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Review

The effects of ageing on cutaneous wound healing in mammals

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ABSTRACT

The dogma that cutaneous wound healing is impaired as a function of age is largely unsubstantiated. This can be attributed to poor experimental design of human studies, the lack of subject characterisation with the exclusion of disease processes, and the study of inappropriate animal models. Structural and functional changes in skin with age have been reported, such as a decrease in dermal thickness, decline in collagen content, a subtle alteration in the glycosaminoglycan profile, and a loss of elasticity, but these reports are subject to the above criticisms in addition to the often-neglected requirement for site specificity. Wound repair can be thought of as a culmination of three major overlapping phases: inflammation, proliferation and remodelling. The inflammatory process has not been studied systematically with respect to age, and despite a reported decline in cellular function and number, there is a confounding increase in the production of specific cytokines involved in the process of repair. The proliferative phase is associated with a loss of cellular responsiveness to specific cytokines with a decline in motility and proliferation; however caution in interpreting these findings is important as, for example, the definition of 'ageing' is used rather loosely with the result that neonatal versus young adult cells are compared instead of young versus old adults. During remodelling, fibronectin and collagen production may increase with age, as may wound contraction; the deposition of elastin has not been assessed and the resulting mechanical properties of the scar are controversial, not least because human in vivo studies have been ignored. The absence of a critical review on the effects of advancing age on would healing has conspired to permit the perpetuation of the belief that well defined tenets exist. This review aims to redress this imbalance and to highlight the need for well designed research into an increasingly important field.

Key words: Ageing; skin; cytokines; wound healing.

INTRODUCTION

Survival of an organism in part depends upon the maintenance of integumental integrity. Dermatosparaxis in animals is an example of a heritable condition in which skin fragility leads to necrotic wounds following minor trauma, and eventual death (Lapiere, 1991). Morbidity associated with delayed wound healing imposes an enormous social and financial burden; the elderly account for a large part of this morbidity and unless improved wound care strategies are developed, the projected relative and absolute increase in the elderly population (Compendium of Health Care Statistics, 1992) will further exacerbate this problem.

It is commonly stated that wound healing is impaired with progression through the human lifespan (Goodson & Hunt, 1979). However, despite age-related chronic wound healing states associated with disease, such as diabetic ulcers, pressure sores and venous stasis ulcers, the axiom that healing rate or quality is adversely affected by age is largely unsubstantiated. In addition to the speculative effects of chronological age, which itself is not a dependable indicator of underlying physiology, the healing process is affected by a number of factors such as the

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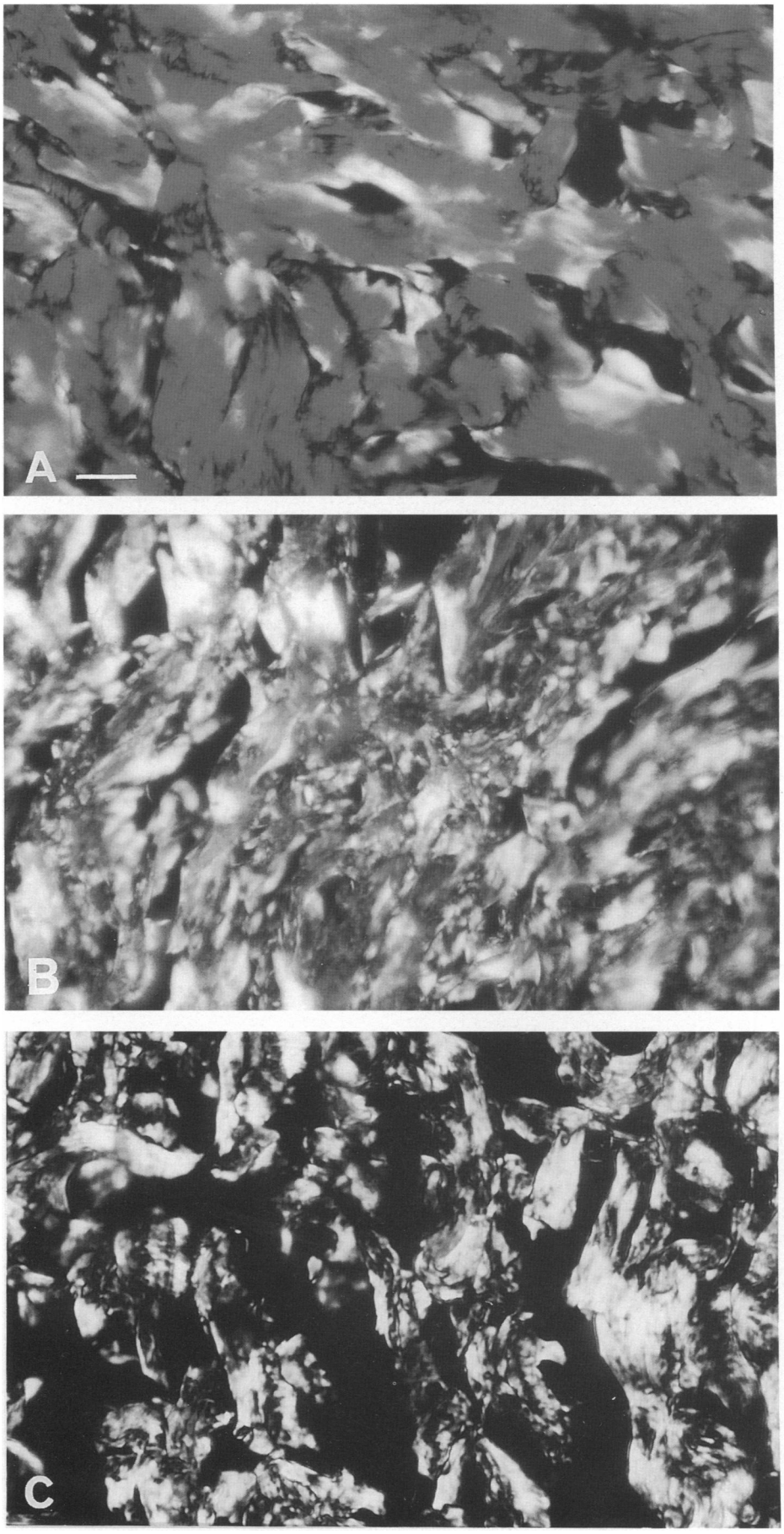
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nutritional status of the subject, the nature and site of the wound, and concurrent morbidity. To a large extent these factors have not been adequately controlled for in the published literature. The assertion that wound healing is impaired with human ageing can be traced to the reports of DuNouy (1916) who followed the rate of closure of wounds in soldiers of different ages during World War I. The nature of the wound, its depth and the degree of contamination were not controlled for, and given the age-range of the subjects (20–40 y) the conclusion that ageing impairs healing should be viewed with considerable scepticism.

Of relevance to wound healing in man is the 'disposable soma' theory of ageing which states that senescence occurs because the level of investment in somatic maintenance is less than that required for indefinite longevity because of the trade-off between investment in reproduction and investment in maintenance (Kirkwood & Rose, 1991). The cellular manifestations would include a reduced cellular lifespan and a reduced responsiveness to growth factors. A subsequent defect in the synthesis of macromolecules would influence homeostasis such that the cell would not be able to maintain responsiveness to external stimuli (Holliday, 1990). Thus it might be expected that wound healing could be modified during ageing, and this is suggested by a number of publications. Halasz (1968) reported the incidence of abdominal wound dehiscence following duodenal surgery in 3000 patients and showed a clear age effect; dehiscence occurred in 1 % of patients aged under 40 y but in 5 % of those over 70 y. However, no attempt was made to control for concurrent morbidity and the wounds were frequently infected. Some studies have reported an increased rate of wound healing in old animals: Cohen (1987) documented an accelerated rate of repair in aged outbred deer mice compared with young animals, and this has been supported by other animal studies (Ershler et al. 1984; Quirinia & Viidik, 1991).

Cutaneous wound healing is a highly orchestrated and elegant process, the understanding of which has advanced enormously over the last 10 years mainly because of advances in cellular and molecular biology. Cytokines are small proteins or glycoproteins that effect a myriad of processes essential to wound repair, including cellular migration, proliferation and extracellular matrix production. Cytokines have emerged in a pivotal role but it is only relatively recently that attention has begun to focus on the differing responses to, and production of, cytokines at different ages. The ancient Greeks ascribed mystical powers, albeit inadvertently, to the action of cytokines. The temple of the god Aesculapius was believed to house serpents that possessed the magical powers of healing if they licked the wound of those afflicted (Reed & Clark, 1985). Animals do indeed lick their wounds and this process may be important in the application of specific salivary hormones such as epidermal growth factor (EGF) to assist healing, particularly as the level of such cytokines is under autonomic control (McGurk et al. 1990).

One emerging theme in ageing research is that a number of changes may not have their origins in age-related cellular changes but rather in the cellular microenvironment. Each can influence the other, in a manner in which cause and effect are difficult to separate. Thus an understanding of age-related changes in the structural microenvironment of the skin constitutes an important factor in any discussion of wound healing and ageing.

Ageing skin

Structural and functional changes caused by intrinsic ageing are now recognised in the skin of elderly individuals, independent of environmental insults (Fenske & Lober, 1986). The thickness of the epidermis probably does not change with age: Whitton & Everall (1973) removed human epidermis from dermis with sodium bromide. After drying, the epidermis was stretched on a template to the original in vivo area, and the results revealed that there were no age or sex differences in thickness. The number of epidermal cell layers remain unchanged as does the ability of the stratum corneum to prevent transepidermal water loss (Leyden, 1990). However the stratum corneum may become a less effective barrier to certain substances: the topical application of 50 % ammonium hydroxide solution to aged skin reveals a decrease in the initial response time characterised by the appearance of tiny follicular vesicles (Grove et al. 1982). Cell replacement in the human stratum corneum slows in men aged over 60 y but not in females (Baker & Blair, 1968) The dermo–epidermal junction flattens which produces an atrophic appearance

Fig. 1. Collagen density and orientation. Polarising microscopy of picrosirius red stained normal human skin, removed from a non-sun-exposed site (upper inner arm) of subjects with defined health status, illustrating the decrease in fibre bundle density with age. The thick, wavy basket-weave collagen bundles in the young become increasingly parallel and less dense with age. Bar 50 μm. *A*, 28-y-old female; *B*, 47-y-old female; *C*, 82-y-old female.

because of retraction of both the epidermal papillae and the microprojections of basal cells into the dermis (Kurban & Bhavan, 1990). This has physiological consequences which may explain the loss of proliferative capacity of the aged epidermis and render the epidermis less resistant to shearing forces (Lavker, 1979). The number of Langerhans cells, which function as antigen-presenting cells within the epidermis, decrease with age by 50% in sun-exposed skin, and by 14% in non-sun-exposed skin from the ages of 24–65 y (Gilchrest et al. 1979; Thiers et al. 1984). The number of dopa-positive melanocytes declines by about 10–20% per decade progression through the lifespan (Quevedo et al. 1969).

The dermis is subdivided into 2 layers: the superficial papillary dermis containing a rich supply of blood vessels, nociceptors and thermoreceptors, and a deep reticular layer of predominantly connective tissue in which fibroblasts are surrounded by a matrix of collagen, elastin and proteoglycans. Skin surface patterns show a loss of orderliness of the sharp angular patterns with age in sun-exposed skin and are thought to reflect changes in dermal architecture; however, in non-sun-exposed skin few age-related differences are observed (Lavker et al. 1980). Collagen is the predominant component of the dermis and elastin comprises only 5% of the dry weight. Collagen has a high tensile strength and prevents tearing from overstretching; elastin maintains normal skin tension but is readily extensible in order to accommodate joint movement. The most abundant form of collagen in skin is type I; type III is widely distributed but at a much lower concentration than type I in adult skin (Lovell et al. 1987). Lavker et al. (1987) reported an apparent increase in collagen density with age in man, most probably secondary to a decrease in space between individual bundles. Collagen also appears as primarily straight, loosely woven fibres as compared with the finding of rope-like bundles of dense fibres in young human dermis (Fig. 1). Skin thickness as assessed by ultrasound and radiographic imaging decreases with age after the 7th decade, and is consistently thinner in females, a phenomenon that becomes exaggerated after the menopause (Shuster et al. 1975; Rigal et al. 1989). Tan et al. (1982), however, reported an increase in skin thickness measured using pulsed ultrasound during maturation until the age of 20 y with a subsequent linear decline with age. The latter is related to a possible decrease in total collagen content with age. For example, the average linear decline in female skin collagen has been reported as 2.1% per postmenopausal year for about 15 y (Brincat et al. 1987). Magnetic resonance imaging of sun-protected human skin revealed significantly more mobile water protons in the upper dermal region of skin of subjects aged over 70 y compared with the upper dermis of subjects under 40 y, an increase which has been related to an increase in total water content and to an apparent decrease in collagen and proteoglycan content (Richard et al. 1993).

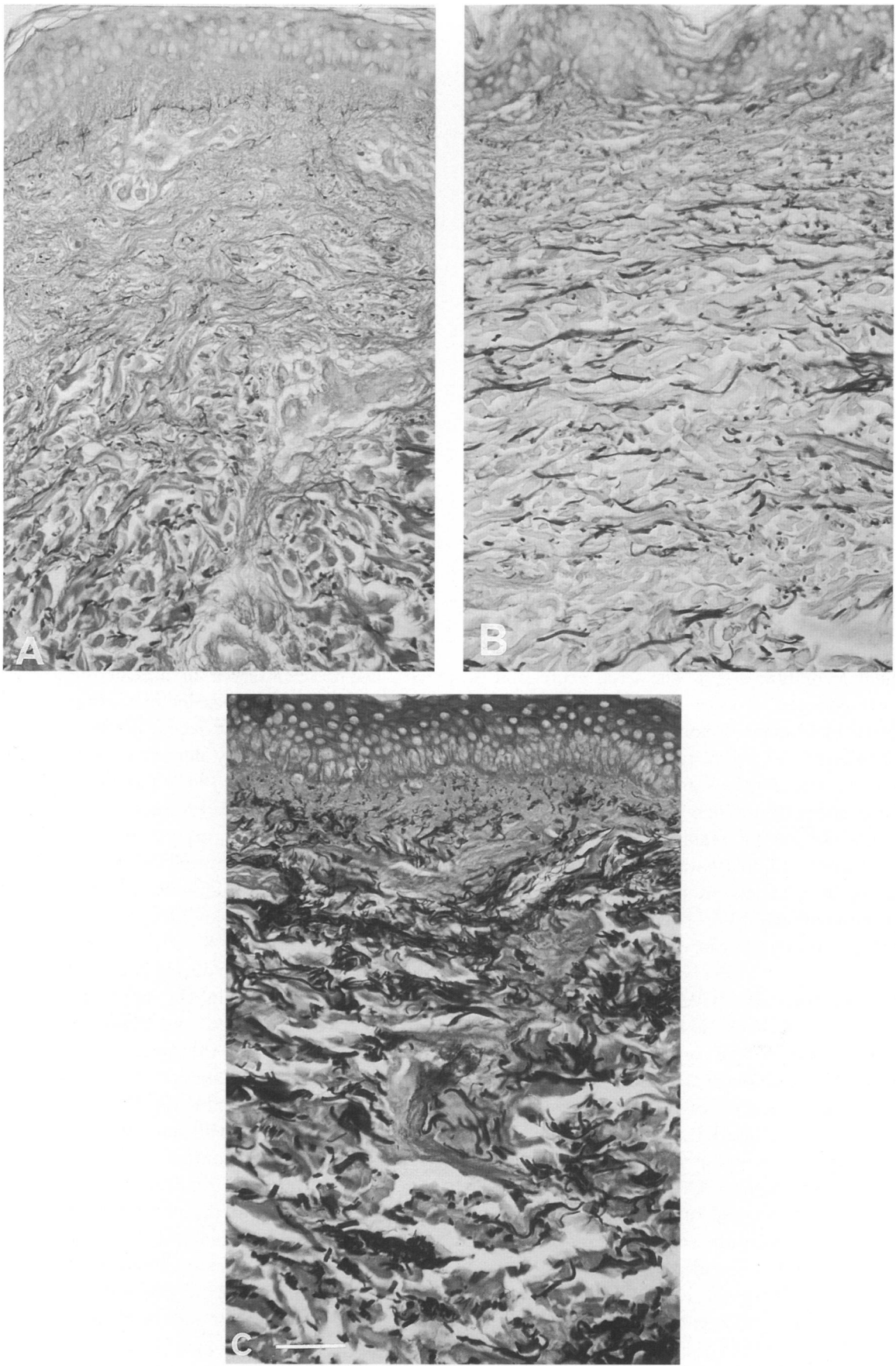
The physical properties of collagen change with age. Skin collagen becomes less soluble: murine and human collagen show an age-associated decline in acetic acid solubility (Maekawa et al. 1970). The crosslinking of collagen changes with age: the divalent reducible cross-links are partly replaced by non-reducible links. In human skin the nonreducible his-hydroxylysinorleucine (HLNL) link increases with age, although in rodent skin there is no detectable his-HLNL (Reiser et al. 1992). One consequence of the absence of lysyl-oxidase derived crosslinks with age could be the exposure of lysine residues to interactions such as nonenzymatic glycation (Reiser et al. 1987). The latter process increases with age with the possible modification of collagen (Miksik & Deyl, 1991; Dyer et al. 1993). In black aged skin, similar features affecting the dermo-epidermal junction and collagen alterations have been described (Herzberg & Dinehart, 1989), but black skin is normally more vascular, and the collagen fibres are more densely arranged (Montagna & Carlisle, 1991).

A reduction in the dermal cell population of human skin occurs (Kurban & Bhavan, 1990) with a decrease in the number and size of fibroblasts and mast cells (Kligman & Lavker, 1988). Elastin production and remodelling appear to increase with age. Serial passage of human skin fibroblasts cultivated from one female aged 25 y showed an increase in elastase-type endopeptidase activity (Homsey et al. 1989). Robert et al. (1988*b*), however, quantified elastin in sun-protected human skin in subjects presenting with hyperlipidaemia and hypertension using image analysis and found no change in the number of elastic fibres per unit area but an increase with age in the relative surface area and length of fibres, suggesting apposition of elastic-type material to pre-existing fibres. Lavker (1979) reported a marked increase in elastogenesis in the papillary dermis with age and the appearance of 8–11 nm microfilaments. Fibroblasts in this area contained extensive amounts of rough endoplasmic reticulum, consistent with active protein synthesis.

In addition to the reported increase in elastin content with age, the site and orientation of elastin within the sun-protected dermis undergoes alterations which may have marked functional consequences. The characteristic candelabra-like organisation of

elastin fibres at the dermo–epidermal junction disappears and thin broad sheets appear in the reticular dermis (Lavker et al. 1987) (Fig. 2). In young rats elastic fibres are arranged in an orderly pattern of relatively straight fibres, whereas collagen is curled randomly within this scaffold. During maturation collagen bundles unravel, become more densely packed, orientate parallel to the direction of tensile load, and in doing so bend and fracture the elastin fibres. Individual elastin fibres become tortuous, and intimately intertwine with collagen, possibly resulting in reduced compliance of the dermis (Lavker et al. 1987; Imayama & Braverman, 1989). Vitronectin (the S protein of complement) is deposited on dermal elastic fibres after the age of 30 y, may be an important substratum for migrating cells, and may protect such cells from complement lysis (Dahlback et al. 1989). The expression of basement membrane components does not appear to change during adulthood: collagen IV gene expression is high in fetal fibroblasts, but declines rapidly in the neonate to remain constant well into old age; laminin B1 and B2 expression do not appear to change with age (Olsen & Uitto, 1989). Type VII collagen is the predominant component of the anchoring fibrils (attachment structures present in the basement membrane zone). The basal expression of type VII collagen in vitro from keratinocytes and fibroblasts of 4 human donors revealed a decline with age; however, the production of collagen VII in response to transforming growth factor-β (TGF-β) remained unchanged (Chen et al. 1994). TGF-β is a low molecular weight family of proteins (TGF-β1, 2, 3) which stimulate collagen and fibronectin formation in a variety of fibroblast cell lines and certain populations of fibroblasts are also stimulated to undergo proliferation in vitro by TGF-β (Roberts et al. 1986).

Glycosaminoglycans (GAGs) are polysaccharides composed of disaccharide units made of amino sugars coupled to uronic acid or a neutral sugar, and subsequently linked to a protein core. They contribute to the maintenance of the essential microenvironment for cell adhesion, migration and proliferation. In the human dermis the most commonly encountered GAGs are hyaluronan (HA), dermatan sulphate in the papillary dermis, and small amounts of keratan, heparan and chondroitin sulphate (Laurent, 1970). Immunostaining has revealed an increase in keratan sulphate from the age of 50 y, a decrease in chondroitin-4-sulphate with age, and a decline in chondroitin-6-sulphate after the age of 60 y in the basal lamina (Willen et al. 1991). The latter is related to the maintenance of dermo–epidermal contact and a lack results in a defect in the basal lamina with associated blister formation (Fine & Couchman, 1989). Dermatan sulphate increases in the papillary dermis after the age of 50 y (Willen et al. 1991). However, in skin biopsies from patients with Werner's syndrome (which is associated with certain manifestations of an ageing phenotype and premature death in humans), a decrease in dermatan sulphate has been reported (Fleischmajer & Nedwich, 1973). Lagier (1960) has shown that the glycosaminoglycan content of human skin decreases up to the age of 10 y, remains constant to 60 y and then increases slightly. Fleischmajer et al. (1972), on the other hand, reported a decrease from middle to old age. Longas et al. (1987) reported a decrease in dermatan sulphate by 48% and hyaluronan by 77% between 19 and 75 y in women undergoing mastectomies and mammoplasties. In human tissue Smith et al. (1962) showed a decrease from childhood to old age of HA and chondroitin sulphate. In rats the content of HA and chondroitin sulphate decreased by 50% until maturity but then remained constant, illustrating the potential difficulties of extrapolating from one species to another (Schiller & Dorfman, 1960). More recently, no significant differences in hyaluronan concentration with age were observed in human thigh skin, but hyaluronan polymers became increasingly covalently linked to collagen with a decline in epidermal staining and increased papillary dermal staining in the 9th decade (Meyer & Stern, 1994). The latter observations were unfortunately made on autopsy specimens where the clinical diagnoses ranged from leukaemia to gastric carcinoma, illustrating the imprecision of most ageing data. In vitro studies using human skin WI-38 fibroblasts have shown an increased incorporation of ^{14}C glucosamine and ^{35}S sulphate into cellular and media heparan sulphate during late population doublings, chondroitin sulphate appeared unchanged whilst dermatan sulphate and hyaluronan decreased in both the cells and the medium during in vitro ageing (Chiarugi & Vannucchi, 1976; Sluke et al. 1981). Hyaluronan production is increased by progeric and Werner's syndrome fibroblasts (Brown, 1989), but caution is required in extrapolating results from premature ageing syndromes to normal human ageing. Expression of the proteoglycan decorin decreases in fibroblasts from infantile progeroid patients (Beavan et al. 1993). Decorin binds via its core protein to collagen fibrils both in vivo and in vitro and is thought to influence the rate of fibril formation and final fibril size: additionally it interacts with fibronectin, inhibiting fibroblast adhesion. Decorin also binds to TGF-β and in vivo inhibits the increased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production of extracellular matrix (Border et al. 1992). Thus the true in vivo age-related glycosaminoglycan changes are difficult to unravel as biochemical, in vitro fibroblast, and immunostaining studies do not always give the same results. A possible consensus is that chondroitin sulphate production decreases with age, keratan and heparan sulphate increase, whilst dermatan sulphate and hyaluronan changes are controversial.

Microvascular wall alterations of widening and atrophy occur with age, and of interest is an identical abnormality in the skin of juvenile diabetics which has been proposed as a model system for the study of aspects of cutaneous ageing (Braverman, 1989). Alkaline phosphatase staining of blood vessels in female genitalia revealed collapse and disorganisation of the microcirculation (Montagna & Carlisle, 1979). Biopsies from buttocks of 80–93 y subjects revealed vessels with abnormally thin walls and basement membranes (Braverman & Fonferko, 1982). Subcutaneous tissue acts as a shock absorber and modulates heat loss. Atrophy of this region with age should be qualified by site: tissue thins in the palms, soles and pretibial regions but remains constant in the triceps region until the age of 70 y (Balin & Kligman, 1989).

The number of hair follicles in human skin in the telogen phase of the growth cycle (resting phase) greatly decreases with age in a generalised manner, but the structure of the follicles remains unchanged apart from the loss of bulb melanocytes (Montagna & Carlisle, 1990). Sebum acts as a protective emollient lipid film and retards water evaporation. The rate of sebum secretion from sebaceous glands declines from the age of 20 y in females, but not until 80 y in males as testosterone is a major influence on secretion (Pochi et al. 1979). The production of sweat from sweat glands also declines with age and active glands are fewer in number (Silver, 1965).

Thus it appears that skin morphology indeed alters with age, with a decline in dermal thickness, a decrease in the number of cells and the number of hair follicles in anagen (growth phase), a flattening of the dermo–epidermal junction and a disorganised microcirculation. Collagen content has been reported to decline and its physical properties change, the glycosaminoglycan composition alters with an overall decrease in content, and elastin quantity may increase. Against this background we can now consider age-related effects on wound healing.

THE HEALING PROCESS

Inflammation

The inflammatory response is an inevitable sequel to cutaneous injury in order to re-establish skin homeostasis. Following an initial insult to the skin, endothelial integrity is disrupted, leading to the extravasation of blood. Platelets aggregate when exposed to subendothelial types IV and V collagen, a process involving the secretion of the von Willebrands factor (Barnes et al. 1980). Platelets induce haemostasis firstly via aggregation, which is enhanced by ADP and thromboxane, secondly by vasoconstriction mediated by ADP and serotonin, and thirdly via phospholipid rearrangement in the platelet membrane facilitating factor V binding, with the subsequent stimulation of the coagulation cascade. The resultant fibrin gel is an important early component of wound repair as judged by impaired wound healing in animals depleted of fibrinogen (Clark and Henson, 1988). Thrombin generated by the cascade recruits additional platelets which release fibronectin, fibrin and thrombospondin which all promote platelet adhesion (Ginsberg et al. 1988). The release from platelet alpha-granules of the cytokines TGF-β, TGF-α and platelet derived growth factor (PDGF) facilitates subsequent cellular chemotaxis, proliferation and extracellular matrix production (Ross et al. 1986; Derynck, 1988; Sporn & Roberts 1992) and the coagulation cascade itself produces mediators such as thrombin and kallikrein which are chemoattractant to circulating leucocytes.

The endothelium expresses specific adhesion receptors that recognise the β-2 integrin family of receptors which are upregulated on circulating leucocytes by chemoattractant factors. The interaction of these cell–cell receptors is responsible for leucocyte margination at the site of injury (Tonneson, 1989; Clark, 1991). Factors promoting neutrophil–endothelial cell adherence include interleukin-1 (IL-1) and tumour necrosis factor (TNF) produced by monocytes and endothelial cells. Diapedesis of neutrophils is encouraged by an increased capillary permeability caused by vasodilators such as serotonin and bradykinin. Fibrin and fibronectin act as a provisional matrix for the influx of monocytes and macrophages. The principle action of neutrophils and macrophages is that of phagocytosis of contaminating organisms and detritus; macrophages in addition produce cytokines

Fig. 2. Changes in elastin density and orientation. Aldehyde fuchsin Gomori staining of normal human skin, removed from a non-sun-exposed site (upper inner arm) of subjects with defined health status, illustrating an increase in elastin fibre density with age. There is an associated loss of fine subepidermal cascades and an increase in randomly orientated small deep dermal fibres. Bar, 100 μm. *A*, 28-y-old female; *B*, 47-y-old female; *C*, 82-y-old female.

such as TGF-α, TGF-β and PDGF. PDGF is the most potent mitogen in serum for cells of mesenchymal origin, which include fibroblasts and smooth muscle cells (Ross et al. 1986). It acts as a potent chemoattractant for fibroblasts, neutrophils and monocytes, and at higher concentrations (20–40 ng/ml) activates leucocytes (Tzeng et al. 1985). Basic fibroblast growth factor (FGF-2) which is mitogenic for fibroblasts and endothelium (Gospodarowicz et al. 1979) and keratinocyte growth factor (FGF-7) which is mitogenic to keratinocytes are upregulated and released (Werner et al. 1992). An increased amount of granulation tissue associated with stimulation of wound healing in rats has been attributed to FGF-2 (Buntrock et al. 1982).

Closely following the entry of the macrophages into the wound site are the lymphocytes. A role for T lymphocytes was inferred from studies showing that lympholytic agents such as cyclosporin A and steroids impaired wound fibroplasia, whilst lymphotrophic agents such as IL-2 increased wound collagen and breaking strength (Barbul, 1988). Secretory products (lymphokines) affect in vitro fibroblast migration, proliferation and protein synthesis: TGF-β, when applied to linear incisions in rats, leads to increased collagen production and fibroblast infiltration (Mustoe et al. 1987). Human recombinant IL-2 administered to rats augments wound breaking strength with a rise in collagen content (Barbul et al. 1986). However, the administration of γ-interferon (γ-IFN) by an osmotic infusion pump to mice resulted in a reduced thickness and collagen production of the fibrous capsule surrounding the subcutaneous pump (Granstein et al. 1989), illustrating the subtle interactions between the lymphokines which determine the eventual wound healing result. Early in the course of wound healing T-helper cells may promote macrophage activity and are activated, in part, by antigen presented by the macrophage. This stimulation mediated by the helper cells is quickly overwhelmed by the T-suppressor cells, with the resulting down-regulation of the system, a lack of which could lead to chronic inflammation (Regan & Barbul, 1991).

There are specific age-related changes in the coagulation and immune systems which may influence wound healing. Ageing increases the ability of collagen to aggregate platelets in rat skin, and thus, in theory, could enhance the initial stages of the inflammatory response (Grigorova-Borsos et al. 1988). Platelet release reaction (as assessed by serotonin release in response to collagen and thrombin) increases between middle-age and old-age in rats (Yonezawa et al. 1989). Granulocyte adherence to nylon fibre increases after the age of 60 y in man, particularly in females (Silverman & Silverman, 1977). The adherence of mouse peritoneal macrophages to fibronectin and collagen type I increases with age, the former being related to the increase in cell surface receptors which recognise the arginine–glycine–aspartic acid–serine (RGDS) peptide sequence within fibronectin (Iiyama et al. 1992). Ageing is associated with a significant reduction in neutrophil respiratory burst activity but has no effect on the ability to phagocytose or kill bacteria (Lipschitz & Udupa, 1986). Despite findings suggestive of possible pro-inflammatory activity, Kligman (1979) has reported that acute inflammatory reactions are slower and less intense in the aged. When ammonium chloride is placed in a cup on young human skin, a blister generally rises within 15 min or less; in aged skin it frequently takes over 60 min for the blister to rise. However, in contrast, Forscher & Cecil (1958) studied the biochemistry of the palatal acute inflammatory response in the rat and reported that 'the tissue reaction to injury in the older animal follows the same metabolic pattern as in the younger animal, with perhaps a slightly reduced responsiveness'. Cohen et al. (1987), in their study of outbred mice, reported that on d 6 and 10 postwounding the subjective scores for the number of inflammatory cells 'generally were higher' in the mature and aged groups than in young *Peromyscus*.

Langerhans cells are reduced in number and undergo functional and morphological changes with age (Thiers et al. 1984; Gilchrest et al. 1987). In one study γ-IFN was injected daily into the thigh skin of young (23–28 y) and elderly (76–88 y) subjects for 3 d and a punch biopsy was taken at d 5. Only the young volunteers showed a marked increase in Langerhans cells in the perivascular infiltrate and dermis, whereas the old keratinocytes showed a decrease in HLA-DR expression (Gilhar et al. 1992). The delayed-type hypersensitivity reaction to recall antigens, reflecting lymphocyte function, is reduced to a significant extent in aged skin, and markedly after the age of 75 y (Mackay, 1972). There is a senescent decline in cytotoxic lymphocytic activity as a consequence of ageing, with a reduction in cell lysis (Bloom, 1991). A major part of the basis for the loss of T-cell function is an inability of T cells to respond to activation signals transmitted through the membrane binding of specific stimulatory signals (Song et al. 1993). T lymphocytes are reduced in absolute number and there is an impaired ability to proliferate when stimulated by a variety of mitogens, and this impairment has been ascribed to a defective production

and response to interleukin-2 (Cossarizza et al. 1989; Nagelkerken et al. 1991). Miller (1984) demonstrated a decrease with age in the number of precursor cells producing IL-2, but no change in the amount produced per responding cell, indicating that only a subset of precursor cells with increasing age are capable of IL-2 production. Evidence indeed exists that at least a proportion of lymphocytes in the aged also do not express IL-2 receptors when exposed to appropriate stimuli (Orson et al. 1989).

Interleukin-1 is involved in promoting lymphocyte proliferation and lymphokine production and there is a marked increase in females of IL-1 following the oestrogen surge at ovulation which is absent post-menopausally (Cannon & Dinarello, 1985). TGF-α is a potent angiogenic factor in wound repair, and IL-6 acts as a helper factor in T-cell activation and may contribute to the fibroblast response to wounding (Kohase et al. 1986). It has been reported that stimulated mononuclear cell production of IL-6 and tumour necrosis factor (TNF)-α and spontaneous IL-6 production are increased in cells from aged human donors (Fagiolo et al. 1992). However, murine alveolar macrophages produce significantly decreased levels of TNF-α in old mice, with a decline in phagocytosis, thus emphasising site-dependence (Higashimoto et al. 1993). CD4+ cells from aged mice produce similar amounts of IL-2 and increased amounts of IL-3, 4, 5 and γ-IFN in early and late cultures compared with cells from young mice (Hobbs et al. 1993). An explanation for the altered profile of cytokine production with age may be attributed to an increase in CD4+ cells compared with naive cells. The notion that alterations in cell population subsets explain age-related changes as opposed to fundamental cellular defects is extremely important. Overall there appear to be complex, site-specific and in some cases gender-influenced, changes in the cytokine profile with age.

Glucose utilisation in lymphocytes and macrophages depends on species and concurrent morbidity and is partly a glucocorticoid-mediated effect. Age decreases the capacity for glucose utilisation in these cell types, which may contribute to a decline in phagocytosis and proliferation (Costa Rosa et al. 1993). Macrophage functional decline may contribute to impaired wound repair in aged mice: young mice injected subcutaneously at a punch-biospy wound site with rabbit antimacrophage serum exhibited delayed closure of the wounds, similar to that of the aged untreated mice (Cohen et al. 1987). The application of peritoneal macrophages from young animals to the cutaneous wounds of the aged mice accelerated the rate of repair to levels almost comparable to those of the young animals (Danon et al. 1989). Similar investigations have not been conducted in humans.

In summary, the literature would suggest an increase in platelet and macrophage adhesion to substrates within the wound with age, but a decline in rodent macrophage function. T-lymphocyte proliferation and function decline, although the production of specific lymphokines involved in the healing process increases. A complex situation may exist with an accumulation of specific cells such as memory T lymphocytes and a polarisation of cells into those expressing specific receptors and those which do not. Obviously, a systematic study of the effects of age on inflammation has not been carried out, yet this has utmost relevance not only to wound repair, but also to a wide variety of physiological and pathological processes.

PROLIFERATION

This phase of wound repair is characterised by the formation of granulation tissue with the migration and proliferation of fibroblasts, the reformation of an epithelial barrier, neovascularisation and the production and deposition of extracellular matrix molecules particularly collagen.

The fibroblast

At approximately 72 h following injury, fibroblasts infiltrate the wound site in response to PDGF, TGF-β, and the provisional fibrin and fibronectin-rich matrix, which guides this cellular migration. Fibronectin receptors of the β-1 integrin family are expressed on fibroblasts under the influence of TGF-β and epidermal growth factor (EGF) (Bellas et al. 1991), and thus the fibronetin matrix functions as a scaffold for cellular movement (Hsieh & Chen, 1983). Fibroblasts then undergo the process of proliferation which begins with the acquisition of 'competence'. The latter is associated with the entry of G0 (quiescent) cells into the early G1 phase of the cell cycle, and is controlled by PDGF, FGF-2 and calcium phosphate. Further progression towards DNA synthesis and mitosis is dependent on the cytokines EGF and insulin-like growth factor-1 (IGF-1). Once activated, the fibroblast produces β-interferon (IFN-β) which acts as an autocrine growth inhibitor. The production of collagen is regulated primarily by TGF-β (Sporn & Roberts, 1992) but

FGF-2 has also been implicated in the production of collagen in normal wound repair (Broadley et al. 1989). PDGF indirectly stimulates collagen synthesis by stimulating fibroblasts to produce TGF-β (Pierce et al. 1989). Large quantities of fibronectin, hyaluronan and collagen (type I and, to a greater extent, type III) are initially deposited to form the immature granulation tissue. Fibronectin plays a role in cell adhesion and may be involved in matrix remodelling in view of its promotion of phagocytosis of particles by macrophages and fibroblasts (Grinnell et al. 1981).

In addtion to its function in extracellular matrix production, the fibroblast is involved in the process of wound contraction. Fibroblasts undergo a phenotypic change with large bundles of actin becoming arranged parallel to the long axis of the cell. These so-called myofibroblasts were first identified by electron microscopy in 1971 by Gabbiani et al. as cells which share phenotypic characteristics of both fibroblasts and smooth muscle cells, and stain for both actin and myosin (Darby et al. 1990). Other studies have questioned this hypothesis, considering the myofibroblast to be a quiescent fibroblast, and suggesting that contraction occurs because of the interaction between the fibroblast and the extracellular matrix (Rudolph et al. 1989).

Ageing is said to affect fibroblast function in a number of ways. The fibroblast in normal human dermis becomes a quiescent cell, with poorly developed endoplasmic reticulum and numerous dense bodies (Pieraggi et al. 1985). There is a reduction in the number and size of fibroblasts, with alterations in the lipid composition of both the fibroblast and mitochondrial membrane as a function of age in man (Schroeder et al. 1984). There is a global decline in all types of cell motility of human fibroblasts when comparing 20 and 66 y subjects, a decrease which is independent of chemotactic gradients; unfortunately the site of origin of the cells was not stated (Pienta & Coffey, 1990). Human skin fibroblasts of fetal and adult origin display distinctive migratory phenotypes resulting from the production of a soluble migration stimulatory factor by fetal cells (Grey et al. 1989), and this may help to explain the decline of fibroblast chemotaxis with age. In fetal tissue hyaluronan is the major component of the extracellular matrix deposited by fibroblasts and is secreted even at confluence in vitro in contrast to adult fibroblasts. A hyaluronan-rich matrix may facilitate the migration of fetal fibroblasts (Chen et al. 1989). The chemotactic response to fibronectin is also reduced in ageing human cells after the 6th decade (Albini et al. 1988). Serum from human subjects aged 60–64 y was shown to be inhibitory to the migration of fetal lung fibroblasts in vitro compared with serum from young subjects, but this difference was only significant in females (Kondo et al. 1989). These purported decreases in motility have not been shown to affect the number of cells within a wound: the number of fibroblasts within a postincisional wound were reported to peak at d 4 in groups of young and old mice with a subsequent decline in young mice. No such decrease was observed in the aged mice (Holm-Pederson et al. 1974).

Fibronectin appears with invading fibroblasts, is intimately associated with bundles of procollagen type III, and decreases when collagen matures into bundles (Kurkinen et al. 1980). Human skin fibroblasts showed an increase in the rate of fibronectin and total protein synthesis per cell in late passage cells, but the turnover rate of protein and fibronectin did not differ between late and early passage (Shevitz et al. 1986). In contrast, Takeda et al. (1992) reported that fibronectin mRNA declines with human fibroblast in vitro ageing but the message from cells of elderly donors actually increased with age, when comparing fetal fibroblasts to those of 51–80 y subjects (the site of cell origin was not stated and the subjects had not been controlled for concurrent morbidity). Despite these reports of increased fibronectin synthesis with age, immunostaining has revealed a decrease of fibronectin associated with collagen in human elderly skin, although site-specificity of the biopsies was not controlled for (Vitellaro-Zucarello et al. 1992).

Altered fibroblast proliferation as a function of age has been postulated. Over 20 y ago, Hayflick (1965) reported that human diploid cells underwent a finite and reproducible number of population doublings in vitro. Schneider (1979) documented a decrease in the in vitro life-span of fibroblasts from aged donors with a decrease in cell population doubling time and a decrease in the percentage of replicating cells. The response of human skin fibroblasts to in vitro wounding demonstrates a delay in the ability to re-establish a confluent monolayer with increasing in vivo donor age; however, only 3 subjects were assessed and the biopsy site for the 2 adults was not specified (Muggleton-Harris et al. 1982). This age-dependent loss of proliferative potential may be genetically programmed: microinjection of senescent fibroblast mRNA into young fibroblasts inhibits the ability of the young cells to synthesize DNA (Lumpkin et al. 1986). Introduction of a normal human chromosome 4 into specific immortal cell lines resulted in the cells losing their immortal phenotype (Ning & Pereira-

Smith, 1991). This could be explained by the defective message being the result of accumulated environmental damage. An inverse relationship has been observed between the donor age of hamster dermal fibroblasts and maximum in vitro proliferative capacity (Bruce & Deamond, 1991). A direct correlation has been made between the latter and the repair efficiency of the biopsy site, as assessed by 'days to closure'; however, these changes were not progressive beyond 12–18 months of age. A reduction in migration and proliferative capacity of rat fibroblasts from cutaneous explants occurred with increasing animal age (Lombard & Masse, 1987). Kondo et al. (1988) reported that serum from aged humans did not inhibit cell proliferation to a greater extent than serum from young donors, and this has been substantiated by Wistrom et al. (1989) who found that human fibroblasts proliferate at the same rate in young or old cow serum. However, Kondo & Namaguchi (1985) demonstrated that old rabbit serum stimulates the proliferation of fetal rabbit skin fibroblasts.

A loss of responsiveness of in vitro aged human fibroblasts to dexamethasone, insulin, EGF and partly purified PDGF from human platelets has been reported (Phillips et al. 1984; Rattan & Derventzi, 1991), and when comparing the response of fibroblasts from 20–27 y and 60–82 y subjects to FGF-7 (Stanulis-Praeger & Gilchrest, 1986). Cells cultured from donors suffering from the progeroid syndrome show a significantly reduced mitogenic response to insulin, serum and EGF (Harley et al. 1981; Colige et al. 1991). Diabetes mellitus is associated with premature senescence of cultured dermal fibroblasts, and an elevated glucose concentration decreases the mean population doublings and inhibits the response to PDGF (Sibbit et al. 1989).

Data have suggested that the G0 exit, or the competence phenomenon, is intact in ageing cells in vitro, and that the proliferative block occurs at a later stage (Shigeoka & Yang, 1990). It could be inferred that a lack of, or abnormal response to, EGF or IGF-1 is involved. The number of EGF receptors remains stable during in vitro cellular ageing, and their degradation process is qualitatively and quantitatively unchanged (Phillips et al. 1984). The internalisation of EGF is equally effective in human foreskin fibroblasts of both early and late passage cultures (Matrisian et al. 1987), and there appears to be no difference in EGF and purified human PDGF stimulated tyrosine-specific phosphorylation of their respective membrane receptors in WI-38 fibroblast lines with in vitro ageing (Gerhard et al. 1991). By contrast, Reenstra et al. (1993) reported a decrease in EGF receptor number and internalisation with age in early-passage newborn versus young versus old adult donors. Unfortunately no description of subject characterisation was given. Young, aged and progeric dermal fibroblasts were analysed with respect to DNA synthesis in response to plasma insulin-like activity, and a reduced response of the aged and progeric cells was noted (Harley et al. 1981). Rosenbloom & Goldstein (1976) found a decline in the number of insulin receptors in cultured human fibroblasts as a function of donor age, whereas insulin binding appears to increase (Hollenberg & Schneider, 1979).

The effects of PDGF were assessed in the same model and an increased concentration of the cytokine was required for maximal proliferation in the aged cells, suggesting that the cells become progressively less sensitive to PDGF with age. Fibroblasts from Werner's syndrome patients show a reduced mitogenic response to PDGF and FGF in vitro, although PDGF binding and receptor number per cell were found to be unaltered (Bauer et al. 1986). Using PDGF-BB which binds with high affinity to both A and B-type PDGF receptors, Winkles et al. (1990) reported that normal and progeric fibroblasts have a similar number of PDGF receptors, and receptor autophosporylation occurs normally. Thus it would appear that the ageing defect may be in both the primary and secondary messenger systems.

The production of cytokines may also be altered with age: in vitro PDGF-BB induction of PDGF-A chain from old donor fibroblasts was reported to be reduced, although PDGF-β receptors were enhanced in old donor strains (Karlsson & Paulsson, 1994). Insulin-like growth factor binding protein 3 is over-expressed in cultures of Werner's syndrome fibroblasts and from a 57-y-old subject, and acts to inhibit IGF-1 mediated DNA synthesis by 70–80% (Moerman et al. 1993). The expression of IL-1, which in turn stimulates protease production such as collagenase, was increased in early passage fibroblasts from 2 aged individuals (Kumar et al. 1993). Of interest is a report observing that although the rate of decline of the growth rate of human fibroblasts was related to donor age, the sensitivity of the human skin fibroblast lines to the growth inhibitory effects of β-IFN was independent of the population doubling, and thus to in vitro age (Tamm et al. 1984).

In summary, fibroblast migration in vitro declines with age, but the number of cells within an acute wound is not altered. Fibroblast proliferation declines with a loss of responsiveness in vitro to specific stimulatory cytokines, but no decrease in response to inhibitors: these changes may however only occur

between young and middle-age. The production of cytokines alters with age: IL-1 increases, possibly affecting matrix remodelling, and PDGF-AA decreases. Serum from aged animals may in some cases stimulate proliferation to a greater extent than serum from young donors, possibly overcoming the decline in cellular responsiveness to cytokines. There is a complete absence of data assessing the function or responses of wound fibroblasts at differing stages of repair in wounds of individuals of different ages.

Contractility

Wound contraction is critical to repair and is thought to be modulated by the fibroblast. At different stages of repair the fibroblast responds in differing ways to growth factors. Cells from granulation tissue at a later stage of repair (21–28 d) contract an in vitro tissue equivalent with enhanced ability compared to cells from the early stages of repair (7–14 d) (Finesmith et al. 1990). TGF-β1 enhances contraction of cells from granulation tissue, with the greatest effect on those fibroblasts from later stages of repair, and FGF-2 at low doses has a modest effect, but at higher doses is inhibitory to all cells. If an application of high-dose FGF-2 is followed by TGF-β1 it actively enhances tissue contraction in highly contractile late stage wound fibroblasts but delays gel contraction by cells from early granulation tissue.

Of interest are the integrin receptors which are intimately involved in cell–matrix interactions. One major family associated with fibroblasts is defined by the alpha-chain of the vitronectin receptor, and binds vitronectin or fibronectin. They also act as collagen receptors when cryptic binding sites are exposed by arginine–glycine–aspartate peptide within the extracellular matrix ligand. These cryptic collagen receptors have been implicated in the contraction of collagen gels, thus highlighting the possible major role of the integrins in wound contraction (Agrez et al. 1991). The cell adhesive properties of fibronectin derived from late passage human foreskin fibroblasts are decreased compared with that of early passage cells (Chandrasekhar & Millis, 1980) and this age-related difference has been shown to be due to a deletion of 5000–10000 Da of the amino-acid sequence at an internal site of the fibronectin molecule (Sorrentino & Millis, 1984).

Kono et al. (1990) observed that the contraction of a type I collagen gel by human dermal fibroblasts diminished from childhood (0–15 y) to early adulthood but not beyond. However, there was no significant difference in the contractile abilities of cell isolates in a hydrated collagen lattice when comparing fibroblasts from 4 month and 30 month rats (Wang et al. 1988). Reed et al. (1994) have reported a similar contractile response of human fibroblasts from donors of varying ages to TGF-β1 in collagen gels. Late passage human diploid fibroblasts displayed an increase in contractile behaviour on a collagen gel, and the f-actin content of high PDL cells was substantially greater than that of low PDL cells. It has been postulated that the elevated f-actin levels in the aged cells could promote increased cellular contractility, via interaction with the fibronectin integrin receptor (Gibson et al. 1989). This claim has been substantiated by Marks et al. (1990) who reported an increase in the ability of postauricular fibroblasts from aged donors (74 y) undergoing rhytidectomy to contract a collagen lattice when compared with fibroblasts from young donors (49 y); subjects were again not assessed for concurrent morbidity.

Epithelialisation

The process of epithelialisation is essential for the reconstitution of cutaneous barrier function. The epidermis is a stratified squamous epithelium, which in the unwounded state is in contact with the basement membrane zone via basal keratinocyte intermediate filaments. The latter insert into electron-dense hemidesmosomes containing the bullous pemphigoid antigen. Extending from the hemidesmosomes are the anchoring filaments which span the lamina lucida, rich in laminin and nidogen, and attach to the lamina densa that contains collagen IV and heparan sulphate. The lamina densa is connected to the dermal interstitial collagen fibres by type VII collagen anchoring fibrils, an absence or abnormality of which is manifested clinically as the blistering disease dystrophic epidermolysis bullosa (Marinkovich, 1993). Type IV collagen and laminin are known to influence epidermal wound closure by supporting epidermal cell attachment (Stenn & Malohtra, 1992). In a matter of hours following injury, prior to epithelial proliferation, cell migration occurs from the wound margin and residual appendages such as hair follicles (Winter, 1962). Migration is under the control of FGF-7 (Tsuboi et al. 1993), TGF-α released from keratinocytes, lymphocytes, eosinophils and monocytes, and IL-1 and TGF-β from keratinocytes, leucocytes and platelets (Coffey et al. 1987; Rappolee et al. 1988; Ju et al. 1993). PDGF-B mRNA is strongly expressed in epithelial cells and fibroblasts at the wound site but is

suppressed at complete epithelialisation (Antoniades et al. 1991). Migration of the basal cells occurs more quickly if the basement membrane remains intact. Closure occurs more slowly if the latter is destroyed, as the migrating epithelium must reconstruct the basement membrane zone. When the basement membrane is disrupted, the epithelial cell comes into contact with fibronectin, dermatan and chondroitin sulphate, and certain unfamiliar collagen types. Woodley et al. (1991) have shown that collagen types I and III and fibronectin induce the locomotion of keratinocytes. EGF and IGF-1 enhance neonatal skin keratinocyte migration, but only when collagen IV and fibronectin are the substrates (Ando & Jensen, 1993). Laminin however inhibits this locomotion and thus could function as a 'stop signal' in the intact basement membrane. The effects of growth factors on the contraction of a collagen lattice by human keratinocytes have been studied: PDGF and EGF have been shown to be the most effective, the keratinocytes extending processes to form a network within the lattice (Assouline et al. 1992).

The substrate of early migrating cells is rich in fibrin and fibronectin (Clark et al. 1982), subsequently the epithelial cells themselves secrete fibronectin and type V collagen and ultimately laminin and type IV collagen, which constitute the newly formed basement membrane (Stenn & Malhotra, 1992). TGF-β and IGF-1 may cause the cells to secrete fibronectin, and TGF-β also stimulates the expression of receptors for the latter, facilitating cell-matrix adhesion (Heino et al. 1989; DeLapp & Dieckman, 1990). Within 1–2 d the epidermal cells behind the migrating front begin to proliferate under the influence of FGF-7, TGF-α and EGF, a process inhibited by TGF-β (Barrandon & Green, 1987), and the fibrin and fibronectin provisional matrix dissipates (Clark et al. 1982). Fibronectin inhibits the terminal differentiation of human keratinocytes, and thus its removal may be a signal for cellular phenotypic change (Adams & Watt, 1989). EGF receptor mRNA is expressed by proliferating keratinocytes within 1 d of wounding in partial thickness injuries in pigs but is suppressed at d 9, which corresponds to complete re-epithelialisation (Antoniades et al. 1993).

A study of aged subjects has revealed antibody staining to laminin and collagen IV are found at the wound edge at d 2 and in a complete linear band at d 14 in human wounds caused by the Simplate instrument; unfortunately the wounds varied greatly in size and the number of subjects was small (Olerud et al. 1988). The rate of epithelialisation of open wounds is slowed in the elderly and in the palatal mucosa of aged rats (Holt et al. 1992; Butcher & Klingsberg, 1963). This phenomenon may be site-dependent, as no appreciable change in the rate of cell proliferation with age occurs in the epidermis of the rat ear (Morris et al. 1989). Holt et al. (1992) reported in human subjects aged 18–55 y and over 65 y that the rate of re-epithelialisation of a 2×2 cm split thickness thigh wound, as assessed visually, was more rapid in the young group. Partial thickness wounds in old mice, assessed by planimetry, showed a delay in re-epithelialisation; however, the definition of 'old' was not given (Cox et al. 1992). The healing of superficial blisters following the application of ammonium hydroxide to human volunteers was assessed by the re-formation of skin surface markings: the elderly group (65–75 y) had a delayed response, although there was considerable interindividual variation in the aged cohort (Grove, 1982).

Early passage in vitro keratinocytes from newborn subjects proliferate more rapidly than early passage adult keratinocytes (Gilchrest, 1983). With age the epidermal labelling index decreases and the epidermal turnover time increases (Grove & Kligman, 1983). Keratinocytes have been reported to show an age-related reduction in mitogenic response to various growth factors (Rattan & Derventzi, 1991) and an increased sensitivity to negative growth modulators. IFN inhibits the growth of human keratinocytes and those from older adult donors are significantly more sensitive to the growth inhibitory effects (Peacocke et al. 1989). Epidermal cell-derived thymocyte-activating factor (ETAF) closely resembles IL-1 in terms of biological properties, stimulates the growth of keratinocytes, and is secreted in significant amounts from keratinocytes. Adult human keratinocytes produce markedly reduced amounts of ETAF compared with newborn but there was no significant difference in the effects of exogenous ETAF on cell proliferation (Sauder et al. 1988), even though aged human cells produce increased amounts of an antagonist to the IL-1 receptor (Garmyn et al. 1992). Conditioned media from newborn, young adult and old adult keratinocyte cultures were used to assess effects on newborn foreskin cells. Keratinocyte growth was significantly reduced with media from increasingly aged cells (Stanulis-Praeger & Gilchrest, 1986). Newborn keratinocyte cultures respond to 10–15 ng/ml of EGF with up to 40% increase in cell yield after 1 wk, yet adult cells remain unresponsive (Gilchrest, 1983) and a hypothalamic extract (FGF-7) produced a 200-fold increase in cell number, total protein and colony size, as compared with less than 75-fold increase for adult keratinocytes. Unfortunately these reports also il-

lustrate problems with the use of 'ageing' to represent newborn and young adults as opposed to young versus old adults, and in comparing cells from differing body sites. By contrast, TGF-β1 mRNA expression increases during in vitro passage of human foreskin keratinocytes and also with increasing age of the donor. Of extreme importance is the finding that the relative amounts of both TGF-β1 and TNF-α message in cultured human keratinocytes varies according to the site of origin (Compton, 1993).

Thus the rate of epithelialisation may decline in rodents and humans with age, although the reports to date can be criticised in terms of subject characterisation and subjective qualitative measures of epithelialisation. In vitro studies have revealed a decline in keratinocyte responsiveness to stimulatory cytokines, an increased response to inhibitory cytokines, and a decline in IL-1 production, although these studies concentrated on neonatal versus young adult cells. One study has shown an increase in site-dependent TGF-β1 message with in vitro ageing; however, the effects of TGF-β1 on aged keratinocytes are unknown and would be extremely interesting.

Angiogenesis

Angiogenesis occurs in connection with the formation of the neomatrix and fibroplasia. Endothelial cells have a number of important functions in wound healing in addition to angiogenesis. As alluded to previously, the expression of specific adhesion molecules facilitates leucocyte diapedesis, and endothelial cells produce both components of the extracellular matrix and matrix proteases, for example collagenase. The latter is important in order to digest basal lamina and extracellular matrix components to permit cellular migration (Gross et al. 1982). Other proteases are also elaborated by endothelial cells such as plasminogen activator (PA) and PA-inhibitor-1. FGF-2 causes a net increase in proteolytic activity and angiogenic stimulation, whereas TGF-β resulted in a net increase in antiproteolytic activity (Pepper et al. 1987, 1992). However, TGF-β has been shown to be angiogenic in vivo (Roberts et al. 1986) and this paradox may be explained by the indirect action of TGF-β to attract cells, e.g. macrophages, which secrete angiogenic factors. Subsequently, migration of the endothelial cells occurs into the injured area. The extracellular matrix is important in migration, fibrin causing endothelial cells to retract and migrate, as does the proteoglycan heparin (Kadish et al. 1979; Azizkham, 1980). Recently, scatter factor (hepatocyte growth factor) secreted by fibroblasts and smooth muscle cells has been shown to stimulate endothelial motility and migration (reviewed in Strain, 1993, and Rosen et al. 1991).

At 24 h following endothelial cell migration, proliferation occurs. Growth control in vitro is constrained by the maintenance of the cell monolayer which, when disrupted, removes the contact-inhibition of movement (Heimarck & Schwartz, 1988). Thrombin may play a role in cell division and the extracellular matrix has been shown to affect proliferation: collagen IV promotes the development and maintenance of a mitotically inactive phenotype whereas purified types I and III collagen promote cell mitosis and migration (Madri & Williams, 1983). Endothelial cells produce at least 2 distinct types of mitogens, one of which is biochemically and immunologically related to PDGF. Macrophage production of FGF-2, TGF-α and TNF-α promotes endothelial proliferation, although significant neovascularisation still occurs with leucocyte depletion (Sholley et al. 1978), as cells such as keratinocytes elaborate proliferative agents such as EGF, PDGF and vascular endothelial growth factor (VEGF) (Brown et al. 1992; Ansel et al. 1993). Formation of a 3-dimensional structure subsequently occurs through which blood flow begins. To stabilise these delicate vessels, human endothelial cells elaborate a basal lamina-like matrix that is ultrastructurally similar to the basal lamina formed in vivo. Cells form capillary tubes more rapidly when plated on the basement components types IV and V collagen, compared with types I and III (Madri & Williams, 1983).

There is a dearth of literature concerning the effects of ageing on angiogenesis. Endothelial cells from different age groups produce extracellular matrices which are similar in ultrastructure but differ in their macromolecular composition (Kramer et al. 1985): thrombospondin was a major constituent of the matrix produced by newborn cells, but was virtually absent in the matrix elaborated by the adult cells. It has been reported that the corneal endothelium retains its proliferative capacity in rabbits but not in cats or primates, and this is of importance when selecting animal models of ageing (Baroody et al. 1987). A significantly reduced proliferative response of endothelial smooth muscle cells occurs with age in the thoracic aorta (Spagnoli et al. 1985). Serum from aged rats has been found to be toxic to endothelial cells in vitro, and an elevated level of very low density lipoprotein (VLDL) appears to be the toxic agent (Arbogast et al. 1984).

Yamaura & Matsuzawa (1980) found that the rate

of capillary growth as assessed visually declined in old rats compared with young rats in a transparent chamber model installed in a dorsal skin flap. Holm-Pederson & Zederfeldt (1973) assessed xenon 133 clearance as a measure of wound blood flow, and showed no difference with age of the animal; however, this is not a sensitive indicator of angiogenesis. Of interest is the report that in the young rat, as in other vertebrates, elements of the microvasculature are periodic acid-Schiff (PAS) negative and become increasingly positive beyond the half-life span, which reflects the increasing carbohydrate content that occurs with ageing. During acute wound repair in the old animals the microvasculature is initially PAS negative, but at 2 months is strongly PAS positive, reproducing the process of rapid ageing, and thus representing a potentially useful model of the latter (Sobin et al. 1992).

Senescent human endothelial cells contain large amounts of IL1α mRNA, IL1α being a potent inhibitor of in vitro proliferation. Treatment of these cells with antisense oligodeoxynucleotides to IL1α transcript prevented cell senescence and extended the proliferative lifespan (Maier et al. 1990). Tumour necrosis factor (TNF) reduces the growth rate and in vitro life-span of endothelial cells, and these effects increase as a function of in vitro cell age (Shimada et al. 1990). TNF inhibits the proliferation of, and increases neutrophil adhesion to, endothelial cells (Stolpen et al. 1986). Phenotypic changes such as an increase in cell size, and a decrease in colony-forming efficiency are attributed to TNF, both of which occur during normal cellular ageing (Gamble et al. 1985). TGF-β reduces the adhesive response of endothelial cells to TNF-α and diminishes the adhesion of neutrophils to endothelial cells. TGF-β may act to inhibit the latter by inhibiting the expression or up-regulation of adhesion-promoting molecules, e.g. TGF-β may down-regulate the cell surface expression of ICAM-1 (Gamble & Vadas, 1988). These authors have reported that the response of umbilical vein endothelial cells to TGF-β is reduced markedly with in vitro age (however 'old' cells were only 9–14 d in culture). Rat aortic smooth muscle cells from aged animals show a normal production of TGF-β1 but fail to respond to the autocrine growth inhibitory effect resulting in increased proliferation (McCaffrey & Falcone, 1993). Thus aged endothelium may exhibit an increased adhesive response to leucocytes, increased response and adhesion to TNF-α, and increased IL-1 production, with a subsequent decline in endothelial cell proliferation, and with increased proliferation of vascular smooth muscle cells.

REPAIR AND REMODELLING

During this extensive phase collagen is produced and remodelled, neovascularisation regresses, and a mature scar is formed. Fibronectin is removed within a few weeks, and hyaluronan is mainly replaced by heparan sulphate in the basement membrane and dermatan and chondroitin sulphate in the interstitium (Clark & Henson 1988). A balance exists between collagen formation and destruction by collagenases, with the ratio of type III collagen to type I collagen decreasing. The collagen in granulation tissue possesses stable aldimine cross-links (derived from hydroxylysine aldehyde) which are present in embryonic tissue but not to any significant extent in normal skin. During healing they are replaced by 2 labile aldimine bonds concomitant with an increase in solubility (Bailey et al. 1973). Collagen synthesis is orchestrated by a number of growth factors. IL-1 and TNF increase collagen synthesis and TGF-β enhances overall collagen deposition both by increasing synthesis and decreasing degradation, e.g. by increasing synthesis of protease inhibitors such as TIMP (tissue inhibitor of metalloproteases) and inhibiting procollagenase synthesis (Overall et al. 1989; Edwards et al. 1992). IFN, TNF-α and prostacyclin inhibit collagen synthesis and a number of factors such as PDGF, EGF, FGF and IL-1 induce the expression of collagenase from fibroblasts and keratinocytes (Mignatti et al. 1988; Jeffrey, 1992; Phillips & Wenstrup, 1992). It should be noted that disorders associated with abnormal or excessive matrix collagen deposition such as keloids and hypertrophic scars do not occur in rodents, a point emphasising that rodent and human wound healing is far from identical (Mast, 1992).

The structure of the extracellular matrix differs with age, and itself affects the production of collagen by fibroblasts. When cultured in a monolayer on plastic or at the surface of a collagen gel, EGF inhibits collagen synthesis regardless of donor age (Colige et al. 1990). However, when embedded in a three-dimensional collagen lattice EGF does not inhibit, and sometimes stimulates, collagen production from young donors, whilst that of the older donor fibroblasts was inhibited. The number of EGF receptors remained unchanged with age, although the site of cell origin was not stated (Colige et al. 1990). Lovell et al. (1987) have reported that the proportion of type III collagen increases to a variable degree from age 65 y and over in human abdominal skin removed at autopsy or laparotomy, and the immunoreactivity of type III also increases with age. In one report the latter only occurred after the age of 79 y (Vitellaro-

Zuccarello, 1992). Uitto (1990) has shown that human skin biopsies from incompletely characterised subjects show a reduced collagen production with age after the age of 30 y, and yet, according to Fleischmajer et al. (1993), collagen dermal content remains the same with age. Others suggest that the content may even increase with age (Clausen, 1962). The putative reduced collagen content of ageing skin observed may be a consequence of both reduced synthesis as shown by the decreased production of mRNA for type I collagen in late passage WI-38 fibroblasts (Furth, 1991; Quaglino et al. 1989), and increased degradation (Mays et al. 1991).

Conflicting results in vitro have questioned the reduction in synthesis observed: fibroblast cultures obtained from donor rats of increasing age showed a direct association between age and collagen production (Mollenhauer & Bayreuther, 1986). In addition, pig fibroblasts aged in vitro produce increased levels of fibronectin and collagen III (Martin et al. 1990). Human fibroblasts from donors aged between 26 and 92 y produce similar quantities of collagen I mRNA in response to TGF-β1 (Reed et al. 1994). However, in vitro studies assessing collagen and proteoglycan synthesis in passaged fetal cells and from donors aged up to 80 y showed decreases of up to 25% with a decrease of both type I and III collagen mRNA (Takeda et al. 1992). Interestingly, cultured Werner's fibroblasts produce significantly raised levels of both type I and III collagen mRNA (Arakawa et al. 1990). Type VI collagen is distributed throughout the dermis and increases in fibrotic diseases such as keloids and scleroderma, and gene expression declined in Werner's syndrome (Hatamochi et al. 1993).

With this confused picture as a baseline, what is known of granulation tissue and collagen production in wounds of subjects of different ages? Holt et al. (1992) reported that age had no effect on collagen synthesis in humans at d 14 in collagen sponges implanted in split-thickness thigh wounds but non-collagenous protein production declined. The amount of granulation tissue in subcutaneously implanted cellulose sponges in rats aged 6 and 14 months (middle-aged) showed no difference at d 4, by d 7 the older animals exhibited greater amounts but at d 14 the situation was reversed (Holm-Pederson & Zederfeldt, 1971*a*). Incisional wounds in aged mice produced significantly more DNA, RNA and protein synthesis at d 14 and d 21 postwounding (Holm-Pederson et al. 1974). Heikkinen et al. (1971) reported no differences with rodent age in hydroxyproline content in granulomas from d 7 to d 60. Up until d 21, the consumption of oxygen, synthesis of protein and proline incorporation into hydroxyproline were greatest in the young, a situation reversed from d 35 to d 60, indicating that collagen breakdown is greatest in the young initially, and greatest in the old from d 35. The rate of maturation of collagen in wounds up to d 21 was greater in old animals as compared with young, as shown by a loss of birefringence with denaturation, a process paralleled by the amount of cross-linkage of the collagen (Holm-Pederson & Viidik, 1972).

Despite these findings, the ability of insulin, EGF, and IGF to stimulate protein synthesis in human lung fibroblasts declines with age in vitro (Ballard & Read, 1985). EGF induces the stimulation of noncollagenous protein synthesis in young fibroblast donors, although ageing fibroblasts do not respond. Studies using fibroblasts from donors suffering from premature ageing syndromes such as Werner's syndrome corroborate these findings: cells have a reduced proliferative response to the effects of FGF and PDGF (Bauer et al. 1986).

Elastin is produced by mesenchymal cells and TGF-β1 and β2 are potent enhancers of elastin mRNA expression (Kahari et al. 1992); this effect is abrogated by TNF-α and FGF-2 (Davidson et al. 1993). Sparse and contradictory evidence exists as to the production of elastin with age. Elastin production is markedly elevated in progeric fibroblasts and the response to TGF-β1 is absent (Mariagabriella & Davidson, 1993). However, tropoelastin production declines in vitro with donor age over 70 y and with passage 30 for fetal fibroblasts (Sephel & Davidson, 1986).

The relative level of elastin mRNA in incisional skin wounds in pigs is low, but TGF-β applied directly to the wound increases mRNA levels (Quaglino et al. 1991). In human scars elastin was reported to be sparsely and randomly distributed, although the subepidermal elastin plexus had reformed. The quantity of elastin decreased from atrophic scars through normal to hypertrophic scars, with no elastin in keloids (Bhangoo et al. 1976). Schwartz (1977) assessed the deposition of elastin in incisional wounds in rodents by light and electron microscopy, and reported that new fibres were deposited 1 wk postwounding in the deep layers, and at 3 wk were present in the dermis with levels even greater than normal at 4 months. The production of elastin in wounds of young and aged human subjects has not been assessed.

Mechanical properties of the scar

The tensile strength of wounds increases with time despite a decrease in the rate of collagen synthesis,

and is thought to reflect the structural modification and augmentation of collagen bundles (Doillon et al. 1985). The ultimate tensile strength of the skin is positively correlated with the mass-average diameter of the collagen fibrils (Parry et al. 1978). The relative quantities of glycosaminoglycans appear to exert an influence: chondroitin sulphate promotes rapid fibril growth with small fibrils, whilst dermatan sulphate promotes slower fibril growth with larger ultimate fibrils, the latter contributing to increased tensile strength because of intrafibrillar cross-links (Hall, 1976). Human skin maintains its extensibility up to the 7th decade as opposed to its elasticity which decreases from an early age and the creep relaxation time which declines linearly with age (Escoffier et al. 1989). At low loads, the same stress produces a greater strain in aged skin, yet at increased loads skin shows increasing stiffness with age (Ridge & Wright, 1966). Wijn (1980) reported decreased stiffness in aged skin but this may reflect the complicated preconditioning used or the different body sites assessed. Using indentometry as a measure, a decline in elasticity with age occurred less rapidly in nuns than in other women, possibly due to hormonal influences or exposure to sunlight (Robert et al. 1988*a*). However, in vitro assessment of cadaver skin elasticity at low loads revealed no age or sex differences (Jansen & Rottier, 1957).

The question as to whether there is an alteration in the mechanical properties of healing wounds with age remains unclear. The rate of wound disruption has been reported to increase in frequency with subject age, but unfortunately again no control for concurrent morbidity was made (Halasz, 1968; Mendoza & Postlethwait, 1970). Holm-Pederson & Zederfeldt (1971*b*) concluded that in vitro skin wound breaking strength did not vary between old and young rats up to d 21 postwounding; however, the breaking strength of the tissue from the aged animals was impaired when the tissue thickness (tensile strength) was taken into account. They concluded that this was a result of the more organised and complex fibre arrangement in the wounds of the young rats. Sussman (1973) found a reduced breaking strength for skin in old rats, but an unchanged tensile strength (as the thickness of the wounds was greater in the young animals), and a significantly increased extensibility of the wounds in younger animals. More recently, Beck et al. (1993) reported a 'reverse in age-related impairment of wound healing' with an increase in breaking strength of incisional wounds in old Fischer 344 rats receiving rTGF-β1 topically, but the numbers of animals used were small (n = 2 for 1 μg or 4 μg application). Cox et al. (1992) assessed the breaking strength of full-thickness incisional wounds in young and old Wistar rats and concluded that at d 14, 21 and 28 post-wounding the 'tensile strength' was greater in the young rats; however, skin thickness was not assessed. TGF-β2 application reportedly enhanced the strength of the wounds of the old animals, most markedly at d 7 compared with later time-points.

In contrast, Quirinia & Viidik (1991) have shown that incisional wounds heal equally well in both young and old rats in mechanical terms, with only an increased stiffness as noted previously. However, when the wounds were made ischaemic, healing of the old animal tissue was impaired by 40–65% as compared with the young animals. The capacity to regain skin strength following surgical laceration and healing by primary intention has been reported to be greater in older mice (Ershler et al. 1984).

Only one study (Sandblom et al. 1953) has assessed the tensile strength of experimental surgical incisions in man, comparing tensile strengths in only 4 individuals over 70 y, and in only 8 under 70 y. Not surprisingly, the subjects had known associated pathology. Less force was required to disrupt the wounds of the older subjects. The belief that wound strength is impaired as a function of age is thus questionable at present.

THE INFLUENCE OF EXTRINSIC FACTORS ON WOUND HEALING

A number of environmental factors affect the process of wound healing specifically related to ageing, and may in part explain the axiom that wound healing is impaired with age, as controls for these factors are often neglected.

Infection

Retardation of wound healing occurs with gross bacterial infection. A number of studies that claim wound healing is impaired as a direct effect of ageing have failed to control for concurrent infection, for example the often-cited studies of DuNouy (1916). It has been stated that ageing may contribute to an increased susceptibility to cutaneous infections because of a reduction in production of epidermal thymocyte-activating factor (Sauder, 1989) and impaired macrophage function, and this could contribute to an indirect association between age and impaired wound healing.

Drug effects

Certain drugs have been reported specifically to affect processes intrinsically associated with wound healing in the aged. Procaine, a local anaesthetic, has no effect on the proliferation of in vitro aged fibroblasts, in contrast to its stimulatory effects on young cells (Pigeolet et al. 1988). Aged human fibroblasts are more sensitive to inhibition of protein synthesis by the aminoglycoside antibiotic paromomycin, which also reduces the lifespan of old cultured fibroblasts (Holliday & Rattan 1984; Luce & Bunn, 1987). These findings could have wide-ranging implications, and further studies looking at the effects of drugs on these aspects of wound healing are required.

Physical stress

Ageing cells are less sensitive to physical stress such as heat shock with a concomitant reduction in heat shock protein production, the latter being important in maintaining homeostasis under stress (Fargnoli et al. 1990). Fibroblasts from old rats are increasingly sensitive to the effects of hypertonicity on the reduction in collagen synthesis (Hata, 1990). Free radicals are produced by cellular metabolism and are mediators of both acute and chronic inflammation, acting as chemoattractants for phagocytes. Free radicals also induce tissue injury by enhancing phagocyte-mediated tissue injury and inducing DNA breaks (Robson & Heggers, 1989). The free radical hypothesis of ageing was proposed over 30 y ago, but there is no established direct causal link between oxidative stress and ageing rates (reviewed by Sohal, 1993). The antioxidant enzyme activities of catalase are reduced with age with an increased sensitivity of ageing cells to oxidants and free radicals (Shindo et al. 1991); however, this latter claim has been disputed (Gutman et al. 1987). A reduction in the activity of skin glutaminase in aged rodents, which is important for nitrogen provision in the process of DNA repair, may be of importance in wound healing (Keast et al. 1989). An age-related decline in the capacity of cells for DNA repair has been reported for mouse fibroblasts (La Belle & Linn, 1984), although this claim has been refuted by some researchers (Mullaart et al. 1989). The flux of oxidised products increases in old fibroblasts, requiring an increased amount of NADPH to maintain cellular glutathione levels, and the ratio of NADPH to NADP has been shown to be reduced in aged cells, indicating that aged cells cannot dispose of oxidised products readily (Jongkind et al. 1987). As the endothelium ages it becomes more susceptible to damage by lipid oxidation products. Chronic oxidative stress decreases the endothelial barrier function, and vitamin E has been reported to delay the cumulative changes in the endothelial barrier caused by the former (Boissonneault et al. 1990).

Oxygen is necessary for all aspects of wound healing. The tensile strength of incisional wounds in rats increases as the ambient oxygen concentration rises (Ninikoski, 1969). Anaemia and hypovolaemia may have profound effects on wound healing with a reported reduction in collagen synthesis. Vasomotor tone plays a critical role, for example beta-blockers and pain cause vasoconstriction with subsequent tissue hypoxia. Ageing reduces the response of cutaneous blood flow in the forearm to the direct effects of heat, and thus has been shown to affect the vasomotor tone (Richardson, 1989). Postischaemic reactive hyperaemia in aged skin is reduced compared with young skin (Hagisawa et al. 1991), and may therefore contribute to tissue hypoxia. Ischaemia has been reported to reduce the mechanical properties of healing wounds in ageing rats by 45–60% compared with young animals (Quirinia & Viidik, 1991).

Advanced glycosylation end-products (AGE products) are accumulated products of the Maillard reaction which alter the structural properties of proteins, reducing their susceptibility to degradation, and are central to the 'glycation hypothesis of ageing' (Dyer et al. 1993). AGE-modified proteins induce human monocytes to generate IGF-1 and PDGF (Kirstein et al. 1992) which have major effects on matrix remodelling and protein synthesis. This finding is often overshadowed by in vitro fibroblast studies.

Nutrition

Malnutrition causes immunosuppression and severe protein deficiency which impairs collagen synthesis. Trace elements and vitamins are also important in wound healing. Vitamin C is necessary for proline and lysine hydroxylation and enhances human dermal fibroblast collagen synthesis in vitro (Gessin et al. 1993). It has been demonstrated that there is an increased urinary excretion of vitamin C in older animals, resulting in reduced retention of this vitamin by body tissues (Abt et al. 1962). The elderly are more likely to be deficient in vitamin C, mainly secondary to poor dietary intake (Olsen & Hodges, 1987).

Zinc is required for RNA and DNA polymerisation. Ageing is accompanied by an increased cellular permeability to zinc, which may contribute to growth

inhibition (Monticone et al. 1987). Copper, thiamine, riboflavin and pyridoxine are required for collagen cross-linkage, and iron is a cofactor for hydroxylation (Ruberg, 1984). Ageing rats have been shown to lose the ability possessed by weanling rats to utilise large quantities of dietary fat efficiently, and gain the ability to use excess amounts of carbohydrate, with a subsequent increase in collagen deposition during wound healing (Hennessey et al. 1991). This may be of relevance to cutaneous wound healing in man.

CONCLUSIONS

The study of wound healing can be seen as a shibboleth for ageing research in many contexts. It is frequently stated that ageing impairs wound healing, but this assertion is usually unsubstantiated. Animal models have often been inappropriate and human subjects have usually not been adequately characterised. Comparisons have often failed to control for wound site and character, and the use of the term 'ageing' has often been confusing. These difficulties must all be addressed. Further research is essential to distinguish between the changes associated with ageing per se, and those which are a result of poor methodology.

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REFERENCES

ABT AF, SCHUCHING SV, MARTINSBURG W (1962) Aging as a factor in wound healing. *Archives of Surgery* **86**, 627–632.

ADAMS JC, WATT FM (1989) Fibronectin has been shown to inhibit the terminal differentiation of human keratinocytes. *Nature* **340**, 307–309.

AGREZ MV, BATES RC, BOYD AW, BURNS GF (1991) Arg–Gly–Asp-containing peptides expose novel collagen receptors on fibroblasts: implications for wound healing. *Cell Regulation* **2**, 1035–1044.

ALBINI A, PONTZ B, PULZ M, ALLAVENA G, MENSING H, MULLER PK (1988) Decline of fibroblast chemotaxis with the age of donor and cell passage number. *Collagen and Related Research* **8**, 23–37.

ANDO Y, JENSEN PJ (1993) Epidermal growth factor and insulin-like growth factor 1 enhance keratinocyte migration. *Journal of Investigative Dermatology* **100**, 633–639.

ANSEL JC, TIESMAN JP, OLERUD JE, KRUEGER JG, KRANE JF, TARA DC et al. (1993) Human keratinocytes are a major source of cutaneous platelet-derived growth factor. *Journal of Clinical Investigation* **92**, 671–678.

ANTONIADES HN, GALANOPOULOS T, NEVILLE-GOLDEN J, KIRITSY CP, LYNCH SE (1991) Injury induces *in vivo* expression of platelet-derived growth factor (PDGF) and PDGF receptor mRNAs in skin epithelial cells and PDGF mRNA in connective tissue fibroblasts. *Proceedings of the National Academy of Sciences of the USA* **88**, 565–569.

ANTONIADES HN, GALANOPOULOS T, NEVILLE-GOLDEN J, KIRITSY CP, LYNCH SE (1993) Expression of growth factor and receptor mRNAs in skin epithelial cells following acute cutaneous injury. *American Journal of Pathology* **142**, 1099–1109.

ARAKAWA M, HATAMOCHI A, TAKEDA K, UEKI H (1990) Increased collagen synthesis accompanying elevated mRNA levels in cultured Werner's syndrome fibroblasts. *Journal of Investigative Dermatology* **94**, 187–190.

ARBOGAST BW, BERRY DL, NEWELL CL (1984) Injury of arterial endothelial cells in diabetic, sucrose-fed and aged rats. *Atherosclerosis* **51**, 31–45.

ASSOULINE M, CHEW SJ, THOMPSON HW, BEUERMAN R (1992) Effects of growth factors on collagen lattice by human keratinocytes. *Investigate Ophthalmology and Visual Science* **33**, 1742–1755.

AZIZKHAM RG (1980) Mast cell heparin stimulates migration of capillary endothelial cells *in vitro*. *Journal of Experimental Medicine* **152**, 931–944.

BAILEY A, BAZIN S, DELAUNEY A (1973) Changes in the nature of the collagen during development and resorption of granulation tissue. *Biochimica et Biophysica Acta* **328**, 383–390.

BAKER H, BLAIR CP (1968) Cell replacement in the human stratum corneum in old age. *British Journal of Dermatology* **80**, 367–372.

BALIN AK, KLIGMAN AM (1989) *Aging and the Skin*, p. 16. New York: Raven Press.

BALLARD FG, READ LC (1985) Changes in protein synthesis and breakdown rates and responsiveness to growth factors with ageing in human lung fibroblasts. *Mechanisms of Ageing and Development* **30**, 11–22.

BARBUL A (1988) Role of T-cell dependent immune system in wound healing. *Progress in Clinical and Biological Research* **266**, 161–175.

BARBUL A, KNUD-HANSEN J, WASSERKRUG HL, EFRON G (1986) Interleukin-2 enhances wound healing in rats. *Journal of Surgical Research* **40**, 315–319.

BARNES MJ, BAILEY AJ, GORDON JL, MACINTYRE DE (1980) Platelet aggregation by basement membrane-associated collagens. *Thrombosis Research* **18**, 375–388.

BAROODY RA, BITO LZ, DEROUSSEAU CJ, KAUFMAN PL (1987) Ocular development and aging. 1. Corneal endothelial changes in cats and in free-ranging and caged rhesus monkeys. *Experimental Eye Research* **45**, 607–622.

BARRANDON Y, GREEN H (1987) Cell migration is essential for sustained growth of keratinocyte colonies: the role of TGF-alpha and EGF. *Cell* **50**, 1131–1137.

BAUER EA, SILVERMAN N, BUSIER DF, KRONBERGER A, DEUEL TF (1986) Diminished response of Werner's syndrome fibroblasts to growth factors PDGF and FGF. *Science* **234**, 1240–1243.

BEAVAN L, QUENTIN-HOFFMAN E, SCHONHERR E, SNIGULA F, LEROY JG, KRESSE H (1993) Deficient expression of decorin in infantile progeroid patients. *Journal of Biological Chemistry* **268**, 9856–9862.

BECK LS, DEGUZMAN L, LEE WP, XU Y, SIEGAL MW, AMENTO EP (1993) One systemic administration of Transforming growth factor-β1 reverses age- or glucocorticoid-impaired wound healing. *Journal of Clinical Investigation* **92**, 2841–2849.

BELLAS RE, BENDORIS R, FARMER SR (1991) EGF activation of vinculin and b1 integrin gene transcription in quiescent Swiss 3T3 cells. Regulation through a protein kinase C-independent pathway. *Journal of Biological Chemistry* **266**, 12008–12014.

BHANGOO KS, QUINLIVAN JK, CONNELLY JR (1976) Elastic fibers in scar tissue. *Journal of Plastic and Reconstructive Surgery* **57**, 308–313.

BLOOM E (1991) Functional importance of CD4+ and CD8+ cells in cytotoxic lymphocyte activity and associated gene expression.

Impact on the age-related decline in lytic activity. *European Journal of Immunology* **4**, 1013–1017.

Boissoneault GA, Hennig B, Wang Y, Wood CL (1990) Aging and endothelial barrier function in culture: effects of chronic exposure to fatty acid hydroperoxides and vitamin E. *Mechanisms of Ageing and Development* **56**, 1–9.

Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD et al. (1992) Natural inhibitor of transforming growth factor-β protects against scarring in experimental kidney disease. *Nature* **360**, 361–364.

Braverman IM (1989) Elastic fiber and microvascular abnormalities in aging skin. *Clinics in Geriatric Medicine* **5**, 69–90.

Braverman IM, Fonferko E (1982) Studies in cutaneous aging. II. The microvasculature. *Journal of Investigative Dermatology* **78**, 444–448.

Brincat M, Kabalan S, Studd JWW, Moniz F, de Trafford J, Montgomery J (1987) A study of the decrease of skin collagen content, skin thickness, and bone mass in the postmenopausal woman. *Obstetrics and Gynaecology* **70**, 840–844.

Broadley KN, Aquino AM, Woodward SC, Buckley-Sturrock A, Sato Y, Rifkin DB et al. (1989) Monospecific antibodies implicate bFGF in normal wound repair. *Laboratory Investigations* **5**, 571–575.

Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF et al. (1992) Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. *Journal of Experimental Medicine* **176**, 1375–1379.

Brown WT (1989) Studies of premature ageing syndromes. In *Aging and the Skin* (ed. A. M. Balin & A. K. Kligman), pp. 93–99. New York: Raven Press.

Bruce SA, Deamond SF (1991) Longitudinal study of *in vivo* wound repair and *in vitro* cellular senescence of dermal fibroblasts. *Experimental Gerontology* **26**, 17–27.

Buntrock P, Jenzsch KD, Heder G (1982) Stimulation of wound healing, using brain extract with fibroblast growth factor (FGF) activity. I. Quantitative and biochemical studies into the formation of granulation tissue. *Experimental Pathology* **21**, 46–53.

Butcher EO, Klingsberg J (1963) Age, gonadectomy and wound healing in palatal mucosa of the rat. *Oral Surgery* **16**, 482–492.

Cannon J, Dinarello E (1985) Increased plasma IL1 activity in females after ovulation. *Science* **227**, 1247–1249.

Chandrasekhar S, Millis AJT (1980) Fibronectin from aged fibroblasts is defective in promoting cellular adhesion. *Journal of Cell Physiology* **103**, 47–54.

Chen WY, Grant ME, Schor AM, Schor SL (1989) Differences between adult and foetal fibroblasts in the regulation of hyaluronate synthesis: correlation with migratory activity. *Journal of Cell Science* **94**, 577–584.

Chen YQ, Mauviel A, Ryynanen J, Sollberg S, Uitto J (1994) Type VII collagen gene expression by human skin fibroblasts and keratinocytes in culture: influence of donor age and cytokine response. *Journal of Investigative Dermatology* **102**, 205–209.

Chiarugi YP, Vannucchi S (1976) Surface heparan sulphate as a control element in eukaryotic cells. *Journal of Theoretical Biology* **61**, 459–475.

Clark RAF (1991) Cutaneous wound repair: a review with an emphasis on integrin receptor expression. In *Wound Healing* (H. Janssen, R. Rooman & J. I. S. Robertson), pp. 7–15. Petersfield: Wrightson Biomedical.

Clark RAF, Dellapelle P, Manseau E, Lanigan JM, Dvorak HF, Oclvin RB (1982) Fibronectin and fibrin provide a provisional cell matrix for epidermal cell migration during wound epithelialisation. *Journal of Investigative Dermatology* **79**, 264–269.

Clark RAF, Henson PM (1988) *The Molecular and Cellular Biology and Wound Repair*, pp. 5–9. New York: Plenum.

Clausen B (1962) Influence of age on connective tissue. *Laboratory Investigations* **11**, 229–234.

Coffey RJ, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL et al. (1987) Production and auto-induction of transforming growth factor-α in human keratinocytes. *Nature* **328**, 817–820.

Cohen BJ (1987) Accelerated wound repair in old Deer mice (Peromyscus maniculatus) and White-footed mice (Peromyscus leucopus). *Journal of Gerontology* **42**, 302–307.

Cohen BJ, Danon D, Roth GS (1987) Wound repair in mice as influenced by age and antimacrophage serum. *Journal of Gerontology* **42**, 295–301.

Colige A, Nusgens B, Lapiere CM (1990) Response in Epidermal Growth Factor of skin fibroblasts from donors of varying age is modulated by the extracellular matrix. *Journal of Cell Physiology* **145**, 450–457.

Colige A, Nusgens B, Lapiere CM (1991) Altered response of progeroid fibroblasts to Epidermal Growth Factor. *Journal of Cell Science* **100**, 649–655.

Compendium of Health Care Statistics (1992) London: Office of Health Economics.

Compton CC (1993) Wound healing potential of cultured epithelium. *Wounds: A Compendium of Skin Research and Practice* **5**, 97–111.

Cossarizza A, Monti D, Bersani F, Paganelli R, Montagnani G, Cadossi R et al. (1989) Extremely low frequency pulsed electromagnetic fields increase interleukin-2 (IL2) utilization and IL2 receptor expression in mitogen-stimulated lymphocytes from old subjects. *FEBS Letters* **248**, 141–144.

Costa Rosa LFBP, Almeida AF, Safi DA, Curi R (1993) Metabolic and functional changes in lymphocytes and macrophages as induced by ageing. *Physiology and Behaviour* **53**, 651–656.

Cox D, Kunz S, Cerletti N, McMaster GK, Burk RR (1992) Wound healing in aged animals: effects of locally applied TGF-β2 in different model systems. In *Angiogenesis – Key Principles* (ed. R. Steiner, P. B. Weisz & R. Langer), pp. 287–295. Basel: Birkhauser.

Dahlback K, Lofberg H, Aluments J, Dahlback B (1989) Immunohistochemical demonstration of age-related deposition of vitronectin (S-protein of complement) and terminal complement complex on dermal elastic fibres. *Journal of Investigative Dermatology* **92**, 727–733.

Danon D, Kowatch MA, Roth GS (1989) Promotion of wound repair in old mice by local injection of macrophages. *Proceedings of the National Academy of Sciences of the USA* **86**, 2018–2020.

Darby I, Skalli O, Gabbiani G (1990) Alpha-smooth muscle actin is transiently expressed by myofibroblasts during experimental wound healing. *Laboratory Investigations* **63**, 21–29.

Davidson JM, Zoia O, Liu JM (1993) Modulation of transforming growth factor-beta 1 stimulated elastin and collagen production and proliferation in porcine vascular smooth muscle cells and skin fibroblasts by basic fibroblast growth factor, transforming growth factor-α, and insulin-like growth factor-1. *Journal of Cellular Physiology* **155**, 149–156.

DeLapp N, Dieckman D (1990) Effect of basic fibroblast growth factor (bFGF) and insulin growth factor type 1 (IGF-1) and type II (IGF-2) on adult human keratinocyte growth and fibronectin secretion. *Journal of Investigative Dermatology* **94**, 777–780.

Derynck R (1988) TGF-alpha. *Cell* **54**, 593–595.

Doillon CJ, Dunn MG, Bender E, Silver FH (1985) Collagen fiber formation in repair tissue: development of strength and toughness. *Collagen and Related Research* **5**, 481–492.

DuNouy P (1916) The relation between the age of the patient, the area of the wound, and the index of cicatrisation. *Journal of Experimental Medicine* **xxiv**, 461–470.

Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR et al. (1993) Accumulation of Maillard reaction products in

skin collagen in diabetes and aging. *Journal of Clinical Investigation* **91**, 2463–2469.

EDWARDS DR, HEATH TK, HOGAN BL, NOMURA S, WILLS AJ (1992) Expression of TIMP in fetal and adult mouse tissues studied by in situ hybridization. *Matrix* **1**, 286–293.

ERSHLER WB, GAMELLI RL, MOORE AL, HACKER MP, BLOW AJ (1984) Experimental tumours and aging: local factors that may account for the observed age advantage in the B16 murine melanoma model. *Experimental Gerontology* **19**, 367–376.

ESCOFFIER C, RIGAL J, ROCHEFORT A, VASSELET R, LEVEQUE JL, AGACHE PG (1989) Age-related mechanical properties of human skin: an *in vivo* study. *Journal of Investigative Dermatology* **93**, 353–357.

FAGIOLO U, COSSARIZZA A, SANTACATERINA S, ORTOLANI C, MONTI D, PAGANELLI R et al. (1992) Aging and cellular defence mechanisms. *Annals of the New York Academy of Sciences* **663**, 490–493.

FARGNOLI J, KUNISADA T, ALBERT FJ, SCHNEIDER EL, HOLBROOK NJ (1990) Decreased expression of heat shock protein 70 mRNA and protein after heat treatment in cells of aged rats. *Proceedings of the National Academy of Sciences of the USA* **87**, 846–850.

FENSKE NA, LOBER CW (1986) Structural and functional changes of normal aging skin. *Journal of the American Academy of Dermatology* **15**, 571–585.

FINE JD, COUCHMAN JR (1989) Chondroitin 6 sulphate proteoglycan but not heparan sulphate proteoglycan is abnormally expressed in skin basement membranes from patients with dominant and recessive dystrophic epidermal bullosa. *Journal of Investigative Dermatology* **92**, 611–616.

FINESMITH TH, BROADLEY KN, DAVIDSON JM (1990) Fibroblasts from wounds of different stages of repair vary in their ability to contract a collagen gel in response to growth factors. *Journal of Cellular Physiology* **144**, 99–107.

FLEISCHMAJER R, PERLISH JS, BASHEY RI (1972) Human dermal glycosaminoglycans and aging. *Biochemica et Biophysica Acta* **279**, 265–275.

FLEISCHMAJER R, NEDWICH A (1973) Werner's syndrome. *American Journal of Medicine* **54**, 111–118.

FLEISCHMAJER R, PERLISH JS, BASHEY RI (1973) Aging of human dermis. *Frontiers of Matrix Biology* **1**, 90–106.

FORSCHER BK, CECIL HC (1958) Some effects of age on the biochemistry of acute inflammation. *Gerontologia* **2**, 174–182.

FURTH JJ (1991) The steady state levels of type I collagen mRNA are reduced in senescent fibroblasts. *Journal of Gerontology* **46**, B122–124.

GABBIANI G, RYAN GB, MAJNO G (1971) Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* **27**, 549–554.

GAMBLE JR, HARLAN JM, KLEBANOFF SJ, VADAS MA (1985) Stimulation of adherence of neutrophils to umbilical vein endothelium by human recombinant tumour necrosis factor. *Proceedings of the National Academy of Sciences of the USA* **82**, 8667–8671.

GAMBLE JR, VADAS MA (1988) Endothelial adhesiveness for blood neutrophils is inhibited by Transforming Growth Factor-β. *Science* **242**, 97–99.

GARMYN M, YAAR M, BOILEAU N, BACKENDORF C, GILCHREST BA (1992) Effect of ageing and habitual sun exposure on the genetic response of cultured human keratinocytes to solar stimulated irradiation. *Journal of Investigative Dermatology* **99**, 743–748.

GERHARD GS PHILLIPS PD, CRISTOFALO VJ (1991) EGF- and PDGF-stimulated phosphorylation in young and senescent WI-38 cells. *Experimental Cell Research* **193**, 87–92.

GESSIN JC, BROWN LJ, GORDON JS, BERG RA (1993) Regulation of collagen synthesis in human dermal fibroblasts in contracted collagen gels by ascorbic acid, growth factors, and inhibitors of lipid peroxidation. *Experimental Cell Research* **206**, 283–290.

GIBSON JM, MILAM SB, KLEBE RJ (1989) Late passage cells display an increase in contractile behaviour. *Mechanisms of Ageing and Development* **48**, 101–110.

GILCHREST BA (1983) *In vitro* assessment of keratinocyte aging. *Journal of Investigative Dermatology* **81**, 184s–189s.

GILCHREST BA, BLOG FB, SZABO G (1979) Effects of aging and chronic sun exposure on melanocytes in human skin. *Journal of Investigative Dermatology* **73**, 141–143.

GILCHREST BA, MURPHY G, SOTER NA (1987) Effect of chronological ageing and ultraviolet irradiation on Langerhan's cells in human epidermis. *Journal of Investigative Dermatology* **79**, 85–88.

GILHAR A, AIZEN E, PILLAR T, EIDELMAN S (1992) Response of aged versus young skin to intradermal administration of interferon gamma. *Journal of the American Academy of Dermatology* **27**, 710–716.

GINSBERG MH, LOFTUS JC, PLOW EF (1988) Cytoadhesins, integrins and platelets. *Thrombosis and Haemostasis* **59**, 1–6.

GOODSON WH, HUNT TK (1979) Wound healing and aging. *Journal of Investigative Dermatology* **73**, 88–91.

GOSPODAROWICZ D, BIALECKI H, THAKRAL TK (1979) The angiogenic activity of the fibroblast and Epidermal Growth Factor. *Experimental Eye Research* **28**, 501–514.

GRANSTEIN RD, DEAK M, JACQUES SL, MARGOLIS RJ, FLOTTE TJ, WHITAKER D et al. (1989) The systematic administration of a gamma interferon inhibits collagen synthesis and acute inflammation in a murine skin wounding model. *Journal of Investigative Dermatology* **93**, 18–27.

GREY AM, SCHOR AM, RUSHTON G, ELLIS I, SCHOR SL (1989) Purification of the migration stimulating factor produced by fetal and breast cancer patient fibroblasts. *Proceedings of the National Academy of Sciences of the USA* **86**, 2438–2442.

GRIGOROVA-BORSOS AM, BARA L, GROCHUKLSKI A, ANDRE J, MOZERE G, PEYROUX J et al. (1988) Aging and diabetes increasing the aggregating potency of rat skin collagen towards normal platelets. *Thrombosis and Haemostasis* **60**, 75–78.

GRINNELL F, BILLINGHAM RE, BURGESS L (1981) Distribution of fibronectin during wound healing *in vivo*. *Journal of Investigative Dermatology* **76**, 181–189.

GROSS JL, MOSCATELLI D, JAFFE EA, RIFKIN DB (1982) Plasminogen activator and collagenase production by cultivated capillary endothelial cells. *Journal of Cell Biology* **95**, 974–981.

GROVE GL (1982) Age-related differences in healing of superficial skin wounds in humans. *Archives of Dermatological Research* **272**, 381–385.

GROVE GL, DUNCAN S, KLIGMAN AM (1982) Effect of ageing on the blistering of human skin with ammonium hydroxide. *British Journal of Dermatology* **107**, 393–400.

GROVE GL, KLIGMAN AM (1983) Age-associated changes in human epidermal cell renewal. *Journal of Gerontology* **38**, 137–142.

GUTMAN R, COHEN MR, MCAMIS W, RAMCHAND CN, SAILER V (1987) Free radical scavenging systems and the effect of peroxide damage in aged human skin fibroblasts. *Experimental Gerontology* **22**, 373–378.

HAGISAWA S, BARBENEL JC, KENEDI RM (1991) Influence of age on postischaemic reactive hyperaemia. *Clinical Physics and Physiological Measurements* **12**, 227–237.

HALASZ NA (1968) Dehiscence of laparotomy wounds. *American Journal of Surgery* **116**, 210–214.

HALL DA (1976) *The Ageing of Connective Tissue*, pp. 69–70. London: Academic Press.

HARLEY CB, GOLDSTEIN S, POSNER BI, GUYDA H (1981) Decreased sensitivity of old and progeric human fibroblasts to a preparation of factors with insulinlike activity. *Journal of Clinical Investigation* **68**, 988–993.

HATA R (1990) Age-dependent changes in collagen metabolism and response to hypertonic culture conditions of rat aortic smooth muscle cells and skin fibroblasts. *Cell Biology International Reports* **14**, 25–33.

HATAMOCHI A, MORI K, TAKEDA K, ARAKAWA M, UEKI H,

YOSHIOKA H (1993) Decreased type VI collagen gene expression in cultured Werner's syndrome fibroblasts. *Journal of Investigative Dermatology* **100**, 771–774.

HAYFLICK L (1965) The limited *in vitro* lifetime of human diploid cell strains. *Experimental Cell Research* **37**, 614–646.

HEIKKINEN E, AALTO M, VIHERSAARI T, KULONEN E (1971) Age factor in the formation and metabolism of experimental granulation tissue. *Journal of Gerontology* **26**, 294–298.

HEIMARCK RL, SCHWARTZ SM (1988) The role of cell–cell interaction in the regulation of endothelial cell growth. In *The Molecular and Cellular Biology of Wound Repair* (ed. R. A. F. Clark & P. M. Henson), pp. 359–369. New York: Plenum.

HEINO J, IGNOTZ RA, HEMLER ME, CROUSE C, MASSAGUE J (1989) Regulation of cell adhesion receptors by TGF-beta. Concomitant regulation of integrins that share a common b1 subunit. *Journal of Biological Chemistry* **264**, 380–388.

HENNESSEY P, NIRGIOTIS JG, ANDRASSY RJ (1991) The effects of age and various fat/carbohydrate ratios on nitrogen retention and wound healing in rats. *Journal of Paediatric Surgery* **26**, 367–373.

HERZBERG AJ, DINEHART SM (1989) Chronologic aging in black skin. *American Journal of Dermatopathology* **11**, 319–328.

HIGASHIMOTO Y, FUKUCHI Y, SHIMADA Y, ISHIDA K, OHATA M, FURUSE T et al. (1993) The effects of aging on the function of alveolar macrophages in mice. *Mechanisms of Ageing and Development* **69**, 207–217.

HOBBS MV, WEIGLE WO, NOONAN DJ, TORBETT BE, MCEVILLY RJ, KOCH RJ et al. (1993) Patterns of cytokine gene expression by CD4+ T-cells from young and old mice. *Journal of Immunology* **150**, 3602–3614.

HOLLENBERG MD, SCHNEIDER E (1979) Receptors for insulin and EGF-urogastrone in adult human fibroblasts do not change with donor age. *Mechanisms of Ageing and Development* **11**, 37–43.

HOLLIDAY R (1990) The limited proliferation of cultured human cells: regulation or senescence. *Journal of Gerontology* **45**, 36–41.

HOLLIDAY R, RATTAN SIS (1984) Evidence that paromomycin induces premature ageing in human fibroblasts. *Developmental Biology* **17**, 221–233.

HOLM-PEDERSON P, ZEDERFELDT (1971 *a*) Granulation tissue formation in subcutaneously implanted cellulose sponges in young and old rats. *Scandinavian Journal of Plastic and Reconstructive Surgery* **5**, 13–16.

HOLM-PEDERSON P, ZEDERFELDT B (1971 *b*) Strength development of skin incisions in young and old rats. *Scandinavian Journal of Plastic and Reconstructive Surgery* **5**, 7–12.

HOLM-PEDERSON P, VIIDIK A (1972) Maturation of collagen in healing wounds in young and old rats. *Scandinavian Journal of Plastic and Reconstructive Surgery* **6**, 16–23.

HOLM-PEDERSON P, ZEDERFELDT B (1973) Respiratory gas tensions and blood flow in wounds in young and old rats. *Scandinavian Journal of Plastic and Reconstructive Surgery* **7**, 91–96.

HOLM-PEDERSON P, FENSTAD AM, FOLKE LEA (1974) DNA, RNA and protein synthesis in healing wounds in young and old mice. *Mechanisms of Ageing and Development* **3**, 173–185.

HOLT DR, KIRK SJ, REGAN MC, HURSON M, LINDBLAD WJ, BARBUL A (1992) Effect of age on wound healing in healthy human beings. *Surgery* **112**, 293–298.

HOMSEY R, PELLETIER-LEBON P, TIXIER JM, GODEAU G, ROBERT L, HORNEBACK W (1989) Characterisation of human skin fibroblast elastase activity. *Journal of Investigative Dermatology* **91**, 472–477.

HSIEH P, CHEN LB (1983) Behaviour of cells seeded on isolated fibronectin matrices. *Journal of Cell Biology* **96**, 1208–1217.

IIYAMA M, SHIMADA Y, KITA T, ITO H (1992) Effect of aging on macrophage adherence to extracellular matrix proteins. *Mechanisms of Ageing and Development* **66**, 149–158.

IMAYAMA S, BRAVERMAN IM (1989) A hypothetical explanation for the aging of the skin. *American Journal of Pathology* **134**, 1019–1025.

JANSEN LH, ROTTIER PB (1957) Elasticity of human skin related to age. *Dermatologica* **115**, 106–111.

JEFFREY JJ (1992) Collagen degradation. In *Wound Healing: Biochemical and Clinical Aspects* (ed. I. K. Cohen, R. F. Diegelmann & W. Linblad), pp. 177–194. Philadelphia: W. B. Saunders.

JONGKIND JF, VERKERK A, POOT M (1987) Glucose flux through the hexose monophosphate shunt and NADP(H) levels during *in vitro* ageing of human skin fibroblasts. *Gerontology* **33**, 281–286.

JU WD, SCHILLER JT, KAZEMPOUR MK, LOWY DR (1993) TGF-α enhances locomotion of cultured human keratinocytes. *Journal of Investigative Dermatology* **100**, 628–632.

KADISH JL, BUTTERFIELD CE, FOLKMAN J (1979) The effects of fibrin on cultured vascular endothelial cells. *Tissue and Cell* **11**, 99–108.

KAHARI V, OLSEN DR, RHUDY RW, CARRILLO P, CHEN YQ, UITTO J (1992) Transforming growth factor-β up-regulates elastin gene expression in human skin fibroblasts. *Laboratory Investigation* **66**, 580–588.

KARLSSON C, PAULSSON Y (1994) Age related induction of platelet-derived growth factor A-chain mRNA in normal human fibroblasts. *Journal of Cellular Physiology* **158**, 256–262.

KEAST D, NGUYEN T, NEWSHOLME EA (1989) Maximal activities of glutaminase, citrate synthase, hexokinase, phosphofructonkinase, and LDH in the skin of rats and mice at different ages. *FEBS Letters* **247**, 132–134.

KIRKWOOD TBL, ROSE MR (1991) Evolution of senescence: late survival sacrificed for reproduction. *Philosophical Transactions of the Royal Society of London* B **332**, 15–24.

KIRSTEIN M, ASTON C, HINTZ R, VIASSARA H (1992) Receptor-specific induction of insulin-like growth factor I in human monocytes by advanced glycosylation end product-modified proteins. *Journal of Clinical Investigation* **90**, 439–446.

KLIGMAN AM (1979) Perspectives and problems in cutaneous gerontology. *Journal of Investigative Dermatology* **73**, 39–46.

KLIGMAN AM, LAVKER R (1988) Cutaneous aging: the differences between intrinsic aging and photoaging. *Journal of Cutaneous Aging and Cosmetological Dermatology* **1**, 5–12.

KOHASE M, HENRIKSEN-DESTEFANO D, MAY LT, VILCEK J, SEHGAL PB (1986) Induction of β-2 interferon by tumour necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell* **45**, 659–666.

KONDO H, NOMAGUCHI TA (1985) Effects of serum from rabbits of various ages on cell proliferation. *Mechanisms of Ageing and Development* **32**, 1–10.

KONDO H, NOMAGUCHI TA, SAKURAI Y, YONEZAWA Y, KAJI K, MATSUO M et al. (1988) Effects of serum from human subjects of various ages on the proliferation of human lung and skin fibroblasts. *Experimental Cell Research* **178**, 287–295.

KONDO H, NOMAGUCHI TA, YONEZAWA Y (1989) Effects of serum from human subjects of different ages on migration *in vitro* of human fibroblasts. *Mechanisms of Ageing and Development* **47**, 25–37.

KONO T, TANII T, FURUKAWA M, MIZUNO N, KITAJIMA I, ISHII M et al. (1990) Correlation between ageing and collagen gel contractility of human fibroblasts. *Acta Dermato-Venereologica Stockholm* **70**, 241–244.

KRAMER PH, FUH GM, BENSCH KG, KARASEK MA (1985). Synthesis of extracellular matrix glycoproteins by cultured microvascular endothelial cells isolated from the dermis of neonatal and adult skin. *Journal of Cell Physiology* **123**, 1–9.

KUMAR S, VINCI JM, MILLIS AJT, BAGLIONI C (1993) Expression of interleukin-1α and β in early passage fibroblasts from aging individuals. *Experimental Gerontology* **28**, 505–513.

KURBAN RS, BHAVAN J (1990) Histologic changes in skin associated with aging. *Journal of Dermatological Surgery and Oncology* **16**, 908–914.

KURKINEN M, VAHERI A, ROBERTS PJ, STENMAN S (1980) Sequential

appearance of fibronectin and collagen in experimental granulation tissue. *Laboratory Investigations* **43**, 47–51.

La Belle M, Linn S (1984) DNA repair in cultured mouse cells of increasing population doubling level. *Mutation Research* **132**, 51–61.

Lagier R (1960) Etude de la composition chimique de tissus humaine de nature. *Gerontologia* **4**, 39–59.

Lapiere CHM (1991). Genetics and tissue repair. In *Wound Healing* (ed. H. Janssen, R. Rooman & J. I. S. Robertson), pp. 3–7. Petersfield: Wrightson Biomedical.

Laurent TC (1970) Structure of hyaluronic acid. In *Chemistry and Molecular Biology of the Intercellular Matrix* (ed. E. A. Balazs), vol. 2, pp. 703–732. New York: Academic Press.

Lavker RM (1979) Structural alterations in exposed and unexposed aged skin. *Journal of Investigative Dermatology* **73**, 59–66.

Lavker RM, Kwong F, Kligman AM (1980) Changes in skin surface patterns with age. *Journal of Gerontology* **35**, 348–354.

Lavker RM, Zheng P, Dong G (1987) Aged skin: a study by light, transmission electron, and scanning electron microscopy. *Journal of Investigative Dermatology* **88**, 44s–51s.

Lavker RM, Zheng P, Dong G (1989) Morphology of aged skin. *Geriatric Dermatology* **5**, 53–67.

Leyden JJ (1990) Clinical features of ageing skin. *British Journal of Dermatology* **122**, 1–3.

Lipschitz DA, Udupa KB (1986) Influence of aging and protein deficiency on neutrophil function. *Journal of Gerontology* **4**, 690–694.

Lombard N, Masse R (1987) Effect of *in vivo* ageing on the growth of rat fibroblasts in cells from cutaneous explants. *Royal Society of Science and Biology* **181**, 267–273.

Longas MO, Russell CS, He XY (1987) Evidence for structural changes in dermatan sulfate and hyaluronic acid with aging. *Carbohydrate Research* **159**, 127–136.

Lovell CR, Smolenski KA, Duance VC, Light ND, Young S, Dyson M (1987) Type I and III collagen content and fibre distribution in normal human skin during ageing. *British Journal of Dermatology* **117**, 419–428.

Luce MC, Bunn CL (1987) Altered sensitivity of protein synthesis to paromomycin in extracts from aging human diploid fibroblasts. *Experimental Gerontology* **22**, 165–177.

Lumpkin CK, McClung JK, Pereira-Smith OM, Smith JR (1986) Presence of high abundance antiproliferative RNA in senescent human diploid fibroblasts. *Science* **232**, 393–395.

Mackay IR (1972) Aging and immunologic functions of man. *Gerontologia* **18**, 285–304.

Madri JA, Williams SK (1993) Capillary endothelial cell cultures: phenotypic modulation by matrix components. *Journal of Cell Biology* **97**, 153–165.

Maekawa T, Rathinasamy TK, Altman KI, Forbes WF (1970) Changes in collagen with age. 1. The extraction of acid soluble collagens from the skin of mice. *Experimental Gerontology* **5**, 177–186.

Maier JA, Voulalas P, Roeder D, Maciag T (1990) Extension of the life-span of human endothelial cells by an interleukin-1 alpha antisense oligomer. *Science* **249**, 1570–1574.

Mariagabriella G, Davidson JM (1993) Familial co-segregation of the elastin phenotype in skin fibroblasts from Hutchinson-Gilford progeria. *Mechanisms of Ageing and Development* **70**, 163–176.

Marinkovich MP (1993) The molecular genetics of basement membrane diseases. *Archives of Dermatology* **129**, 1557–1565.

Marks MW, Morykwas MJ, Wheatley MJ (1990) Fibroblast-mediated contraction in actinically exposed and actinically protected aging skin. *Journal of Plastic and Reconstructive Surgery* **86**, 255–259.

Martin M, Nabout RE, Lafuma C, Crechet F, Remy J (1990) Fibronectin and collagen gene expression during *in vitro* ageing of pig skin fibroblasts. *Experimental Cell Research* **191**, 8–13.

Mast BA (1992) The skin. In *Wound Healing: Biochemical and Clinical aspects* (ed. I. K. Cohen, R. F. Diegelmann and W. Lindblad), pp. 344–355. Philadelphia: W. B. Saunders.

Matrisian LM, Davis D, Magun BE (1987) Internalization and processing of Epidermal Growth Factor in aging human fibroblasts in culture. *Experimental Gerontology* **22**, 81–89.

Mays PK, McNulty RJ, Campa JS, Laurent GJ (1991) Age-related changes in collagen synthesis and degradation in rat tissues. *Biochemical Journal* **276**, 307–313.

McCaffrey TA, Falcone DJ (1993) Evidence for an age-related dysfunction in the anti-proliferative response to Transforming Growth Factor-β in vascular smooth muscle cells. *Molecular Biology of the Cell* **4**, 315–322.

McGurk M, Hanford L, Justice S, Metcalfe RA (1990) The secretory characteristics of epidermal growth factor in human saliva. *Archives of Oral Biology* **35**, 653–659.

Mendoza CB, Postlethwait RW (1970) Incidence of wound disruption following operation. *Archives of Surgery* **101**, 396–398.

Meyer LJM, Stern R (1994) Age-dependent changes of hyaluronan in human skin. *Journal of Investigative Dermatology* **102**, 385–389.

Mignatti P, Welgus HG, Rifkin DB (1988) Role of degradative enzymes in wound healing. In *The Molecular and Cellular Biology of Wound Repair* (ed. R. A. F. Clark & P. M. Henson), pp. 497–523. New York: Plenum.

Miksik I, Deyl Z (1991) Changes in the amount of e-hexosyllysine, UV absorbance, and fluorescence of collagen with age in different animal species. *Journal of Gerontology* **46**, B111–116.

Miller RA (1984) Age-associated decline in precursor frequency for different cell-mediated reactions with preservation of helper or cytotoxic effect per precursor cell. *Journal of Immunology* **132**, 63–68.

Moerman EJ, Thweatt R, Moerman AM, Jones RA, Goldstein S (1993) Insulin-like growth factor binding protein 3 is over-expressed in senescent and quiescent human fibroblasts. *Experimental Gerontology* **28**, 361–370.

Mollenhauer J, Bayreuther K (1986) Donor age related changes in the morphology, growth potential, and collagen biosynthesis in rat fibroblast subpopulations *in vitro*. *Differentiation* **32**, 165–172.

Montagna W, Carlisle K (1979) Structural changes in aging human skin. *Journal of Investigative Dermatology* **73**, 47–53.

Montagna W, Carlisle K (1990) Structural changes in ageing skin. *British Journal of Dermatology* **122**, 61–70.

Montagna W, Carlisle K (1991) The architecture of black and white facial skin. *Journal of the American Academy of Dermatology* **24**, 929–937.

Monticone RE, Nick RJ, Eichorn GL (1987) The effects of cellular age on zinc levels in untreated and zinc-treated human diploid fibroblasts. *Journal of Inorganic Biochemistry* **30**, 291–298.

Morris GM, Hamlet R, Hopewell JW (1989) The cell kinetics of the epidermis and follicular epithelium of the rat: variations with age and body site. *Cell Tissue Kinetics* **22**, 213–222.

Muggleton-Harris AL, Reisert PS, Burghoff RL (1982) *In vitro* characterisation of the response to a stimulus (wounding) with regard to ageing in human skin fibroblasts. *Mechanisms of Ageing and Development* **19**, 37–43.

Mullaart E, Roza L, Lohman PHM, Vijg J (1989) The removal of UV-induced pyrimidine dimers from DNA of rat skin cells *in vitro* and *in vivo* in relation to aging. *Mechanisms of Ageing and Development* **47**, 253–264.

Mustoe TA, Pierce GF, Thomasen A, Gramates P, Sporn MB, Deuel TF (1987) Accelerated healing of incisional wounds in rats induced by transforming growth factor-β. *Science* **237**, 1333–1336.

Nagelkerken L, Hertogh-Huijbregts A, Dobber R, Drager A (1991) Age-related changes in lymphokine production related to a decreased member of CD45RBhi CD4+ RT cells. *European Journal of Immunology* **21**, 273–281.

NING Y, PEREIRA-SMITH OM (1991) Molecular genetic approaches to the study of cellular senescence. *Mutation Research* **256**, 303–310.

NINIKOWSKI J (1969) Effect of oxygen supply on wound healing and formation of experimental granulation tissue. *Acta Physiological Scandinavica* **334**, 1–72.

OLERUD JE, GOWM AM, BICKENBACH J, DALE B, ODLAND GF (1988) An assessment of human epidermal repair in elderly normal subjects using immunohistochemical methods. *Journal of Investigative Dermatology* **90**, 845–850.

OLSEN DR, UITTO J (1989). Differential expression of type IV procollagen and laminin genes by fetal vs adult skin fibroblasts in culture: determination of subunit mRNA steady-state levels. *Journal of Investigative Dermatology* **93**, 127–131.

OLSEN JA, HODGES RE (1987) Recommended dietary intakes (RDI) of vitamin C in humans. *American Journal of Clinical Nutrition* **45**, 693–703.

ORSON FM, SAADEH CK, LEWIS DE, NELSON DL (1989) Interleukin-2 receptor expression by T cells in human aging. *Cellular Immunology* **124**, 278–291.

OVERALL CM, WRANA JL, SODEK J (1989) Independent regulation of collagenase, 72 kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-β. *Journal of Biological Chemistry* **264**, 1860–1869.

PARRY DAD, BARNES GRG, CRAIG AS (1978) A comparison of the size distribution of collagen fibrils in connective tissue as a function of age and a possible relation between fibril size distribution and mechanical properties. *Proceedings of the Royal Society of London* B **203**, 305–321.

PEACOCKE M, YAAR M, GILCHREST BA (1989) Interferon and the epidermis: implications for cellular senescence. *Experimental Gerontology* **24**, 415–421.

PEPPER MS, VASSALI JD, MONTESANO R, ORCI L (1987) Urokinase-type Plasminogen Activator is induced in migrating capillary endothelial cells. *Journal of Cell Biology* **105**, 2535–2541.

PEPPER MS, SAPPINO AP, MONTESANO R, ORCI L, VASSALLI JD (1992) Plasminogen Activator Inhibitor-1 is induced in migrating endothelial cells. *Journal of Cellular Physiology* **153**, 129–139.

PHILLIPS C, WENSTRUP RJ (1992) Biosynthetic and genetic disorders of collagen. In *Wound Healing: Biochemical and Clinical Aspects* (ed. I. K. Cohen, R. F. Diegelmann & W. Lindblad), pp. 152–176. Philadelphia: W. B. Saunders.

PHILLIPS PD, KAJI K, CRISTOFALO VJ (1984) Progressive loss of the proliferative response of senescing W1-38 cells to platelet-derived growth factor, epidermal growth factor, insulin, transferrin and dexamethasone. *Journal of Gerontology* **39**, 11–17.

PIENTA KJ, COFFEY DS (1990) Characterisation of the subtypes of cell motility in ageing human skin fibroblasts. *Mechanisms of Ageing and Development* **56**, 99–105.

PIERAGGI MJ, BOUISSOU H, ANGELIER C, UHART D, MAGNOL JP, KOKOLO J (1985) Le fibroblaste. *Annals of Pathology* **5**, 65–76.

PIERCE GF, MUSTOE TA, LINGELBACH J, MASAKOWSKI VR, GRIFFIN GL, SENIOR RM et al. (1989) Platelet-derived growth factor and transforming growth factor-β enhance tissue repair activities by unique mechanisms. *Journal of Cell Biology* **109**, 429–440.

PIGEOLET E, RAES M, HOUBION A, REMACLE J (1988) Effect of procaine on cultivated human W1-38 fibroblasts. *Experimental Gerontology* **23**, 87–96.

POCHI PE, STRAUSS, J, DOWNING DT (1979) Age-related changes in sebaceous gland activity. *Journal of Investigative Dermatology* **73**, 108–111.

QUAGLINO D, KENNEDY R, FORNIERI C, NANNEY L, RONCHETTI IP, DAVIDSON JM (1989) Matrix gene expression during the aging process revealed by in situ hybridisation. *Journal of Histochemistry and Cytochemistry* **37**, 933.

QUAGLINO D, NANNEY LB, DITESHEIM JA, DAVIDSON JM (1991) Transforming growth factor-beta stimulates wound healing and modulates extracellular matrix gene expression in pig skin: incisional wound model. *Journal of Investigative Dermatology* **97**, 34–42.

QUEVEDO WC, SZABO G, VIRKS J (1969) Influence of age and UV on the population of dopa + melanocytes in human skin. *Journal of Investigative Dermatology* **52**, 287–290.

QUIRINIA A, VIIDIK A (1991) The influence of age on the healing of normal and ischaemic incisional skin wounds. *Mechanisms of Ageing and Development* **58**, 221–232.

RAPPOLEE DA, MARK D, BANDA MJ, WERB Z (1988) Wound macrophages express TGF-α and other growth factors *in vivo*: analysis by mRNA phenotyping. *Science* **241**, 708–712.

RATTAN SIS, DERVENTZI A (1991) Altered cellular responsiveness during ageing. *BioEssays* **13**, 601–606.

REED BR, CLARK RAF (1985) Cutaneous tissue repair: practical implications of current knowledge. II. *Journal of the American Academy of Dermatology* **13**, 919–941.

REED MJ, VERNON RB, ABRASS IB, SAGE EH (1994) TGF-beta 1 induces the expression of type I collagen and SPARC, and enhances contraction of collagen gels, by fibroblasts from young and aged donors. *Journal of Cellular Physiology* **158**, 169–179.

REENSTRA WR, YAAR M, GILCHREST BA (1993) Effect of donor age in epidermal growth factor processing in man. *Experimental Cell Research* **209**, 118–122.

REGAN M, BARBUL A (1991) Regulation of wound healing by the T-cell dependent immune system. In *Wound Healing* (ed. H. Janssen, R. Rooman & J. I. S. Robertson), pp. 21–31. Petersfield: Wrightson Biomedical.

REISER K, HENNESSY SM, LAST JA (1987) Analysis of age-associated changes in collagen cross-linking in the skin and lung in monkeys and rats. *Biochimica et Biophysica Acta* **926**, 339–349.

REISER K, MCCORMICK RJ, RUCKER RB (1992) Enzymatic and nonenzymatic cross-linking of collagen and elastin. *Journal of FASEB* **6**, 2439–2449.

RICHARD S, QUERLEUX B, BITTOUN J, JOLIVET O, IDY-PERETTI I, LACHARRIERE O et al. (1993) Characterisation of the skin *in vivo* by high resolution magnetic resonance imaging: water behaviour and age-related effects. *Journal of Investigative Dermatology* **100**, 705–709.

RICHARDSON D (1989) Effects of age on cutaneous circulatory response to direct heat on the forearm. *Journal of Gerontology* **44**, 189–194.

RIDGE MD, WRIGHT V (1966) The aging of the skin: a bio-engineering approach. *Gerontologia* **12**, 174–192.

RIGAL J, ESCOFFIER C, QUERLEUX B, FAIVRE B (1989) Assessment of ageing of human skin by *in vivo* ultrasound imaging. *Journal of Investigative Dermatology* **93**, 621–625.

ROBERT C, BLANC M, LESTY C, DIKSTEIN S, ROBERT L (1988*a*) Study of skin ageing as a function of social and professional conditions: modification of the rheological parameters measured with non-invasive method: indentometry. *Gerontology* **34**, 284–290.

ROBERT C, LESTY C, ROBERT A (1988*b*) Ageing of the skin: study of elastic fiber network modifications by computerised image analysis. *Gerontology* **34**, 291–296.

ROBERTS AB, SPORN MB, ASSOIAN RK, SMITH JM, ROCHE NS, WAKEFIELD LM et al. (1986) Transforming Growth Factor type beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proceedings of the National Academy of Sciences of the USA* **83**, 4167–4171.

ROBSON MC, HEGGERS JP (1989) Eicosanoids, cytokines and free radicals. In *Wound Healing: Biochemical and Clinical Aspects* (ed. I. K. Cohen, R. F. Diegelmann & W. Lindblad), pp. 292–304. Philadelphia: W. B. Saunders.

ROSEN EM, GRANT D, KLEINMAN H, JAKEN S, DONOVAN MA, SETTER E et al. (1991) Scatter factor stimulates migration of vascular endothelium and capillary-like tube formation. *Experientia*, (Suppl.) **59**, 76–88.

ROSENBLOOM, AL, GOLDSTEIN S (1976) Insulin binding to cultured human fibroblasts increases with normal and precocious aging. *Science* **193**, 412–415.

ROSS R, RAINES EW, BOWEN-POPE DF (1986) The biology of PDGF. *Cell* **46**, 155–169.

RUBERG RL (1984) Role of nutrition in wound healing. *Surgical Clinics of North America* **64**, 705–714.

RUDOLPH R, VANDE BERG J, EHRLICH HP (1989) Wound contraction and scar contracture. In *Wound Healing: Biochemical and Clinical Aspects* (ed. I. K. Cohen, R. F. Diegelmann & W. Lindblad), pp. 96–115. Philadelphia: W. B. Saunders.

SANDBLOM PH, PETERSON P, MUREN A (1953) Determination of the tensile strength of the healing wound as a clinical test. *Acta Chirurgica Scandinavica.* **105**, 252–257.

SAUDER DN (1989) Effect of age on epidermal immune function. *Clinics on Geriatric Medicine* **5**, 149–160.

SAUDER DN, STANULIS-PRAEGER BM, GILCHREST BA (1988) Autocrine growth stimulation of human keratinocytes by epidermal cell-derived thymocyte-activating factor: implications for skin aging. *Archives of Dermatological Research* **280**, 71–76.

SCHILLER S, DORFMAN A (1960) Effect of age on the heparin content of rat skin. *Nature* **185**, 111–112.

SCHNEIDER EL (1979) Aging and cultured human skin fibroblasts. *Journal of Investigative Dermatology* **73**, 15–18.

SCHROEDER F, GOETZ I, ROBERTS E (1984) Age-related alterations in cultured human fibroblast membrane structure and function. *Mechanisms of Ageing and Development* **25**, 365–389.

SCHWARTZ D (1977) The proliferation of elastic fibres after skin incisions in albino mice and rats: a light and electron microscopic study. *Journal of Anatomy* **124**, 401–411.

SEPHEL GC, DAVIDSON JM (1986) Elastin production in human skin fibroblast cultures and its decline with age. *Journal of Investigative Dermatology* **86**, 279–285.

SHEVITZ J, JENKINS CSP, HATCHER VB (1986) Fibronectin synthesis and degradation in human fibroblasts with aging. *Mechanisms of Ageing and Development* **35**, 221–232.

SHIGEOKA H, YANG HC (1990) Early kinase C dependent events in aging human diploid fibroblasts. *Mechanisms of Ageing and Development* **55**, 49–59.

SHIMADA Y, ITO H, KAJI K, FUKUDA M (1990) Tumour necrosis factor reduces lifespan of human endothelial cells *in vitro*. *Mechanisms of Ageing and Development* **55**, 245–254.

SHINDO Y, AKIYAMA J, YAMAZAKI Y, SAITO K, TAKASE Y (1991) Changes in enzyme activities in skin fibroblasts derived from persons of various ages. *Experimental Gerontology* **26**, 29–35.

SHOLLEY MM, GIMBRONE MA, COTRAN RS (1978) The effects of leucocyte depletion on corneal vascularisation. *Laboratory Investigation* **38**, 32–40.

SHUSTER S, BLACK MM, MCVITIE E (1975) The influence of age and sex on skin thickness, skin collagen and density. *British Journal of Dermatology* **93**, 639–643.

SIBBIT WL, MILLS RG, BIGLER CF, EATON RP, GRIFFEY RH, VANDERJAGT DL (1989) Glucose inhibition of human fibroblast proliferation and response to growth factors is prevented by inhibitors of aldose reductase. *Mechanisms of Ageing and Development* **47**, 265–279.

SILVER A (1965) The effect of age on human eccrine sweating. In *Advances in Biology of the Skin*, vol. 6 (ed. W. Montagna), pp. 129–149. Oxford: Pergamon.

SILVERMAN EM, SILVERMAN AG (1977) Granulocyte adherence in the elderly. *American Journal of Clinical Pathology* **67**, 49–52.

SLUKE G, SCHACHTSCHABEL DO, WEVER J (1981) Age-related changes in the distribution pattern of glycosaminoglycans synthesized by cultured human diploid fibroblasts. *Mechanisms of Ageing and Development* **16**, 19–27.

SMITH JG, DAVIDSON EA, MITCHELL W, CLARK RD (1962) Alterations in human dental connective tissue with age and chronic sun damage. *Journal of Investigative Dermatology* **39**, 347–350.

SOBBIN SS, BERNICK S, BALLARD KW (1992) Acute wound repair in an aged animal: a model for accelerated aging of the microvasculature. *Journal of Gerontology* **47**, B121–125.

SOHAL RS (1993) The free radical hypothesis of aging: an appraisal of the current status. *Aging in Clinical and Experimental Research* **5**, 3–17.

SONG L, HO KIM Y, CHOPRA RJ, PROUST JJ, NAGEL JE, NORDIN AA et al. (1993) Age-related effects in T-cell activation and proliferation. *Experimental Gerontology* **28**, 313–321.

SORRENTINO JA, MILLIS AJT (1984) Structural comparisons of fibronectins isolated from early and late passage cells. *Mechanisms of Ageing and Development* **28**, 83–97.

SPAGNOLI LG, SAMBUY Y, PALMIERI G, MAURIELLO A (1985) Age-related modulation of vascular smooth muscle cells proliferation following arterial wall damage. *Artery* **13**, 187–198.

SPORN MB, ROBERTS AB (1992) Transforming growth factor-β: recent progress and new challenges. *Journal of Cell Biology* **119**, 1017–1021.

STANULIS-PRAEGER BM, GILCHREST BA (1986) Growth factor responsiveness declines during adulthood for human skin-derived cells. *Mechanisms of Ageing and Development* **35**, 185–198.

STENN KS, MALHOTRA R (1992) Epithelialisation. In *Wound Healing: Biochemical and Clinical Aspects* (ed. I. K. Cohen, R. F. Diegelmann & W. Lindblad), pp. 115–127. Philadelphia: W. B. Saunders.

STOLPEN AH, GUINAN EC, FIERS W, POBER JS (1986) Recombinant tumor necrosis factor and immune interferon act singly and in combination to reorganize human vascular endothelial cell monolayers. *American Journal of Pathology* **123**, 16–24.

STRAIN AJ (1993) Hepatocyte growth factor: another ubiquitous cytokine. *Journal of Endocrinology* **137**, 1–5.

SUSSMAN MD (1973) Aging of connective tissue and physical properties of healing wounds in young and old rats. *American Journal of Physiology* **224**, 1167–1171.

TAKEDA K, GOSIEWSKA A, PETERKOFSKY B (1992) Similar, but not identical, modulation of expression of extracellular matrix components during *in vitro* and *in vivo* aging of human skin fibroblasts. *Journal of Cellular Physiology* **153**, 450–459.

TAMM I, KIKUCHI T, WANG E, PFEFFER LM (1984) Growth rate of control and β-interferon treated human fibroblast populations over the course of their *in vitro* life span. *Cancer Research* **44**, 2291–2296.

TAN CY, STATHAM B, MARKS R, PAYNE PA (1982) Skin thickness measurement by pulsed ultrasound: its reproducibility, validation and variability. *British Journal of Dermatology* **106**, 657–667.

THIERS H, MAIZE JC, SPICER SS, CANTOR AB (1984) The effect of aging and chronic sun exposure on human Langerhans cell populations. *Journal of Investigative Dermatology* **82**, 223–226.

TONNESON MG (1989) Neutrophil–endothelial cell interactions: mechanisms of neutrophil adherence to vascular endothelium. *Journal of Investigative Dermatology* **93**, 535–585.

TSUBOI R, SATO C, KURITA Y, RON D, RUBIN JS, OGAWA H (1993) Keratinocyte growth factor (FGF-7) stimulates migration and plasminogen activator activity of normal human keratinocytes. *Journal of Investigative Dermatology* **101**, 49–53.

TZENG DY, DEUEL TF, HUANG JS, BAEHNER RL (1985) Platelet-derived growth factor promotes human peripheral monocyte activation. *Blood* **66**, 179–183.

UITTO J (1990) A method for studying collagen biosynthesis in human skin biopsies *in vitro*. *Biochimica et Biophysica Acta* **201**, 438–443.

VITELLARO-ZUCCARELLO L, GARBELLI R, ROSSI VDP (1992) Immunocytochemical localisation of collagen types I, III, IV and fibronectin in the human dermis. *Cell and Tissue Research* **268**, 505–511.

WANG SY, MERRILL C, BELL E (1988) Effects of ageing and long-

term subcultivation on collagen lattice contraction and intralattice proliferation in three rat cell types. *Mechanisms of Ageing and Development* **44**, 127–141.

WERNER S, PETERS KG, LONGAKER MT, FULLER-PACE F, BANDA MJ, WILLIAMS LT (1992) Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proceedings of the National Academy of Sciences of the USA* **89**, 6896–6900.

WHITTON JT, EVERALL JD (1973) The thickness of the epidermis. *British Journal of Dermatology* **89**, 467–476.

WIJN P (1980) *The alinear viscoelastic properties of human skin in vivo for small deformations*. Ph.D. thesis, Catholic University of Nijmegen, The Netherlands.

WILLEN MD, SORRELL JM, LEKAN CC, DAVIS BR, CAPLAN AI (1991) Patterns of glycosaminoglycan/proteoglycan immunostaining in human skin during aging. *Journal of Investigative Dermatology* **96**, 968–974.

WINKLES JA, O'CONNOR ML, FRIESEL R (1990) Altered regulation of Platelet-derived Growth Factor A-chain and c-fos gene expression in senescent progeric fibroblasts. *Journal of Cellular Physiology* **144**, 313–325.

WINTER GD (1962) Formation of the scab and the rate of epithelialisation of superficial wounds in the skin of the young domestic pig. *Nature* **193**, 293–294.

WISTROM C, FENG J, VILLEPONTEAU B (1989) Proliferative capacity of human fibroblasts when cultured in serum from young or old cows. *Journal of Gerontology* **44**, B160–163.

WOODLEY DT, SARRET Y, O'KEEFE EJ (1991) Human keratinocyte locomotion on extracellular matrix. In *Wound Healing* (ed. H. Janssen, R. Rooman & J. I. S. Robertson), pp. 91–97. Petersfield: Wrightson Biomedical.

YAMAURA H, MATSUZAWA T (1980) Decrease in capillary growth during aging. *Experimental Gerontology* **15**, 145–150.

YONEZAWA Y, KONDO H, NOMAGUCHI TA (1989) Age-related changes in serotonin content and its release reaction of rat platelets. *Mechanisms of Ageing and Development* **47**, 65–75.