.11) (IV, Fig. 1).
Fig. 10. Blood flow after blister induction in diabetic patients and control persons. Box plots show range (vertical line), median (horizontal line) and values between the 25th and 75th percentiles (box height). Circles indicate the outlying variables (IV, Fig.1).
5.4 Microcirculatory response of skin in patients with diabetes (V)

The microvascular reactions in diabetic patients were studied using benzoic acid and methyl nicotinate as test substances. The dilution series were used to find also weak and marginal reactions. There were no differences in the maximal hyperaemic responses between the controls and diabetic patients. The diabetic patients had stronger blood flow responses than the control patients with the lowest concentration of test substances: 31 and 15 mM benzoic acid (BA), 1 and 0.2 mM methyl nicotinate (MN). The difference was significant at a 0.05 level in the analysis of variances between the following groups (V, Fig. 2):

![Box plot showing blood flow on the eighth day of healing in diabetic patients and control persons.](image)

Fig. 11. Blood flow on the eighth day of healing in diabetic patients and control persons. The box plot is interpreted as figure 10 (IV, Fig. 1)
5.5 Effect of resolution of neoplastic obstructive jaundice on blister wound healing (VI)

There were no differences in the restoration of the epidermal barrier function in the blister wounds between the jaundiced and control patients (WE 19±11 g/m²h in jaundice vs. 20±6g/m²h in controls, non-significant) or blood flow (157±54 (arbitrary unit) in jaundice vs. 191±61 in controls, non-significant).

There were no changes in the restoration of the epidermal barrier function as a consequence of successful drainage. Water evaporation from the wound on the fourth healing day was 17.4 g/m²h before drainage and 18.8 g/m²h after it (p=0.6, non-significant) (VI, Fig. 1A)

Blood flow tended to increase as a result of biliary drainage, although there was some variability in the results. Blood flow was 151±58 before drainage and 173±55 after it (arbitrary units). The 95% confidence interval for the difference was from –13 to 58 (p=0.188) (VI, Fig. 1B).

5.6 Effect of resolution of neoplastic obstructive jaundice on skin collagen synthesis and serum collagen propeptide levels

The baseline skin collagen synthesis was measured determining levels of collagen propeptides in the suction blister fluid. The first set of blisters was induced when the patients were jaundiced and the second set after its resolution. The comparisons were made between the jaundiced and control patients to find out whether jaundice itself has effect on skin collagen synthesis. Another comparisons were made before and after the resolution of jaundice to find out whether resolution of jaundice has effect on skin collagen synthesis. The serum collagen propeptide levels were measured at same time points with blister induction. Corresponding experiments were done to control patients.

The levels of PINP and PIIINP were lower in the suction blister fluid in the jaundiced patients than in the control patients (PINP: 85 µg/L±91 in jaundice vs. 222 µg/L±227 in controls, p=0.004 Mann-Whitney U-test; PIIINP: 72µg/L±94 in jaundice vs. 184 µg/L±284 in controls, p=0.028 Mann-Whitney U-test) (VI, Fig. 2A and 2B)

There was an increase in the blister fluid levels of PINP as a result of biliary drainage (from mean 82 µg/L to 123 µg/L, p=0.032, t-test for paired samples) (VI, Fig 3A).
The PIIINP values tended to increase as well (from mean 90 to 118 µg/L, p=0.066, t-test for paired samples) (VI, Fig. 3B).

The serum levels of PIIINP decreased towards the normal level as a result of successful drainage (from mean 13.340 to 6.926 µg/L, the normal reference interval being 1.7-4.2 µg/L, and the average decrease was 6.4 µg/L, 95%CI 3.240 to 9.587, p <0.001) (VI, Fig. 4) Serum PINP was unchanged. PINP and PIIINP did not change in the control patients during follow-up.
6 Discussion

6.1 Methodological considerations

The suction blister method was used in two ways to study wound healing: in histological studies of re-epithelization and in physical follow-up based on water evaporation and blood flow in the wound.

The histological method has been commonly used in studies of re-epithelization. It appears to be a reliable way to study re-epithelization on the partially intact basement membrane \textit{in vivo}, and plenty of information has been gained by using it. It is a rather non-invasive and standard wound model. The most invasive part of the study is the biopsy, which leaves a small scar and poses a minor risk for wound infection. The biopsy has to be taken under local anaesthesia. In a single biopsy, it is possible to study keratinocytes at the different phases of healing: normal keratinocytes are found around the wound, hyperproliferative keratinocytes occur at the wound edge, and migrating ones are seen on the wound bed. The wounds can be biopsied at different phases, e.g. on the second or fourth day. Interest in cell kinetics, signalling and morphogenesis is expanding, and the suction blister method is a reliable tool for studying cell migration and proliferation \textit{in vivo} (Leivo \textit{et al.} 2000, Oikarinen \textit{et al.} 1982).

The suction blister itself is non-invasive and painless, does not leave scar and is almost without complications. I have seen one minor infection after blister induction in approximately two hundred patients studied. There are no reports of complications in the literature. Thus, the follow-up of wound healing in non-invasive ways would enable studies of larger groups of patients and would minimise the inconvenience involved in the study. These are the main advantages why others and we decided to use follow-up of water evaporation from the wound and blood flow in the wound (Svedman \textit{et al.} 1991b, Levy \textit{et al.} 1995). The epidermis forms a barrier between the individual and the environment, and one chief function of the barrier is to regulate water passage (Oestmann \textit{et al.} 1993). Water evaporation from an epidermal defect is appr. 10-fold compared to intact skin, and it decreases exponentially when the wound is covered by new epithelium. The observed exponential decrease is predictable based on the circular shape of the wound and the steady rate of the migrating epithelial sheet. The other barrier-restoring
element in suction blister wounds is the fibrin plug. In the wound, the plug is stable during the first week if it is not mechanically removed, and it is therefore most likely that the barrier restoration observed upon decreasing water evaporation is a result of epidermal migration and the formation of tight junctions at the leading edge (Clark 1996, Roop 1995). The measurement of decreasing water evaporation does not provide a direct measure of re-epithelization, but indicates the rate of restoration of the epidermal barrier function. The advantage of evaporation measurement is that it gives a numeric value to the epidermal barrier.

One of the most intriguing biological phenomena is inflammation. The impaired control of inflammation is related to diseases, e.g. inflammatory bowel and joint, skin and bladder diseases, and it is also related to dissemination of malignant neoplasm, (Balkwill & Mantovani 2001) Local inflammation is essential for wound repair, but systemic inflammation, e.g. sepsis and systemic inflammatory response syndrome, are harmful (Kim & Deutschman 2000). The measurement of the level of inflammation is not a straightforward task because the inflammation is created by a number of different cytokines and other mediators, which have overlapping functions and quickly alternating quantities.

The symptoms and signs of systemic inflammation can be recorded as physical parameters, such as body temperature and hemodynamics. A septic patient has hemodynamics characterised by increased cardiac output and low blood pressure. Increased blood flow is another measurable feature of local inflammation, and determination of the blood flow rate gives a numeric value for the inflammation. In the present work, blood flow was measured using a laser Doppler flowmeter, which is based on the Doppler shift of laser light reflected from moving blood cells. The numeric value measures the amount and speed of moving blood cells beneath the probe (Oberg et al. 1984). The value was here used as a parameter of local inflammation.

The suction blister model can also be used in studies of connective tissue metabolism of the skin. Suction blister fluid can be regarded as a sample of interstitial fluid because only a small fraction of proteins is derived directly from serum. The amount of proteins derived from serum decreases as their molecular weight increases (Kiistala 1976). Previously, the method has been used in studies of collagen synthesis in normal skin and after surgical procedures, in skin diseases and after corticosteroid treatments. It has turned out to yield valid data of skin collagen synthesis in vivo (Ihlberg et al. 1993, Oikarinen et al. 1992, Vermeer et al. 1979).

Microvascular responsiveness in patients with diabetes was studied using benzoic acid and methyl nicotinate test substances, which cause non-immunologic contact reactions. The mediator of these reactions has been concluded to be prostaglandin D₂ (PGD₂). Prostaglandins cause vasodilatation via nitric oxide, thus the above mentioned substances can be used to study nitric oxide-mediated microvascular reactivity (Di Rosa et al. 1996, Downard et al 1995).
6.2 Cytokeratins and tight junctions in regenerating epidermis

The observed shift towards lower molecular weight cytokeratins during re-epithelization is most likely to be related to the migrating and proliferating phenotype of keratinocytes. A changed profile of epithelial keratins has been seen previously in inflammatory skin disorders where the turnover of cells is increased and in carcinoma cells, i.e. in the situations characterised by enhanced cell kinetics (Fuchs & Cleveland 1998, Leigh et al. 1995). Keratins are intermediate-sized filaments of cytoskeleton, which are connected to desmosomes. Desmosomes are strong cell junctions considered to give mechanical strength to epithelium (Garrod et al. 1996). The keratin filament-desmosome network is the structural scaffolding of epithelial sheets. The keratin scaffolding is also connected to hemidesmosomes, which mediate the contact between the epithelium and the basement membrane. Mutations in keratin genes cause blistering skin diseases (Geiger & Karsenti 1997).

The rigid connections between the frameworks of cells must be released to enable cell movement in wound repair. Desmosomes have actually been shown to be dynamic structures allowing cell movements during development and healing (Jacinto et al. 2001). Due to this fact that and the fact that activated keratinocytes do not undergo normal terminal differentiation, it is predictable that the cytokeratin profile of wound keratinocytes is changed.

In this and some other studies, it has been suggested that wound keratinocytes express K16 (Paladini et al. 1996). In the present study, K10 in suprabasal keratinocytes was replaced by K14 and, most likely, by K16. The primitive keratins K13 and K18 appeared in all layers of regenerating epidermis. Replacement of mature keratins by immature ones is related to the immaturity of newly formed epidermis. The effects mediated via changes remain to be resolved in detail, but there is emerging evidence to suggest that keratins have specific functions during wound repair. Transgenic mice overexpressing keratin 16 express features of healing epidermis, e.g. cell hypertrophy and dissociation of desmosomes, and K6 null mice show delay in epithelization. It is thus possible that epithelial proliferation and migration are controlled via activation of certain keratin genes. It has also been shown that the expression of K6 and K16 coincides with the new arrangement of keratins at juxtanuclear locations, probably contributing to migration (Paladini et al. 1996, Wong & Coulombe 2003).

The recent studies have emphasized the importance of tight junctions in the regulation of epidermal permeability. Genetic ablation of the claudin-1 gene, a tight junction protein, in mice causes neonatal death from dehydration. Similarly, mice overexpressing claudin-6 die soon after birth. It has also been shown that fetal epidermis, which lacks a corneal layer, is rich in tight junctions, which are thus most likely responsible for the early barrier (Furuse et al. 2002, Pummi et al 2001, Turksen & Troy 2002). The aim of the present study of tight junctions in regenerating epidermis was to resolve whether there are tight junctions in the migrating epidermal sheet creating an early barrier in the absence of stratum corneum and epidermal lipids. The components of tight junctions were, indeed, present in the two uppermost layers of epidermal cells, resembling the expression of fetal epidermis. As it was found that the barrier was partly restored (water evaporation was decreased but more abundant than normal), and that partial re-
epithelization without stratum corneum was present, it is likely that the restoration seen here is due to the formation of tight junctions in the neoeipidermis.

The unique expression of the tight junction protein in the hyperproliferative zone cannot be interpreted straightforwardly as expression in the migrating epidermal sheet. Some expression was also detected in the spinous layer, and it was thus more widely spread than would have been necessary for a barrier. The hyperproliferative zone is a place where new cells and the epithelial phenotype are created. Over the past years, there has been a lot of research on the regulation of the epithelial phenotype. The process is not yet fully understood, but it is known that signalling pathways are activated by cell contacts. The cadherin-mediated adherens junction formation is postulated to activate the Rho family of small GTPases and thereby to control the differentiation and growth of cells. The Rho small GTPases control the actin microfilaments responsible for cell movement (Tapon & Hall 1997). Cadherin-dependent adhesion is concluded to be a prerequisite for the formation of desmosomes, gap junctions and tight junctions because of the cadherin-mediated cell stabilization and activation of pathways mentioned above. In recent studies, tight junctions have been recognized as active spots of signalling, and it can be postulated that increased expression in the hyperproliferative zone could be connected to signalling (Chou \textit{et al.} 1997, Welch \textit{et al.} 1997).

6.3 Restoration of epidermal barrier and microcirculatory responses in patients with diabetes

The healing of a suction blister wound on healthy-looking abdominal skin of a diabetic patient is free from the most obvious causes of impaired healing in diabetes, e.g. problems related to neuropathic foot and infection. It is also likely that autonomic neuropathy, which is a cause of microvascular dysfunction, does not affect abdominal microvessels to the same degree as foot microvessels. The patients did not have any other diseases, they had good metabolic balance, and they had no nephropathy. However, wound healing was still delayed. The difference between the diabetic patients and the controls was most marked on the fourth day of healing. It is also noteworthy that diabetic patients had lower blood flow after wounding. The immediate inflammation is controlled by neuropeptides, e.g. substance P and calcitonin gene-related peptide and histamine and cytokines (IL-1, IL-6 and TNFa) (Clark 1996, Schaffer \textit{et al.} 1998). This and other results of the impaired microvascular reaction to trauma suggest impaired immediate inflammation in patients with diabetes (Rayman \textit{et al.} 1986). Little is known about the mediators of inflammation in diabetes. In a recent study, the level of endopeptidase (endopeptidases degrade substance P) was increased in diabetic patients (Antezana \textit{et al.} 2002). In another study, a decreased level of inducible nitric oxide synthase was found in patients with diabetes (Stallmeyer \textit{et al.} 2002). It can be concluded that the impaired inflammatory response is related to impairment of the later phases of wound healing.

Several studies have revealed a lack of growth factors in diabetic wounds. Decreased levels of VEGF, PDGF, FGF and KGF mRNA and proteins have been recorded in diabetic experimental animals, and PDGF is used clinically for the treatment of diabetic
foot ulcers (Brown et al. 1997, Frank et al. 1995, Greenhalg et al. 1990, Matsuda et al. 1998, Werner et al. 1994). Defects in the function of macrophages and neutrophils and in the formation of leukocyte infiltrate have been found in diabetes. Several aspects of the wound healing deficit in diabetes can be linked to the impaired function of macrophages and particularly the impaired cytokine production (Greehalgh 2003b). In a recent study, IGF1 was found to be absent from the basal keratinocytes of diabetic patients (Blakytny et al. 2000). In another study of diabetic mice, the proliferation of diabetic epidermis was delayed (Wertheimer et al 2001). It has also been reported that a high glucose concentration inhibits keratinocyte proliferation in cell culture (Spravchikov et al 2001). It is thus suggested that growth factor expression and proliferation are impaired in the keratinocytes of diabetic patients.

Non-immunologic immediate contact reactions (NIICR) were used as a model of microvascular reaction. There were no differences in the maximal hyperaemic responses, but diabetic patients responded to lower concentrations of test substances. The mediators of NIICR are not known in detail, but benzoic acid induces biosynthesis of PGD₂ in the skin. Thus, the reactions are possibly mediated via PGD₂. The effect of PGD₂ is mediated by NO (Downard et al. 1995, DiRosa et al. 1996). Because NO has been found to have a diminished effect on the microvessels of diabetic patients, the microvasculature is not likely to be more sensitive to the vasodilatory effects of PGD₂ (Elliott et al. 1993). A more probable explanation is that either the sensitivity of the cells synthesising PGD₂ to benzoic acid and methyl nicotinate is increased or that the synthesis of PGD₂ in diabetes is enhanced. There is some evidence of increased synthesis of PGD₂ in vas deferens and cardiac microsomes in diabetic rats (Durante et al. 1989, Peredo et al. 1984). The increased levels of prostaglandins are also related to renal vasodilatation and hyperfiltration in diabetes (Harvey et al. 1992).

6.4 Effect of biliary drainage on blister wound healing, local inflammation and local collagen synthesis

The surgery of patients with obstructive jaundiced has been associated with increased postoperative complication, e.g. sepsis syndrome and wound dehiscence. In recent studies, these are concluded to be a consequence of a proinflammatory state resulting from portal and systemic endotoxemia. Endotoxemia is due to disturbed bowel barrier function, which causes an absence of bile in the bowel, or to a disturbed ability of Kupffer cells to clear endotoxins from blood (Kimmings et al. 2000). In experimental studies, obstructive jaundice has been associated with decreased wound and anastomotic bursting pressure (Arnaud et al. 1981, Cömert et al. 2000). Over the recent years, there has been some controversy as to the usefulness of preoperative biliary drainage, since contrary to earlier evidence, biliary drainage has recently been associated with increased postoperative septic complications and death (Sewnath et al. 2002). Preoperative biliary drainage is still the most frequently used policy, since the waiting time for the operation is usually several weeks, and a long period of jaundice would cause inevitable hepatic and renal problems. At present, the widely adopted practice is to relieve the jaundice
preoperatively if there is no possibility to operate the patient within a couple of weeks. (Pisters et al. 2003)

In the present study, there were no changes in the rate of restoration of the epidermal barrier function as a result of biliary drainage. The barrier was restored at the same rate as in the control patients. This indicates that obstructive jaundice does not disturb epidermal cell proliferation and migration or the formation of tight junctions in the skin. There was a trend towards an initially weaker inflammatory response in jaundiced patients, which could be related to the suppressive effect of systemic inflammation on local inflammation.

The collagen synthesis was decreased in jaundiced patients, which is in accordance with the earlier finding (Grande et al. 1990). The inter-individual variation in skin collagen propeptides was large. The current and previous studies have revealed that the baseline collagen synthesis between individuals varies, but that within one individual, the levels are reasonable steady (Autio et al. 1994). Synthesis was slightly improved by biliary drainage. It is possible that systemic endotoxemia causes decreased cell protein synthesis. It is also possible that bilirubin or bile acids have direct toxic effects on skin fibroblasts. The interval between drainage and the second measurement was short, which is a probable reason for this slight improvement.

The results suggest that cell proliferation and migration, which play a major role in human homeostasis, are unaffected by a few weeks of jaundice. Collagen synthesis is decreased by obstructive jaundice.

### 6.5 Effect of resolution of jaundice on serum collagen propeptides

The levels of type I collagen propeptides were unaffected by the treatment of jaundice. Most PINP derives from bone, where collagen synthesis is apparently unaffected by treatment of jaundice (Risteli et al. 1995). The PIIINP levels began to normalise after biliary drainage. Increased PIIINP in serum may result from decreased uptake of PIIINP in the endothelial cells of the liver or the increased production of PIIINP as a result of fibrosis (Risteli & Risteli 1995). It is quite possible that the clearance of PIIINP in the liver is impaired because of obstruction. However, if this had been the sole reason, it would have been predictable that PINP values would also have changed, because propeptides are cleared via the same receptor type. Increased amounts of type III collagen mRNA have been seen soon after bile duct ligation in experimental animals (Desmouliere et al. 1997). For this reason, it is possible that increased PIIINP mirrors early hepatic fibrosis.
7 Summary and conclusions

1. The suction blister wound healing model can be used to study the basic biology of wound healing and healing disorders.
2. Cytokeratins in regenerative epidermis differ from normal epidermis. In the suprabasal layer, K10 is replaced by K14 and, most likely, by K16. K18, which is not present in normal epidermis, but is present in regenerative epidermis. The alterations mirror differentiation and may enable motility.
3. The tight junction proteins ZO-1 and occludin are present in regenerating epidermis. Tight junctions may create a barrier in the newly formed epidermis.
4. The restoration of epidermal barrier function is delayed in patients with diabetes, which is most likely due to a slower re-epithelization rate.
5. Microvascular reactivity of diabetic skin to non-immunological contact irritants is increased.
6. Obstructive jaundice does not delay blister wound healing. The resolution of jaundice appeared not to have an effect on healing when examined with the present method.
7. Skin collagen synthesis was decreased in obstructively jaundiced patients. Biliary drainage slightly improved the synthesis. Jaundiced patients had higher than normal serum PIIINP levels. Biliary drainage lowered serum PIIINP values. An increase in PIIINP can be related to increased production of type III collagen in liver fibrosis.
References


