

Ageing and its influence on wound healing

Given the constantly rising percentage of older people in the population and the huge cost of treating wounds in this patient group, the importance of examining the relationship between ageing and wound healing cannot be underestimated. This article will examine current thinking in this area. Despite early reports of delays in wound healing associated with age and changes in cutaneous structure, it is now thought that wound healing in healthy older people is essentially normal but factors commonly associated with old age, such as ischaemia may account for any differences observed.

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KEY WORDS

Skin

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The percentage of older people in the UK is rising. Based on population estimates from mid-2000, 18.1% of the population in the UK is over the retirement age (currently 65 years for men and 60 years for women) and the projected percentage for 2021 is 19.1% (Office for National Statistics, 2002). The health services spend more than £1bn per year on the treatment of acute wounds, chronic wounds and delayed wound healing in older people (Ashcroft, 2002).

Delays in wound healing associated with age were first reported in the 1920s (Carrel and DuNuoy, 1921). It has also been reported that complications of wound healing, such as wound dehiscence, are more common in older people (Halasz,

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1968). Given the significant social and economic cost of treating these wounds it is important to consider the relationship between old age and wound healing. This article will examine current thinking on the issue.

Ageing and changes to the skin

The way the skin ages is effected by chronological ageing and environmental factors (Rogers and Gilchrest, 1990). The structure and the function of the skin are intimately related (Desai, 1997a) and it is important to compare the structure of the skin when considering wound healing rates between young and old. Indeed it has been shown that structural, physiological, clinical and pigmentation differences do exist between these groups (Cerimele et al, 1990; Leyden, 1990; Montagna and Carlisle, 1990; Ortonne, 1990).

Structural changes in the human epidermis that occur with age include flattening of the dermo-epidermal junction, a decrease in the number of melanocytes and Langerhans cells, and cytoarchitectural disarray (Desai, 1997a). Some authors dispute that all of these changes are merely age-related. For example, Montagna and Carlisle (1990) examined several hundred skin specimens and concluded that the prominence of the cytoplasmic extensions of the basal cell layer of the epidermis into the

dermis is an individual characteristic and may be blunted even among the young (Desai, 1997b).

It is also critical to consider the fact that ageing is largely an individual process and this has to be taken into account when generalisations are made about age groups. Comparing people by age may not be appropriate and it can become even more misleading in animal experiments as it is difficult to define and compare young and old across different species (Ashcroft et al, 2002).

Dermal changes associated with old age include reduction in thickness, elastic fibre, changes to dermal ground substance and decreased vascularity and density (Desai, 1997a). Age-related cellular defects are shown by a decrease in the absolute number of cells, the number of hair follicles in growth phase, the diminished production of macromolecules which results in a loss of collagen from the dermis and the in-vitro reduction in doubling capacity of mesenchymal cells.

Changes in the microenvironment/local structural architecture (outside the cells), such as a decline in glycosaminoglycan and collagen content, the alteration in physical properties of collagen and elastic fibres and the disorganised microcirculation may be just as important as the age-related cellular defects, as they 'may lead to

modified cellular responses resulting from altered matrix binding of growth factors, impaired cellular migration/proliferation in response to changes in matrix structure, quantity, and/or adhesion molecules expression, and altered signalling between matrix and cells leading to downstream changes in gene expression' (Ashcroft, 2002).

Wound healing in older people

Foetuses exhibit scarless repair and the transition to adult-type scarring occurs around the 24th week of gestation (Dang et al, 2003). This corresponds with the time that foetal skin becomes histologically similar to adult skin. Foetal wound healing appears to be fundamentally different to postnatal healing (Desai, 1997a) because it involves less differentiated tissue. Wound healing in older people is a process of repair and involves the formation of a scar. Unlike foetal wound healing there is not a definite age where healing becomes impaired. It may be that any differences found between different postnatal age groups studied depends on the extent of impairment of the skin's structure and function caused by associated conditions.

In a small study by Carrel and DuNuoy (1921), the authors experimented with dogs of various ages and produced a 'cicatrisation index' formula that calculates the rate of change of the surface area of an open wound over time. A larger index indicates a wound that is healing faster. This and DuNuoy's 1937 study have been criticised for experimental errors (Ashcroft et al, 1998b). Among these errors — acknowledged by the authors — is the fact that dog skin does not adhere to the aponeurosis and is very mobile, causing the size of the wound to become modified with slight changes in the position of the animal. Furthermore, correlation of the effects of ageing in wound healing between animal models and humans remains to be established (Swift et al, 2001). DuNuoy's (1937) study showed a significant delay to healing of open wounds associated with age in humans. Despite using uniformly sized 40cm² wounds and a large number of patients in the study, there was no

control for the site, nature, depth, and presence of infection (Ashcroft 1998b). In addition, the oldest group examined were aged 40 years and can hardly be regarded today as old.

The current thinking is that the effect of age after controlling known associated factors is not marked and that wound healing in healthy older people is essentially normal (Ashcroft et al, 1998b) despite alterations to individual processes. However, systemic, regional and local factors commonly associated with old age may impair wound healing (Van De Kerkhof et al, 1994) and all the phases of wound healing have been shown to alter with age (Hardy, 1989).

The impact of ageing on the phases of wound healing

Inflammatory phase

Although when challenged with ammonium chloride the acute inflammatory reactions of the skin appear to be less intense in older people (Kligman, 1979), evidence to date including work with people defined as healthy according to the SENIEUR protocol (Lighthart et al, 1984), confirms a marked pro-inflammatory response with age (Ashcroft et al, 2002). These subjects were not taking medication, they did not smoke (or had not smoked for 20 years and had smoked less than five cigarettes per day), and had no previous medical history of note. In addition, all subjects over 40 years of age had normal electrocardiograms and chest X-rays, as well as biochemical, lipid, and glucose profiles. A contributing factor may be the increased ability of dermal collagen to aggregate platelets as shown in experimentation on older rats (Grigoroვა-Borsos, 1988), perhaps causing the wounds to exhibit an early excessive inflammation response.

There is increasing evidence that oxidative stress underlies the ageing process. Also, research strongly suggests that calorie restriction reduces age-related oxidative stress and has anti-inflammatory properties (Chung et al, 2002). However, the effect of calorie restriction on wound healing remains unknown. Platelet release increases with age in rats (Yonezawa, 1989). In humans,

granulocyte adherence increases (Silverman and Silverman, 1977), and in vivo studies in humans (Ashcroft et al, 1998a) have shown an early influx of neutrophils and an altered ratio of mature to immature macrophages with an increased number of mature macrophages in older people. This is of potential importance because the phase of macrophage differentiation can dictate the expression of specific cytokines. However, the precise significance of this, apart from being another element in the altered inflammatory response in older people, is not yet known.

In a defined excisional wound model, Swift et al (2001) compared the inflammatory response between young and old mice. They found that the content of neutrophils in the wounds were similar but macrophage levels were 56% higher in aged mice who intriguingly, had lower phagocytic capacity, both in percentage and numbers of particles consumed by each cell. It may be that the increase in numbers is a compensatory effort for the decrease in phagocytic ability. The authors also showed an altered chemokine profile. These results appear to agree with the hypothesis presented by Stout and Suttles (2005) that the age-associated 'dysfunction' of macrophages is the result of their functional adaptation to age-associated changes in tissue environments.

A weakness of the study by Swift et al (2001) is that the definition of old age in mice and its correlation to old age in humans is arbitrary. Of extreme importance is the fact that alterations in cell populations rather than cellular defects alone are considered (Ashcroft, 2002) as this emphasises that alterations in cell populations subsets, rather than fundamental cellular defects, explain age-related changes. This provides an alternative focus of attention of research, not only in the field of wound healing but also in other pathophysiological conditions (Ashcroft, 2002).

Lymphocytes appear in the wound soon after macrophages (Ashcroft, 1998b). As lymphotropic agents, such as IL-2, and lympholytic agents e.g.

cyclosporine A, enhance and impair final wound strength respectively (Ashcroft, 1998b), cytokines produced by lymphocytes are likely to be important in this regard. In the study by Swift et al (2001), wounds of aged mice exhibited a delay in T-cell infiltration but with a higher eventual peak. This represents another alteration in inflammatory cell content in older subjects suggesting that chemokine production might also be altered with age.

Thivolet and Nicolas (1990), Makinodan et al (1991) and Gupta (2005) all explored some aspects of immune changes of the skin associated with ageing. Thivolet and Nicolas (1990) provide an overview of the relation between skin ageing and immune competence, including age-related changes in epidermal immune functions, such as morphological and functional changes in Langerhans cells and decreased production of various cytokines. Makinodan et al (1991) found that cutaneous delayed-type (type IV) hypersensitivity is also markedly attenuated with ageing, an intrinsic, at least in part, change in the immune system. Among immune functions, a decline in T-cell functions during ageing predominates and Gupta (2005) reviewed apoptosis in various subsets of T-cells and discussed the role of apoptosis in immune senescence.

Proliferation phase

Among the earlier researchers to study the effect of age in fibroplasia and wound strength during wound healing in rats, Howes and Harvey (1932) concluded that fibroplasia in the young begins earlier and results in more rapid healing. However, their work has several methodological imperfections, such as the absence of statistical analysis and standardisation of the surgical incisions studied.

Fibroblasts, the key cells responsible for matrix deposition, have been shown to change with age, demonstrating a decline in motility, proliferative capacity and chemotactic response (Ashcroft et al 1997b, Ashcroft et al 1998b). Using an incisional wound healing model in an ageing mouse colony, Ashcroft

(1997a) provided evidence of reduced deposition of specific extracellular matrix components, associated with ageing.

Growth factors and their receptors have also been found to change significantly with age both in vitro and in vivo. In a study of one patient with Werner's syndrome, Bauer (1986) demonstrated a diminished response of fibroblasts to platelet-derived growth

Despite alterations in the normal and wounded skin of older people, there is no evidence to suggest that advanced age impairs wound healing per se. However, the changes associated with age may predispose older people to the deleterious effects of ischaemia and other factors that impair wound healing.

factor (PDGF) and fibroblast growth factors (FGF). This corresponds well with the delayed and lower peaks of PDGF isoforms and their receptors within acute incisional wounds with increasing age in a mouse colony reported by Ashcroft et al (1997b). A similar finding was evident with epidermal growth factor (EGF) and EGF receptor. On the contrary, basic FGF (bFGF) had a statistically significant earlier and higher peak in the wounds of older mice. Reenstra et al (1993) showed a significant decrease in the number of EGF receptor number, affinity and internalisation with age, which corresponds well with Ashcroft et al's findings (1997b).

The effect of ageing on angiogenesis in wound healing is largely unknown (Ashcroft et al, 2002). In a small, assessor-blinded study in humans, Gilchrist et al (1982) showed that chronological ageing alters the inflammatory response mounted when the skin is exposed to ultra-violet light. Despite the small number of patients

(four subjects aged 22–26 years and seven subjects aged 62–86 years) statistical significance was shown in a number of variables in this generally well-conducted study. A decrease in the venular cross-sectional area in normal skin was shown to occur with age and a histological endothelial cell reaction to the UV light occurs later and is less intense in the older subjects compared with the younger group. This corresponds well with the study in rats by Ashcroft et al (1997a) who also found a delay in angiogenesis. In this study the degree of angiogenesis was ultimately increased, suggesting that it is unlikely to be a major factor in impaired wound healing in older people. An early decrease of macrophage production of the angiogenic vascular endothelial growth factor (VEGF), as shown by Swift et al (2001), is a reasonable explanation of the initial delay in angiogenesis in older people. Differences in degree and rate of angiogenesis may be due to the study model used (incisional versus excisional wounds), the age selected as representative of old and the time selected for quantification of angiogenesis (Ashcroft et al, 2002).

Keratinocytes from older donors show reduced response to most proliferative stimuli (Ashcroft et al, 1998b), including hypoxia (Xia et al, 2001), and increased sensitivity to inhibitors of proliferation such as interferon (Peacocke et al, 1989). In addition to providing a mechanism for the delay in epithelialisation observed in older people, these studies also demonstrate the influence of environmental factors on individual cell responses. In the same context, oestrogen appears to have a positive effect on keratinocytes (Roth et al, 1981, Ashcroft et al, 1997a) and serum from older donors has a negative effect on migration (Kondo et al, 1989).

As a later process in the phase of proliferation, wound contraction in older people has not been investigated extensively. The delayed wound healing demonstrated in aged rats by Ballas and Davidson (2001) was associated with increased contraction

by skin fibroblasts, not with differences in apoptotic or myofibroblast cell populations. It was also associated with increased collagen gel remodelling, which has been examined more extensively by various authors (Ashcroft et al, 1997c; Khorramzadeh et al, 1999).

Maturation phase

A lower rate of synthesis and higher rate of degradation of collagen with age has been reported (Mays, 1991). More recently, and in healthy people screened by the SENIEUR protocol (Lighthart et al, 1984) aged between 19 and 96 years of age, Ashcroft et al (1997c) showed upregulation of matrix metalloproteinase (MMP) -2 and -9 expression and activity, and down regulation of tissue inhibitor of metalloproteinase (TIMP) -1 and -2 expression (Ashcroft, 1997d). These lead to increased proteolytic activity, thus reducing matrix deposition possibly via local cytokine regulation, such as the observed decreases in TGF- β and PDGF and increase in bFGF (Ashcroft et al, 1997b).

More recently, Benanti et al (2002) have shown induction of transcription of the extracellular matrix remodelling genes MMP1 and plasminogen activator inhibitor 2 by the fibroblast senescence-associated protein APA-1, suggesting that this identified novel zinc finger protein is a transcription factor that regulates expression of matrix-remodelling genes during fibroblast senescence. The role of this protein in wound healing in older people remains to be defined.

In their review based on earlier experimental studies by various authors, Goodson and Hunt (1979) concluded that open wounds contract more slowly and incised wounds gain strength more slowly. Tissue remodelling leads ultimately to the appearance of a fine scar with reduced mechanical strength (Ashcroft et al, 1997a;d) and in older people the final scar resembles the regenerative scars found in foetal wound healing.

With regards to wound-breaking strength, animal models have produced

contradictory results (Ashcroft et al, 2002), partly because of the failure to adequately characterise these animal models. In a murine incisional cutaneous wound healing model, Quirinia and Viidik (1991) followed up their study long enough (up to 20 weeks) and showed equal mechanical properties at the end despite earlier lag (throughout the first three weeks of healing) in the wound strength between young and old animals. Furthermore, with the introduction of ischaemia by using an H-shaped double skin flap and testing the wound in the horizontal line of the H that has been shown previously to be ischaemic, they showed a greater degree of impairment (40–65%) in the old compared with the younger animals. The study showed that the ischaemic microenvironment affected older subjects significantly more than the young and suggested that re-establishment of microvascular competence should be a priority for injured tissues. In a similar model, Quirinia and Viidik (1996) have subsequently shown that treatment with hyperbaric oxygen is much more effective in older rats despite the pronounced effect of counteracting ischaemia in younger animals as well.

In a more recent experimental study of rats, Mogford et al (2004) examined the effect of ischaemia and ageing in cutaneous wound healing in young and old animals. A distinctive phenotype of wound healing was demonstrated by the combination of age and ischaemia that represents a common clinical scenario: granulation tissue formation was affected less and epithelialisation was affected more by age than ischaemia.

Conclusion

The main features of cutaneous wound healing in older people include: an altered inflammatory response; decreased collagen synthesis and increased degradation; delayed angiogenesis; and slower epithelialisation. Despite alterations in the normal and wounded skin of older people, there is no evidence to suggest that advanced age impairs wound

Key Points

- ▶ Differences in structure and the cellular microenvironment may be important considerations when comparing wound healing rates between young and old.
- ▶ The current thinking is that the effect of age after controlling for associated factors is not marked and wound healing in healthy older people is essentially normal.
- ▶ Ageing is associated in particular with increased inflammation, delayed epithelialisation and matrix degradation.
- ▶ Systemic, regional and local factors commonly associated with old age may impair wound healing.

healing per se. However, the changes associated with age may predispose older people to the deleterious effects of ischaemia and other factors that impair wound healing. **WUK**

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