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From wound to scar

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Cutaneous wound repair is a complex, multistep physiological, biochemical, cellular and molecular process still not fully understood. The complexity of the biological mechanisms leading to the healing of a cutaneous wound are related to its pathogenesis, the extent of damage, the physical and functional characteristic of the involved structures and the response of the host to the local aggression.

Skin, the largest organ of the body, is also a very complex multistructural and multifunctional organ with substantial regional variations. It is composed of two distinct layers of different embryonal origin, the ectodermal avascular epidermis and the vascularized mesodermal dermis, lying over a fibroadipose connective tissue which in turn attaches the skin to the underlying musculoskeletal system. Separating, but at the same time binding the epidermis and dermis, there is a zone of attachment referred to as the basement membrane or epidermal dermal junction. This biological complex zone (basal membrane zone) is composed of both ectodermal and mesodermal elements forming a specialized bridge that integrates epidermis and dermis; it contains at least three major matrix molecules (laminin, type IV collagen and bullous pemphigoid antigen) and provides a functional supporting structure for cellular and extracellular activities¹.

Therefore, the restoration of skin continuity after injury would include ectodermal as well as mesodermal repairing processes involving epithelial resurfacing, synthesis of connective tissue and active centripetal movements aimed at diminishing the tissue's gap.

Vertical cutaneous injuries, such as the surgical incisions which have a minimal loss of tissue, will essentially heal through the formation of a blood clot, rapid epithelialization and fibroblast proliferation. In cutaneous wounds with a predominant horizontal loss of tissue such as ulcerations, lacerations or burns, the healing will proceed through a variety of mechanisms according to the extent and level of the involved structures.

Lesions involving epidermis and superficial dermis, such as donor sites or superficial partial-thickness burn, heal by the migration of epithelial cells from the edge of the wound and from intact skin appendages. The scarring will be minimal or absent, especially in areas where skin appendages are numerous as in the scalp. A critical level of damage is the deep partial-thickness lesion in which

epidermal-dermal healing takes between 2 and 6 weeks and frequently results in abnormal scarring.

In full-thickness skin loss where all epithelial sources are destroyed, the re-epithelialization occurs only from the edges. Obviously, if the extent of the burn is too large it may take months to resurface or it may be impossible; therefore, the use of skin grafts or skin equivalents is mandatory. The cutaneous repair after burning is essentially similar to the healing of other traumatic lesions, but has some particular differences.

The ischaemic, hypoxic and highly oedematous burn wound follows a slow course in comparison to other types of wounds, mainly because of the severe oedema, large amount of necrotic tissue, progressive secondary damage, the large 'lag phase', the delayed inflammatory reaction, delayed epithelial migration and intercellular communications, and the possible interference with the normal sequence of specific mediators. The unique characteristics of the burn injury require a thorough knowledge of normal wound healing in order to exert an adequate control of the healing process, minimize complications, and to achieve the best possible therapeutic results.

The immediate first response to skin injury is to control the bleeding. Platelets play the central role in this period. The initial neurohumoral and immune response is followed by release of mediators by macrophages within the wound which are the central component of the inflammatory phase of tissue repair and are essential for normal wound healing. With the active intervention of the host environment, cellular and extracellular elements will converge to achieve epidermal and mesenchymal closure of the defect^{2,3}. The continuous, intermixed sequence of cellular, extracellular and biomolecular events, within the frame of the commonly designated phases of inflammation, proliferation and reparation, is followed by a lengthy period of remodelling or maturation, which may last several months or years⁴. The final results may be a normal or abnormal scarring such as hypertrophic, hypotrophic or atrophic healing.

The haemostatic process

As stated above, the immediate first response is to control the damage produced to the vascular system. The haemostatic process involves blood vessels, platelets and blood coagulation⁵. These three mechanisms, vasoconstriction, the platelet haemostatic plug formation and blood coagulation, are essential for normal haemostasis and they are

interrelated⁶. The sequence of events in the haemostatic process may be divided into the following steps:

1. *Contact phase* – A break in the continuity of a blood vessel exposes subendothelial elements such as collagen and laminin. Platelet adhesion to these subendothelial elements is facilitated by the bridging action of von Willebrand multimers, which attach to the activated receptor glycoprotein-1b-IX on the platelet surface. The activated platelets produce thromboxan A2 which causes vasoconstriction and platelet aggregation. Released ADP also modulates platelet aggregation. At the same time vessel wall damage leads to the expression of tissue factor on the surface of the affected cells which form a complex with factor VIIa.
2. *General activation* – While aggregating platelets continue to form the platelet plug, the tissue factor (factor VII), a complex, as well as the intrinsic pathway, activate factors IX and X, which is a key step in the haemostatic process. Factor X-a bonds to its specific receptor V-a on activated platelets at the wound site. This action serves to focus haemostasis at the specific required site.
3. *Stabilization* – Thrombin (factor IIa) binds to its specific receptor to convert fibrinogen to fibrin which is, in turn, cross-linked and stabilized by factor XII-a. Endothelial cells somewhat removed from the wound site release prostacyclin which blocks further platelet aggregation and also antagonizes thromboxane induced vasoconstriction. A stabilized haemostatic plug has now sealed off the wound site.
4. *Remodelation* – Once a stable plug has formed, the vessel must be remodelled and the clot removed to restore vessel patency. Plasminogen, a plasma protein, binds to fibrin. Tissue plasminogen activator from endothelial cells and other cells adjacent to the process also bind to fibrin, and this ternary complex leads to the activation of plasmin. Plasmin then lyses the clot and generates fibrin degradation products. Fibrinolysis is modulated by two mechanisms: plasminogen activator inhibitor-1 inhibits tissue plasminogen activator and alpha-2-antiplasmin inhibits fluid-phase plasmin.

Platelets not only have a central role in primary haemostasis but also trigger the healing process by releasing a variety of local and circulating biological factors. Platelet has two major forms of granules, the alpha granules and the electron-dense granules, and also contains lysosomes⁵.

Alpha granules contain platelet-specific proteins such as beta-thromboglobulin, thrombospondin, bactericidal factor, chemotactic factor and platelet basic protein, as well as some proteins which normally circulate in plasma at relatively high concentrations, such as fibrinogen, fibronectin, albumin, factor V, factor VIII related antigen (von Willebrand) and protease inhibitors. They also contain growth factors (PDGF, PFA, TGF, FGF). The electron-dense granules contain a pool of nucleotides, ionized calcium, serotonin and other catecolamines. Lysosomes provide a full complement of enzymes such as proteases, hydrolases and cathepsins. The liberated ADP will recruit additional platelets while fibrinogen, fibronectin and thrombospondin act as ligands for platelet aggregation. von Willebrand factor facilitates the adhesion to fibrillar collagen and, with the release of several growth factors, such as TGF alpha and beta and PDGF, will perform important activities. Transforming growth factor (alpha and beta) stimulates fibroblasts, facilitates epithelialization

and facilitates neovascularization. Transforming growth factor beta serves as a chemotactic factor for macrophages, stimulates the production of other growth factors and retards the synthesis of H₂O₂ to protect fibroblasts. PDGF serves as chemotactic and mitogenic for fibroblasts and facilitates the formation of procollagen and collagen fibrils.

The resting platelet is maintained in its discoidal shape by energy-requiring mechanisms. On stimulation, a number of responses can be recognized, among them four basic phenomena: shape change, adhesion, aggregation and secretion. These may not necessarily occur in the same order⁵.

Activated platelets rapidly change from the circulating discoid form to an irregularly shaped cell with pseudopodial projections. This is achieved by the activity of several contractile and cytoskeletal proteins. The change of shape facilitates the adhesion to various elements of the injured vessel wall.

As we already described, the platelets aggregate, actively participate in the coagulation cascade, and secrete an ample variety of biological molecules which trigger the healing process.

The liberated peptides will provide the signals for chemotactic attraction of neutrophils, monocytes, lymphocytes and mast cells, and also provide the amino acids for the protein synthesis in the reparative process.

Polymorphonuclear leucocytes form the first line of defense against local bacterial contamination and initiate wound debridement by liberating proteolytic, collagenolytic and fibrinolytic enzymes⁷. Within 24–72 h they are gradually superseded by macrophages. However, neutrophils are non-essential to the wound healing process if the wound is not infected⁸.

Lymphocytes are also part of the healing process. Although wound healing can progress in the absence of T-lymphocytes, an intact T-cell system is essential for a normal outcome. Lymphocytes modulate wound healing and may provide regulatory influence over macrophage-induced activities. The lymphocytic infiltration in the healing process is a dynamic event which peaks by the end of the first week after wounding^{9–11}.

Mast cells are integrally involved in the process of dermal wound repair, storing and producing a number of inflammatory mediators¹². Primary mediators are contained within the granules and include histamine, heparin, chemotactic factors and enzymes. Secondary mediators are generated by a complex series of reactions in the mast cell membranes. The generated arachidonic acid will produce prostaglandins and leukotrienes.

Extensive mast cell degranulation occurs within several hours after skin injury. The local release of chymase may lead to the accelerated demise of injured or dying cells. Chemotactic factors attract inflammatory cells and the proliferation of fibroblasts, collagen synthesis and angiogenesis may be encouraged by the release of histamine, heparin or tumour necrosis factor-alpha.

Macrophages are the central component of the inflammatory phase of tissue repair and are essential for normal wound healing^{13,14}. They can tolerate the low oxygen tension at the leading edge of the wound, have a relatively long life span, and appear during the first 5 days. Wound repair suffers dramatically in the absence of monocytes (macrophages).

Macrophages have a central role in a number of activities such as inflammation and fever, lymphocyte

activities, tissue reorganization, tissue damage, and tumoricidal and microbicidal activities. An extensive number of growth factors are released by macrophages, among them chemoattractants for many cells such as PDGF (polymorphonuclear leucocytes, fibroblasts, monocytes, smooth muscle cells), TGF beta (fibroblasts, epithelial cells) and EGF (endothelial cells).

They also release stimulators of angiogenesis (TGF alpha and beta, bFGF, EGF, IL-8); stimulators of collagen synthesis and collagenase secretion (PDGF, TGF beta, TNF alpha, IL-1), keratinocyte activities (TGF alpha, bFGF, EGF, IGF-1, IL-1) and stimulation of granulation tissue (TGF beta, bFGF, EGF, IGF-1, PDGF). The formation of extracellular matrix is enhanced by the release of TGF alpha, EGF, TNF alpha, IL-1 and bFGF.

Also several inhibitors are released which inhibit fibroblast proliferation (TGF beta, TNF alpha, IFN beta and gamma), inhibit epithelial cells (TGF beta) and inhibit collagen synthesis (EGF, IFN gamma).

Beginning of the provisional matrix

Within 48 h, the blood clot is remodelled into a matrix which includes fibrin and hyaluronic acid. The wound clot is not merely a plug but is also a complex macromolecular substrate for cell migration. Hyaluronic acid seems to be essential for the physical stabilization of the matrix and the platelets not only participate in the formation of the haemostatic plug but also actively intervene in the formation of the matrix releasing growth factors and other phlogogenetic agents¹⁵.

The newly constructed matrix within the wound site serves both a structural and a regulatory function for subsequent cell migration. Neutrophils, monocytes, lymphocytes and fibroblasts migrate, the budding of capillaries into the matrix is initiated, and the formation of granulation tissue starts.

Granulation tissue

Granulation tissue is the framework for the repair process and the provisional support for the resurfacing epithelium^{2,3,4}. Macrophages, fibroblasts and blood vessels embedded in a loose, gel-like, extracellular matrix of collagen, hyaluronic acid, fibronectin and other glycosaminoglycans move into the wound as a unit, suggesting an interdependence of these elements. Fibronectin precedes the appearance of collagen and gives direction to migrating processes¹⁶.

The granulation tissue is basically a high cellular, vascular, functional structure directed primarily 'to clean' the damaged area, 'to fill' the existent gap and 'to feed' the growing, active new mesenchymal tissue.

The wound space is hypoxic, hypoglycaemic, hypercarbic, hyperlactic, acidotic and hyperkalaemic. This means that at a time when the connective tissue needs more nutrition than ever, there is a great deficiency of all elements needed to cover all its needs. The tissue tries to solve this crisis by sending emergency signals toward the nearest functional blood vessels in order to obtain the growth of new capillaries into the damaged area¹⁷.

Fibroblasts, working with very limited nutrition, will also receive signals sent by macrophages and platelets, thereby producing enough collagen to support the budding capillaries. Once a new route of circulation has been

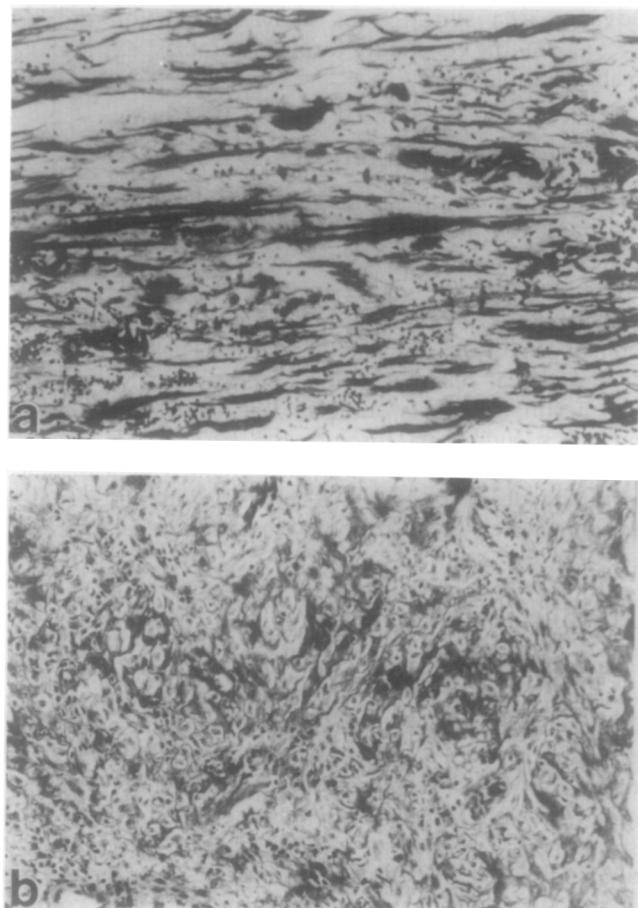


Figure 1. Granulation tissue. **a**, Parallel collagen fibres in a wound leading to normotrophic healing. **b**, disorganized collagen network (whorls) in a wound leading to a hypertrophic healing. (Masson's trichrome, orig. magn. $\times 200$.)

established, the nutrition rapidly becomes more adequate for macrophages and fibroblasts.

The fibroblasts, which were previously working very hard in hypoxia, can now make more collagen and more extracellular matrix, and the macrophages can advance more. When the deficit is reached again, new signals start and the process is repeated until the wound space is filled.

The structure of the granulation tissue is characteristic. In the beginning, the collagen fibres run in a parallel orientation with respect to the neovessels. However, as the granulation tissue matures, the newly formed collagen fibres tend to be orientated parallel to the surface and perpendicular to the vessels (Figure 1a).

Toward fibroplasia

The hallmarks of fibroplasia are fibroblast proliferation and extracellular matrix production. On the way to the wounded area, the Golgi apparatus and the endoplasmic reticulae of the fibroblasts take a perinuclear location and the cells form actin filaments.

In the wound space, there is a cytoplasmic dispersion of the endoplasmic reticulae and the Golgi apparatus, and a great production of collagen starts. Some of the fibroblasts will align actinomyosin filaments in a parallel fashion to each other (in the direction of the contraction of the wound), constituting the myofibroblasts described by Gabbiani in 1971¹⁸. However, fibroplasia would not

advance if a synchronous neovascularization did not accompany the constantly forming fibroblast extracellular matrix complex.

Initiation of angiogenesis

The first step towards angiogenesis is the degradation of the basement membrane. Fibroblast growth factor stimulates endothelial cells to release plasminogen activator and procollagenase which, in turn, liberate plasmin and collagenase. These enzymes degrade the basement membrane beneath the stimulated endothelial cells¹⁹⁻²¹.

Endothelial cell chemoattractants such as heparin and fibronectin fragments stimulate the endothelial cells to project pseudopodia through the basement membrane defect. Once the capillary bud has extended some distance from the vessel of origin, a lumen forms between the endothelial cells and a tube for blood flow is established.

The extracellular matrix

The extracellular matrix, a dynamic complex of macromolecules that underlies epithelia and surrounds connective tissue cells is a very important component of the total process. It provides tissues with structural support and modulates important processes such as: development, migration, attachment, differentiation and repair. The strength of the wound and the properties of the scar ultimately depend on the deposition of an adequate extracellular matrix. This matrix has five major components: collagen, basement membranes, structural glycoproteins, elastic fibres and proteoglycans.

Collagen

Although the best known function of collagen is physical support, it is a very active element of the extracellular matrix, thus becoming a crucial element for wound repair²²⁻²⁴.

Collagen modulates cell proliferation, migration and specific gene expression; it is a major determinant of tissue biomechanical properties, is important in the wound contraction process, and promotes re-epithelialization by constituting the substratum, along with fibrin and fibronectin.

Elastic fibres

Elastic fibres provide the ability to recoil after transient stretching, limit elasticity and maintain tissue integrity. They are rich in glycine and proline like collagen, but contain practically no hydroxylated amino acids.

Basement membranes

Basement membranes provide physical support to structures resting on or enclosed by them, function as a site for cell attachment, have significant tensile strength and serve as a filter. They are synthesized by the cells resting on them.

Structural glycoproteins

A variety of non-collagenous extracellular proteins play important roles in many cell-surface interactions especially in the adhesive, antiadhesive activities. Some of the well-known glycoproteins are fibronectin, entactin, laminin, chondronectin, mesonectin, anchorin, GP 148 and 69, vitronectin, tenascin, epinectin, fibrillin, thrombospondin,

SPARC (secreted protein acidic and rich in cysteine), osteopontin and sialoprotein²⁵⁻²⁷.

The adhesion is mediated by cell surface receptors. The primary class of these receptors is a family of transmembrane proteins known as integrins. There are 13 alpha and eight beta subunits known at this time.

The integrins bind to extracellular matrix protein (i.e. fibronectin) at specialized cell attachment sites that often have the tripeptide sequence RGD (arginine, glycine, aspartic acid) as the target sequence for the integrin binding.

Other known receptors are the cadherins and the IgG superfamily. Cells bind to ECM to anchor, to derive traction for migration or to receive signals for growth and differentiation. Naturally, the mechanisms for adherence must be both controllable and reversible, but what is important because it opens a wide field for therapeutic intervention, is that the bond can be inhibited by monoclonal antibodies against receptor subunits, or by synthetic proteins (peptides) containing the RGD sequence.

Proteoglycans

Proteoglycans are very versatile and striking molecules with essential functions. They function as cell surface receptors, ligands and antagonists for growth factors and extracellular signals for cellular growth migration and differentiation²⁸.

Proteoglycans are necessary for stable assembly of the extracellular matrix and functional cell-ECM interaction, and consist of a core protein to which one or more glycosaminoglycan chains are covalently attached²⁹.

Proteoglycans participate with many other elements in cell proliferation, cell adhesion and migration, coagulation and metabolism³⁰.

They can be classified either on the basis of their core protein or on the basis of their glycosaminoglycan chain³¹. In general, there are three groups of core proteins: extracellular proteins, (large aggregating proteoglycans, basement membrane proteoglycan, leucine-rich repeat family, alpha 2 collagen), membrane associated proteins (syndecan, thrombomodulin, betaglycan, glypican, fibroglycan, CD44, NG2) and intracellular granules (serglycin, chromogranin A).

There are six different types of glycosaminoglycan chains: two galactosaminoglycans (chondroitin sulphate and dermatan sulphate) and four glucosaminoclycans (heparan sulphate, heparin, keratin sulphate and hyaluronic acid).

Proteoglycans interact with growth factors prolonging their action, localizing them to the immediate cell environment and influencing the intensity of response to a single factor.

Contraction

Within 1 week postinjury when fibrillar collagen type I starts to build up the strength of the fibrous tissue, another mechanism commences. Along with the efforts to epithelialize the denuded area, the organism tries also to achieve a spatial reduction by centripetal movements of the surrounding skin.

This active physiological process of contraction is in contrast to the pathological deforming contracture which is a late end result. The contractile forces are suggested to be produced by fibroblasts and its functional and structural phenotypic modulated version, the myofibroblast^{18,32}.

The process of re-epithelialization

Within hours after injury, epithelial cells from the wound margins or adnexal structures will initiate a series of specific mechanisms directed to cover the new surface. (In burns, this epithelial response may be delayed up to several days³³⁻³⁵.)

The mechanisms involved in the epithelial movement are controversial and include the 'leap frog model', whereby cells above and behind the leading cell stream over the latter to attach to the wound bed³⁶, or the formation of a chain of cells advancing but always maintaining its original position in the chain³⁵. The initial stimulus is largely unknown. The migration may be induced by loss of the attachment to neighbouring damaged cells (free edge effect), active contact guidance or the presence of soluble mediators such as fibronectin, chalones or epibolin.

In normal skin, the epidermal layer attaches to basement membrane, a complex zone containing a variety of different components such as laminin, type IV collagen and heparan sulphate proteoglycan, with the hemidesmosomes as the primary adhesion structures. After injury, the epidermal basal cells lose their desmosomes and hemidesmosomes and express fibronectin receptors³⁵. The cells, without hemidesmosome links start their lamellipodial crawling over a provisional matrix of fibronectin, fibrin, fibrinogen and tenascin while secreting plasminogen activators and collagenases, to open their way through desiccated tissue.

The cells migrate on fibronectin using integrin receptors³⁷. Fibronectin promotes keratinocyte mobility while laminin, an adhesion factor in intact epidermis, is lost after wounding¹. Epithelial cells advance more rapidly if the wound bed has adequate humidity^{38,39}. The epithelial migration ceases when the advancing epithelium meets its counterpart growing from the opposite direction. The cells which were migrating in a lateral motion across the wound surface will now regain the normal vertical direction toward the surface to become cornified cells. The same migrating cells will reconstitute the basement membrane⁴⁰.

The beginning of the permanent scar

When the migrating epithelium has completed the resurfacing of the new connective tissue matrix, the building up of the granulation tissue stops. The granulation tissue becomes a true scar tissue, inelastic and somewhat brittle. The remodelling includes an increased crosslinking of collagen, breakdown activity of collagenase, a decrease in glycosaminoglycans, regression of the neovascularity, and adequate I/III collagen ratio and the reorientation of collagen fibres in response to mechanical stress⁴¹.

Clinically, the original redness, elevation and firm consistency of the new scar tissue gradually evolves into a flat, softer scar tissue, level with the adjacent skin surface. This phase of 'maturation' includes gradual replacement of the original scar tissue over a period of at least 6 months.

Wound healing interferences

It is obvious that any factor capable of disturbing the complex mechanisms of tissue repair will interfere with adequate wound healing. The more common problems are associated with local factors such as: necrosis, oedema, tissue perfusion, oxygen tension, wound pH, exudation,

infection, wound dressing, haematoma, recurrent trauma, mechanical stress, topical agents, temperature (blood flow), foreign bodies, denervation and surgical technique, or some systemic conditions such as: age, obesity, malnutrition, anaemia, renal diseases, liver diseases, sepsis, drugs and pre-existing diseases (i.e. diabetes, arteriosclerosis, neoplasia, immunodiseases, collagenosis^{42,43}).

Toward solutions

Some of the complicating factors in the early phase of healing are hypoxia, hypoperfusion, infection, a delayed inflammatory phase or impaired nutrition. A controlling approach would provide an adequate systemic haemodynamia, debridement of necrotic tissue, optimization of local environment, adequate nutritional support and adequate wound dressing, and indicated pharmacotherapy.

Platelets, as is indicated above, are an important part of the acute response and may have congenital disorders involving aggregation (Glanzmann's thrombasthenia), activation (impaired receptor availability), coagulation, secretion or adhesion (von Willebrand's syndrome, Soulier's syndrome). They may have also acquired disorders involving aggregation, activation, coagulation, secretion and adhesion, many of them triggered by physiopathological conditions (i.e. hepatic or renal dysfunction) or medication (antibiotics, corticoids, aspirin). A careful monitoring of platelet function is strongly indicated in all severe burns.

The fibroplasia-fibrosis problem

The basic conditions for development of fibrosis are: proliferation of fibroblasts, production of collagen and expression of genes encoding the appropriate cytokines. There is an increased evidence that lymphocytes, mainly T cells, are major modulators of cells involved in wound healing^{7,44,45}. Several studies have shown that T cells may play a role in the development of fibrosis⁴⁶. Cytokines from T cells may activate macrophages to release mediators that induce fibroblasts' proliferation and collagen synthesis.

The induced fibroplasia may proceed to develop fibrosis. Thus, it is possible that by blocking the effects of some cytokines, a therapeutic antifibrotic effect may be obtained. A variety of lymphokines have been shown to inhibit and stimulate migration and proliferation of fibroblasts and collagen synthesis. Because of this dual activity it seems logical to think that an imbalance could result in wound failure or in excessive fibrosis⁴⁶.

Controlling fibroblasts and the extracellular matrix

Extensive research and a variety of clinical approaches are being used to manipulate the direction of the wound healing process by influencing fibroblasts and the extracellular matrix. The control of stimulation and migration of fibroblasts is being approached by the use of chemoattractants (fibronectin, collagen and elastin fragments, PDGF, interleukins or C5a)⁴⁷⁻⁴⁹, trying to eliminate the stimuli (actin, corticoids), neutralizing the membrane receptors (blockade) or controlling locomotion (colchicine)⁵⁰. The control of proliferation may be achieved by the use of corticoids, antimetabolites, antimicrotubules or anti-histaminics⁵⁰⁻⁵², while contraction may be influenced by troponin (smooth muscle antagonist), colchicine, vinblastine or ATP blockers^{50,53-54}. The synthesis of extracellular matrix may be influenced by colchicine, beta-

aminopropionic nitrate (BAPN), penicillamine, collagenase or IFN gamma⁵⁷⁻⁶².

Growth factors

The availability of purified growth factors may provide an exciting tool for altering wound healing.

EGF is a chemotactic and mitogenic factor and has been shown to influence positively the wound healing process^{57,63}.

Adolph showed the pro-inflammatory and fibrotic activity of TGF-beta. Using rabbits he demonstrated that fetal wounds known to heal without scarring when treated with TGF-beta develop an increased inflammatory and fibrotic response similar to that observed in adults⁶⁴.

TGF beta has beneficial effects such as: chemoattraction, induction of angiogenesis, control of the production of cytokines and other inflammatory mediators, auto-induction, induction of increased deposition of ECM, and increased expression of integrins, thus facilitating the adhesion to matrix. There are also adverse effects: the increased deposition of extracellular matrix can lead to excessive scarring and fibrosis, and its ability for auto-induction may be conducive to chronic, progressive scarring and fibrosis.

TGF-beta is one of the best characterized fibrogenic agents and also has bifunctional activity: anti-inflammatory and pro-inflammatory. Adequate levels may lead to normal healing while insufficient levels may impair healing and excessive levels may produce fibrosis.

An interesting experiment was performed by Shah et al⁶⁵. It suggests a new approach to the control of scarring using neutralizing antibodies (NA) to TGF-beta. Immunocytochemical and biochemical analysis showed that 7 days after wounding, NA-treated wounds contained much less collagen than did other wounds in the same animal. Surprisingly, the advantageous effects on scarring in the NA-treated wounds were not accompanied by a delay in wound healing or a reduction in wound strength.

The beneficial effects of applying TGF-beta, not only topically but also systemically, were demonstrated by Beck et al.⁶⁶.

It seems that a combination of growth factors, rather than the use of single ones, is necessary to obtain a better response. Lynch noted that the combination of PDGF and IGF-1 were more potent stimulators of healing than the use of single factors⁶⁷.

The administration of recombinant human growth hormone seems to improve the healing time of donor sites in burned children and also to accelerate the healing of extensive burn wounds^{68,69}.

Growth factors have opened new ways to approach wound healing, however questions still exist about their multifunctional properties, the wound environment, the appropriate therapeutic concentration, the appropriate sequence and timing, the appropriate delivering system, the appropriate time of exposure, the stability of the product, the possible adverse effects and the relationship with atypical growth.

Growth factors may accelerate the process in wound healing but cannot replace state of art debridement, control of infection, proper nutrition or adequate positioning. Also, it is necessary to remember that the inhibitors are as important as the growth factors.

Possibly the greatest potential for the use of GF is the treatment of hypotrophic, chronic, open wounds and not the acceleration of closure of normally healing wounds. A

very exciting prospect is the use of growth factors to reduce the interval between harvests in burn donor sites.

Epithelialization

Several approaches are being sought to improve epithelialization. Among them, the use of fibronectin which promotes cell adhesion, spreading, migration, chemotaxis, phagocytosis, matrix and basement membrane organization, secretion of growth factors and increase in wound-breaking strength^{49,70}. Other approaches include the use of epibolin (serum) which enhances epidermal cell spreading and migration, EGF which accelerates epithelialization, and the RGD peptide (arginine-glycine-aspartic acid) which enhances epithelialization and may stimulate early formation of the basement membrane^{57,71,72}.

Fetal wounds

An interesting fact is that fetal wounds heal without scarring. This is an exciting area of research and may help us to better understand the normal and abnormal process of adult wound healing. This scarless wound may be attributed to the organization of a peculiar extracellular matrix (ECM). The early appearance and sustained presence of a hyaluronic acid-rich matrix together with the presence of a special glycoprotein (hyaluronic acid-stimulating activity (HASA)), facilitates cell migration, cell proliferation, and regeneration⁷³⁻⁷⁶.

The scar

The end result of healing may be a normotrophic healing or several abnormalities ranging from hypertrophic scars to chronic ulcers.

The healing of the wound requires a delicate equilibrium of opposite actions: cell proliferation versus cell necrosis, collagenesis versus collagenolysis, angiogenesis versus angiolytic. If an adequate equilibrium is not reached, the resulting scar may be depressed, hypotrophic, atrophic, hypertrophic or present other abnormalities.

Chronic, open wounds are a very difficult problem. Despite numerous approaches, the therapeutic results are still very limited. Among the measures to be taken toward solutions are: a rapid and accurate aetiological diagnosis, the optimization of macro- and microenvironment, the optimization of systemic adequacy (haemodynamics, nutrition, etc.), and the maximization of research using adequate animal models (impaired healing, i.e. diabetes), to achieve a better understanding of the complexities of the wound healing process and the optimal exploration of therapeutic possibilities⁷⁷.

Hypertrophic healing

The end of the scarring continuum is occupied by the hypertrophic healing process. The events differentiating normal healing from hypertrophic healing actually start during the development of the granulation tissue and it is fairly evident between 3 and 5 weeks after injury. One of the most notable differences is the orientation of collagen fibres. If the healing process will result in a hypertrophic healing, it is possible to see in the granulation tissue a tendency for the collagen fibres to run in a haphazard direction with a tendency to a whorl-like pattern characteristic of hypertrophic scars^{78,79} (Figure 1b).

At the same time, although the cell population of the granulation tissue, as seen under light microscopy, does not show qualitative differences between normal and

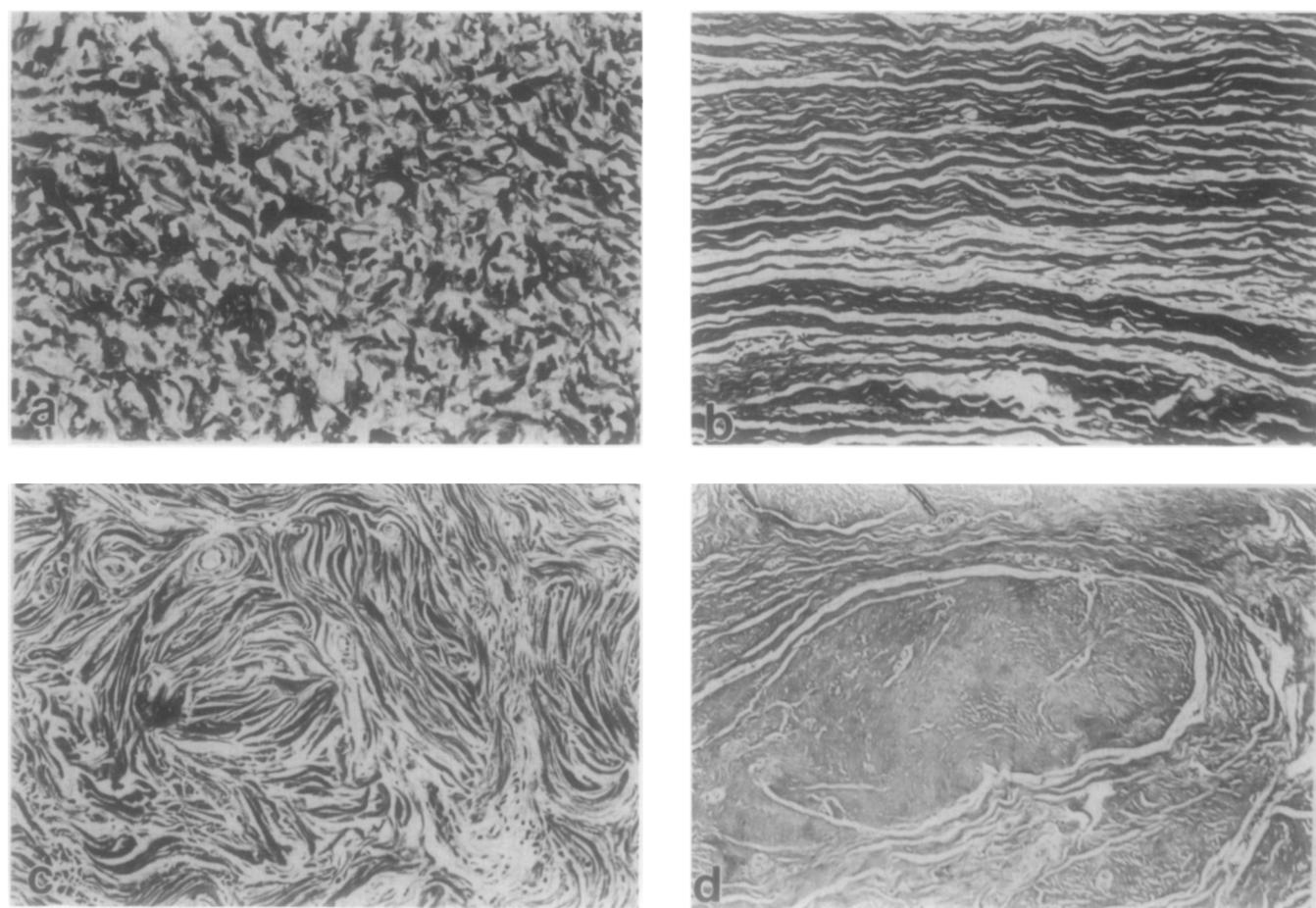


Figure 2. Collagen patterns to compare. **a**, Orthogonal disposition in normal dermis. **b**, Parallel orientation in normotrophic healing. **c**, Whorl-like arrangement (**c**), and nodular pattern (**d**) in hypertrophic healing. (Masson's trichrome, orig. magn. $\times 100$ (**b,d**) and $\times 200$ (**a,c**).)

hypertrophic development, there is a marked difference in their cyclic evolution. The structural characteristic of immature connective tissue, which normally disappears within several weeks will persist for several months if hypertrophic healing develops. In many cases, a chronic inflammatory reaction of variable degree may persist. The collagen pattern, persistent cellularity, abundance and composition of proteoglycans and the prolonged chronic inflammatory reaction are the most common morphological features distinguishing hypertrophic healing from normal healing.⁸⁰⁻⁸¹

Hypertrophic scars are depicted as tumour-like lesions raised above the level of the skin with a wide spectrum of variations in shape, size, colour and consistency⁸². These characteristics usually correspond to the site and extension of the injury, time of evolution and individual susceptibility. In certain areas (chest, shoulders, lobules of the ears) the hypertrophic healing tends to be prominent, nodular, pedunculated or semipedunculated, round or oval, smooth and shiny, hard and somewhat elastic. In other areas, it usually has an irregular surface and is rarely elevated more than 15–20 mm above the level of the surrounding skin.

The edges are usually prominent and end abruptly, sometimes with finger-like or chain-like prolongations. Initially the scar is red or pink (so-called immature), but after a period of time, months or even years, the scar flattens, softens and blanches (maturation).

In immature scars, redness and turgidity is due to increased active vascularity. Immature scars sometimes

display a minute pinkish, bluish or purplish superficial net of small blood vessels. The scar does not adhere to the subcutaneous tissue and appears to grossly exceed the limits of the original injury.

Histologically the appearance depends on the evolutionary state in which the sample was taken. Also, scars from different anatomical sites display histological differences. Those located on ears or shoulders show very broad, thick collagen fibres more frequently than scars from other sites. It is also possible to observe that there is no 'invasion' of surrounding normal tissue but that the overgrown healing tissue actually pushes apart the surrounding normal tissue without invading it. The gross anatomy appearance of the lesions exceeding the limits of the original injury is seen to be produced by tumour-like growth pushing the epithelium upward. This suggests that the clinical appearance will depend in part on the forces generated by the opposing superficial layers of the skin in the area and the skin resistance to deformation.

The histological appearance of the epidermis is also variable. It may appear hyperplastic with visible rete ridges or thin and smooth. The typical histological features of the hypertrophic healing reside in the reticularis layer of the dermis. In the early stages, there is hypercellularity (fibroblasts, myofibroblasts) and hypervascularity. The collagen fibres run in a curvilinear, whorl-like arrangement which in most cases progresses to distinct nodular forms. Whorls and nodules may be seen in the same histological section⁷⁸ (Figure 2).

These structures never involve the subcutaneous tissue, from which they are often separated by somewhat compact parallel bands on connective tissue with nearly normal characteristics. The increased cellular population is composed of young, active fibroblasts and myofibroblasts. Mast cells appear increased over the number observed in normal skin. Plasma cells and lymphocytes are usually present at the beginning of the formation of the scar and form a perivascular cuff, which may persist for a long time as scattered foci or chronic inflammation. The vascular structure is prominent and the capillary network appears to follow the arrangement of the collagen fibres. The skin appendages are atrophic, destroyed or displaced by the scar. At times they do not slough, but do elicit a foreign-body reaction. Usually, no elastic tissue can be found except in the papillary areas of the dermis and in the normal dermal areas surrounding the scar. The interstitium shows a significant amount of ground substance (glycosaminoglycans), which has been shown to contain an elevated amount of chondroitin-4-sulphate⁸³.

With time, the thickened hyaline fibres of nodules and whorls become elongated and the elastic fibres reappear. This is indicative of a regression pattern, which proceeds towards 'mature' scar. The reorientation of the collagen fibres in a parallel arrangement, as found with non-hypertrophic healing, is accompanied by a progressively reduced number of fibroblasts and myofibroblasts, and a pronounced decrease in vascularization, which corresponds to the flattening, blanching and softening of the mature scar described above, although the tissue structure never recovers its original appearance⁸².

Some aetiopathogenic mechanisms

The biological mechanisms responsible for the deviation of the normal healing process toward an excessive reparative response are still elusive. The lack of a reproducible laboratory animal model adds a very frustrating factor. However, the increasing technology and the firm interest from many researchers are providing, piece by piece, the elements which hopefully will solve the hypertrophic healing puzzle.

Evidently, the most visible feature is the excess of collagen deposition suggesting that the essential balance between collagen synthesis and degradation is missing. An increased proline hydroxylase activity and an increased collagenase activity insufficient to counteract the exuberant collagen synthesis have been described by many authors⁸⁴⁻⁸⁶. Despite equal or increased levels of activity of lysyloxidase, it seems that hypertrophic scars have a lesser content of highly cross-linked collagen and also an increased amount of soluble collagen⁸⁷⁻⁸⁹. A proper degradation of collagen may be inhibited by the action of alpha 1-antitrypsin and alpha 2-macroglobulin, which were found in the extracellular matrix of hypertrophic scars^{84,90}. Some workers reported a significant increase in the I/III collagen ratio, while others described just the opposite⁹¹⁻⁹³.

An intriguing observation was made by Abergel et al. that the increase in collagen synthesis was not a constant characteristic of keloids⁹¹. It was speculated that the heterogeneous population of normal skin fibroblasts, which can strongly differ in the amount of collagen synthesized among them, may also be reflected in keloid fibroblasts cultures. This may result in keloids with a normal or very high production of collagen^{94,95}.

Although the growth of keloid fibroblasts is normal, the

cells seem to be modulated to some specific function such as the production of more collagen⁹⁶. Cultures with an excessive production of collagen may also have high levels of type I collagen-specific mRNA, which suggests a loss of regulatory control at the transcriptional level^{91,94}.

Two conflicting reports of similar studies raise some speculation about the possibility of racial factors and also reflect the concept that production of collagen is not the sole problem in hypertrophic healing. Procollagen synthesis was found to be increased in five of nine keloid fibroblast cultures from black patients⁹¹ while, despite increased levels of intracellular prolyl-4-hydroxylase and glucosyltransferase, a normal synthesis was found in eight of nine keloid fibroblasts cultures from white patients⁹⁵. The initial rate of collagen synthesis in hypertrophic scars is about twice that of normotrophic scars and fell to the same level as in normal scars about 2-3 years after injury⁹⁷.

The glycosaminoglycan/proteoglycan factor

Proteoglycans influence the aggregation of collagen, and there is evidence from a variety of studies that glycosaminoglycans' and proteoglycans' composition in wound healing and in hypertrophic healing differs from normal skin. While in normal skin human dermis shows a much greater amount of decorin than chondroitin sulphate proteoglycans, the reverse occurs in granulation tissues and scars^{91,98}.

In particular, chondroitin-4-sulphate has been identified as the main contributor to the striking increase of proteoglycan levels in hypertrophic scars^{99,100}.

It has also been demonstrated that the strong association between collagen and proteoglycans in hypertrophic scars may prevent collagenase from breaking down collagen. The excessive presence of chondroitin-4-sulphate may contribute to the overabundance of collagen deposition which is characteristic of hypertrophic scars^{81,101}.

Furthermore, it has been observed that in hypertrophic scars there is a persistent presence of a perivascular cuff of variable density, mainly composed of lymphocytes^{78,82}, and that the proteoglycan associated with this perivascular T-cell infiltration is mainly chondroitin-4-sulphate¹⁰².

Fibroblasts derived from hypertrophic scars incorporated proportionally more radiolabelled precursors into chondroitin sulphate and hyaluronic acid in vitro than did normal skin fibroblasts¹⁰³. The immunological explanation has been explored by several workers¹⁰⁴.

Also, fibroblasts from hypertrophic healing were shown as having an increased fibronectin production, suggesting the presence of specific mechanisms controlling fibronectin expression¹⁰⁵. A heavy fibronectin deposition was found to be predominantly located in the nodular structures of hypertrophic healing sections¹⁰⁶.

A recent study showed an increased production of IL-6, TNF- α and IFN- β in hypertrophic healing, suggesting that the altered levels of immunoregulatory cytokines may play a significant role in the overproduction of collagen¹⁰⁷. It has also been suggested that the excessive and inappropriate action of TGF-beta may promote abnormal healing¹⁰⁸⁻¹¹¹.

Therapeutic approach and the future

Therapeutic solutions to hypertrophic scars have been met with the same degree of confusion and controversy as their morphological and aetiopathogenic descriptions. Proposed solutions have been innumerable but in most instances frustration has been the net result.

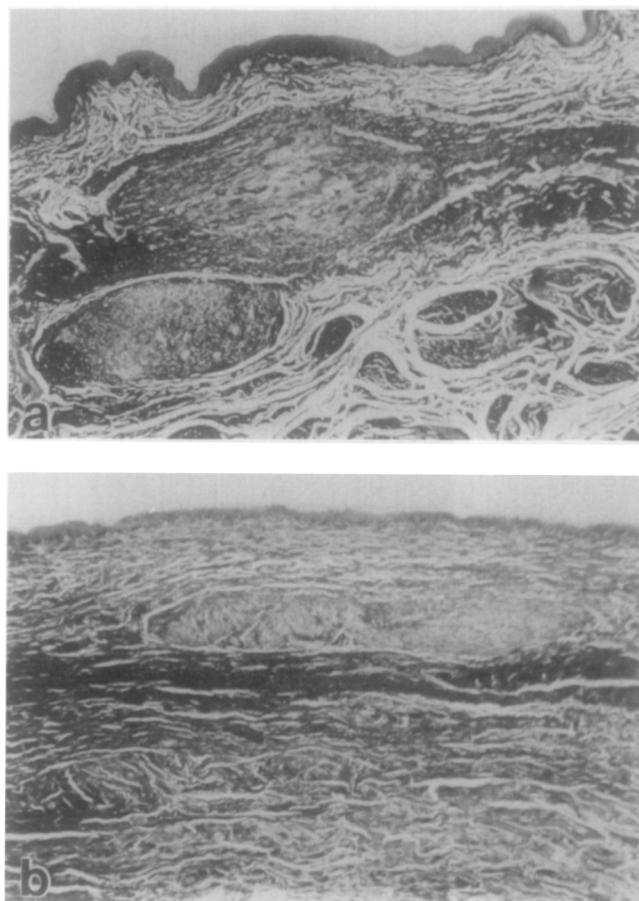


Figure 3. Pressure treatment. **a**, No pressure, 4 years postburn. **b**, Pressure treatment, 4 months postburn. Note the parallelization of the collagen fibres. (Masson's trichrome, orig. magn. $\times 40$.)

Unfortunately, the present state of knowledge of aetiopathogenic mechanisms responsible for the hypertrophic healing is still inadequate to formulate a sound biological basis for a routinely effective treatment. However, it is possible to achieve if not ideal, at least satisfactory, results with severe different approaches using surgical, pharmacological and physical methods. Surgery, corticoids and pressure alone or in combination are currently the most universally used methods of treatment¹¹²⁻¹¹⁶ (*Figure 3*).

Recent progress in molecular biology has shown that many cell functions are brought about by an interaction among macromolecules. The binding between macromolecules, therefore, has far-reaching physiological implications. The study of proteoglycans in hypertrophic scars can be of great interest because the organization of collagen fibrils in tissues may be related to amounts and kinds of proteoglycans, and because the binding of proteoglycans to collagen can influence fibril formation.

The research should include the study of the factors that influence the structure, migration, function and modulation of the cells that control the synthesis and degradation of the intercellular matrix. The immunological and genetic interference should be thoroughly explored. Biochemical analysis and structural events could be important in determining the role of the sulphate glycosaminoglycans in the development and aggregation of collagen fibres.

Several possible mechanisms might be involved in producing the excessive amount of collagen deposition

that is characteristic of these scars: an actual increase in the rate of collagen synthesis; the presence of inhibitors for the enzymatic breakdown of collagen and/or glycosaminoglycans; the absence or defective production of the specific enzymes; the presence of physical barriers opposing a proper enzymatic attack; growth activity promoted by sulphated proteoglycans; or other causes, yet unknown⁶².

The distinction between keloids and hypertrophic scars

The differentiation between true, genuine, spontaneous or idiopathic keloid, and false, spurious, traumatic or cicatricial keloid was introduced by Alibert almost 200 years ago, leading to the controversy that still continues¹¹⁷. According to Alibert, true keloids have a lancinating, pungent and burning pruritus with a painful sensation, while false keloids result only from inflammation of a scar after burns or ulcerations. Although this division was generally accepted in the beginning, the false keloid started to be designated as cicatrix, warty tumour of the cicatrix or vegetations of the cicatrix. By the end of the nineteenth century, 'hypertrophic scar' became a differentiating term. It is in Kaposi's chapter on keloids, in 1874, that the differentiation among 'true keloid, cicatricial keloid and hypertrophic scar' was first made¹¹⁸. He stated that scars 'which remain within the limits of the loss of substance of the skin represent so-called hypertrophied scars, looking, however, exactly like keloid. Does this kind of scar belong also to the false, or cicatricial keloid? Or is it probable that the so-called spontaneous keloid is only hypertrophied scar?'

Unfortunately, quoted perfunctorily from paper to paper, from book to book, the definition, differential diagnosis and concepts about keloids vs. hypertrophic scars have undergone an endless series of misconceptions, mutations and permutations throughout almost two centuries. Repeated efforts have been made to solve such a controversial issue. Perhaps two of the most currently used criteria of differentiation are that keloids exceed the limits of the initial injury as defined by Kaposi in 1874, and that keloids have conspicuous thick, glassy, faintly retractile collagen bundles, as described by Blackburn and Cosman in 1966¹¹⁹. However, histological studies will show that the growing of scars over the limits of the wound is related not only to the activity of the scar itself but also to the structure and opposing forces of the surrounding normal tissue⁸². This 'trespass' of boundaries may be seen in clinically diagnosed hypertrophic scars as well as in scars clinically classified as keloids. Also, thick, glassy, faintly retractile collagen bundles may be seen in the same histological section of scar sharing large areas with collagen bundles of opposite characteristics. Several biochemical profiles of the skin and scar samples have shown that hypertrophic scar and keloid are not distinct pathological entities but similar aberrations, with keloids showing greater quantitative deviations from normal healing.

Toward a rational and dynamic approach to hypertrophic healing research

The apparent discrepancies and conflicting opinions regarding hypertrophic healing may be a reflection of the complexities and unknowns surrounding this abnormal process. The criteria for the classification of the specimens or the rational use of particular techniques are so diverse that, not surprisingly, the research results elicit also an

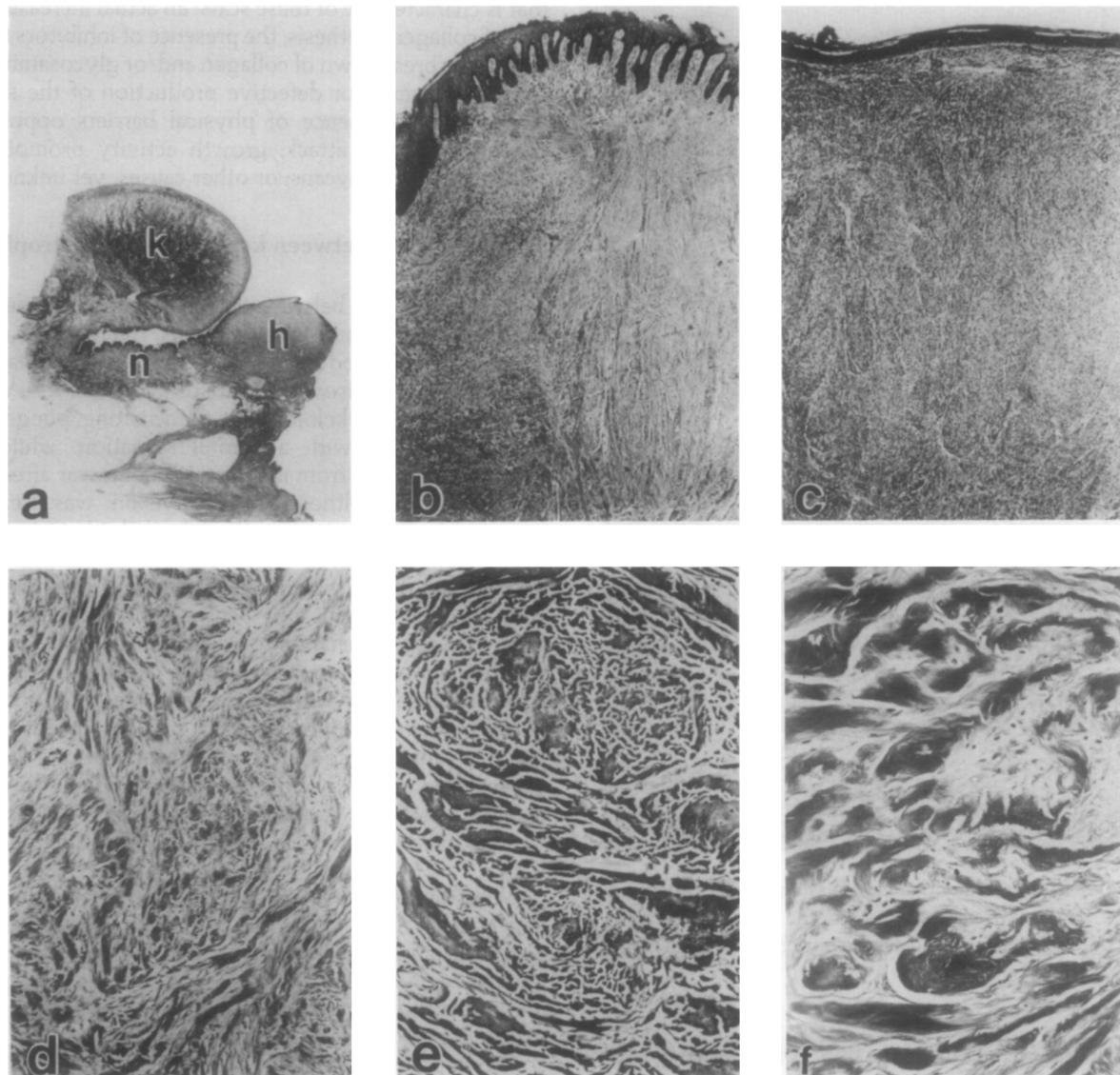


Figure 4. Sections from the same scar. (see description in the text). **a.** Gross anatomy with three different areas k,h,n. **b.** Areas of epithelium with rete ridges. **c.** Areas of epithelium without rete ridges. **d.** Whorl-like arrangement of the collagen. **e.** Nodules of collagen with thin fibres. **f.** Nodules of collagen with thick fibres. (Masson's trichrome, orig. magn. 1:1 (a), $\times 40$ (b,c), $\times 200$ (d-f).

ample variety of interpretations. In too many biomolecular, biochemical or immunological studies, the specimens to be studied have arrived in the laboratories labelled as hypertrophic scars or keloids according to their clinical appearance or criteria, and are processed basing their differential diagnosis on those clinical characteristics, overlooking the clear morphological heterogeneity depicted by every scar under proper histological examination. The histomorphology of each scar, which is also related to their physiopathological characteristics, not only differs in many instances from patient to patient, but also substantial tissue differences may be noted among scars from the same patient and more interesting yet, among areas of the same scar. Figure 4a shows the gross anatomy of a hypertrophic healing with three distinct areas: an exuberant scar, exceeding the limits of the original wound, which may be clinically classified as a keloid by some workers (k) is separated by a bridge of normal skin (n) from another scarring area which may be clinically classified as a hypertrophic scar (h). Figure 4b-f shows areas of the same

scarring tissue (Figure 4a) with clearly distinctive morphology. In many hypertrophic scars (keloids), compact nodules of collagen are seen side by side with looser collagen structures or collagen fibres arranged in a whorl-like pattern. In other specimens, abnormal collagen arrangement in the upper areas of the dermis is clearly different from the collagen arrangement of deeper areas showing great morphological variations (Figure 5). In many cases 'active' nodules with considerable amounts of extracellular matrix and fibroblasts are seen next to areas of clear 'regressive' characteristics, with scanty fibroblasts, reduced amount of proteoglycans and the appearance of elastic fibres. Obviously, with such a morphological structure-function-related diversity, the chance for discrepancies and conflicting opinions among researchers, are greatly increased. For example, fibroblast cultures, fairly popular in many laboratories, are highly critical not only because of the scar tissue diversity but specifically, because even normal dermal fibroblasts are heterogeneous. Cultured fibroblasts may show differences merely based on

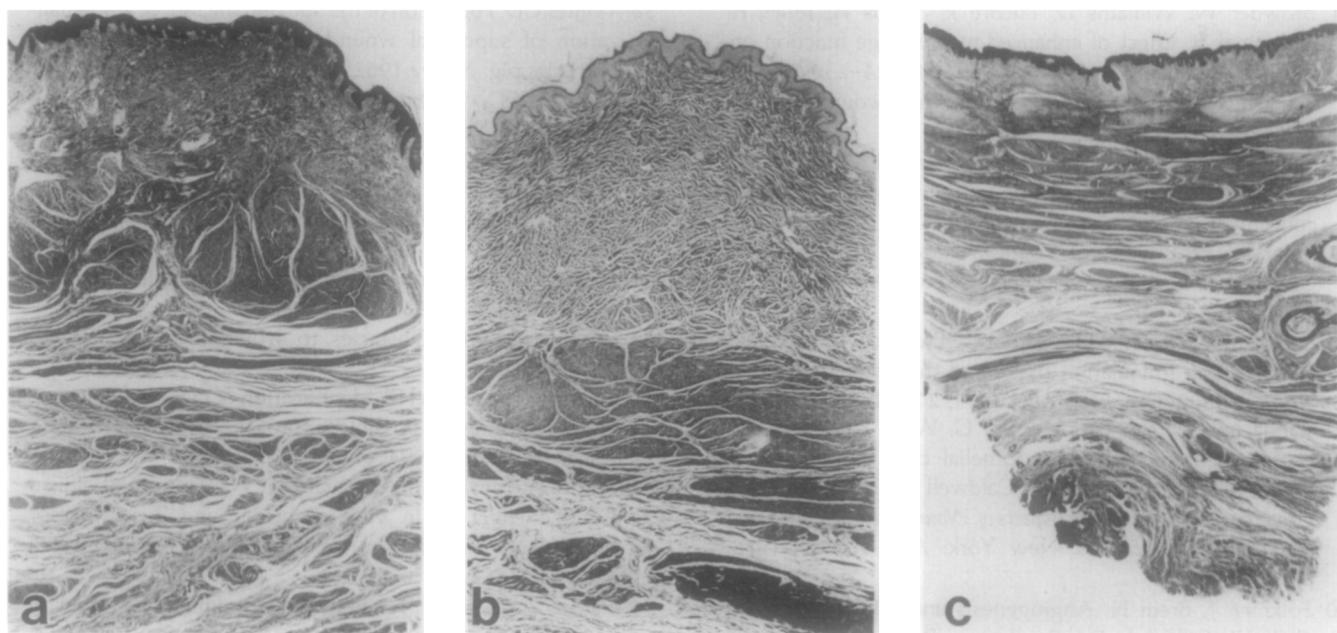


Figure 5. Hypertrophic scars with different collagen patterns. **a**, Nodules in a predominant middle and upper dermis location. **b**, Nodules in a predominant lower dermis location. **c**, Nodules at different levels of the dermis. (Masson's trichrome, orig. magn. $\times 20$.)

the histocellular characteristics of the area from where they were harvested and do not truly represent 'in toto' the characteristics of the abnormal healing. It is unfortunate that the heterogeneity of hypertrophic scar tissue and fibroblasts has been largely neglected^{120–122}. It is also true that quantitative assays should be used, but we believe that it is essential that microscopic analysis should always be planned to determine, as accurately as possible, which areas of the scars are exhibiting the assayed element. In our opinion, hypertrophic healing research would have maximal chances of success if it is approached in a multidisciplinary team effort, involving, at least, histomorphology, immunohistochemistry, biochemistry, immunology and cell cultures. The best specimens are those provided by human burn wounds and scars due to therapeutical retrieval or by patient consent. Granulation tissues taken at different times of evolution may be compared with the scars developed in the same patient, thus establishing a truly dynamic (longitudinal) approach to the healing process. The ability to heal depends on an adequate balance between the components of a complex, interactive and interdependent biomolecular network which includes cells, fibres and a variety of interrelated mediators. Therefore, rather than measure isolated elements in time and space, a truly multidisciplinary longitudinal study should be performed in order to investigate why, when and how an imbalance is occurring, and to evaluate the possibilities to correct it. The best technical approach is to perform microdissections of the hypertrophic tissue to provide similar areas to all researchers, always including histomorphological control. Thus, the chances of obtaining comparable results will be enhanced. We support the concept that the differences are essentially quantitative, that the post-injury scars called 'keloids' are extreme variants of hypertrophic healing, and that the aetiopathogenesis is related to a chronic, immunoinflammatory process associated with cell-cell and cell-matrix miscommunications.

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The British Burn Association is pleased to announce that the 1996 James Laing Essay will be entitled
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