Review

The mechanism of skin graft contraction: An update on current research and potential future therapies

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Abstract

Skin graft contraction is a common and intractable problem. The current treatments focus on mechanical opposition of contractile forces using splints and on compression of the grafted skin with pressure garments. For the patients, this causes significant morbidity with restriction of joint mobility and often requires multiple episodes of corrective surgery. Despite 50 years of research in this area, treatment and prevention of graft contraction have progressed very little and understanding of the underlying mechanism remains poor. This article reviews the clinical problem and the approaches used to prevent or treat graft contracture. It also considers to what extent we currently understand the cellular basis of graft contracture, based on in vitro models of skin contraction and in vivo observation of patients.

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1. Introduction

The treatment of patients with extensive burns or traumatic skin loss usually involves large areas of skin grafting. Contraction of human skin after grafting is a distressing post-operative complication that often necessitates further corrective surgery. In the United Kingdom, 13,000 people per annum are admitted to hospital for burn injuries and 1000 of these have severe burns requiring fluid resuscitation [1]. In the developing world, burns are much more common and globally, in excess of 6 million patients require grafting for burns each year. At least 30% of patients requiring grafting will go on to suffer hypertrophic scarring and graft contraction in the subsequent months, often reducing joint mobility [2–4]. Leblebici et al., recently showed that joint contracture significantly impacts burn patients’ quality of life, especially with respect to “Physical functioning, physical role limitations, bodily pain, and vitality” [5]. Despite the huge morbidity that graft contraction causes, relatively little is known about its aetiology and how to prevent it. Here we summarise what is known about graft contraction and discuss recent advances toward greater understanding of the underlying mechanisms.

2. The clinical problem of graft contraction

Wound contraction is a normal physiological phenomenon that reduces the area of a full-thickness wound and therefore expedites its closure. Loose-skinned animals possess a subcutaneous muscle layer called the panniculus carnosus which enables the skin to glide smoothly over the underlying tissues [6]. However, in man, this muscular layer is absent and which enables the skin to glide smoothly over the underlying subcutaneous muscle layer called the panniculus carnosus expedites its closure. Loose-skinned animals possess a layer that reduces the area of a full-thickness wound and therefore expedites its closure. Suggested models less than ideal for the study of wound and graft contraction.

It is widely recognised that application of a split-thickness skin graft to a wound reduces wound contraction and hypertrophic scarring compared with full-thickness wounds left to heal by secondary intention alone [7]. However, skin grafts can also contract, with restriction of joint mobility [8]. Established contractures often require surgical release with further skin grafting to the released area. This causes significant morbidity for the patient and financial cost to the NHS. Figures for the number of patients currently receiving treatment for graft contracture are not readily available on a national level in the United Kingdom. However, in a prospective study of 985 consecutive adult burn patients from 1993 to 2002 in Boston, USA, 38.7% had developed at least one contracture by hospital discharge, with a mean of three contractures in each of these patients [4]. The shoulder was the most frequently contracted joint (38%), followed by the elbow (34%) and knee (22%) [4].

Skin graft contraction occurs in two stages. When the skin is first harvested from its donor site, it undergoes immediate reduction in size, referred to as primary contraction. Primary contraction has been found to range from 9 to 22% dependent on the thickness of the graft and is thought to be due to passive recoil of elastic fibres in the dermis [9]. When harvested from the same region of the body, full-thickness grafts exhibit the greatest degree of primary contraction, split-thickness grafts contain a lower volume of elastin-containing dermis and hence exhibit less contraction and pure epidermal grafts fail to contract [9].

When a skin graft is placed on its recipient bed it then undergoes secondary contraction. This contraction reduces both the size of the graft at the interface with its recipient bed and the circumference of the graft at its periphery, with each edge of the graft moving towards the centre [10]. Again, the propensity for contraction varies with the graft thickness, but in the case of secondary contraction it is the split-thickness grafts that contract more than the full-thickness grafts [11]. This is the reverse of the findings noted for primary contraction and may be due to differences in cellular or matrix composition between the dermal layers contained within the graft [12]. Epidermal hyperplasia and dermal fibrosis are less prominent in full-thickness grafts than split-thickness grafts at 4 weeks after grafting [12].

The graft bed exerts an influence on the degree of contraction. Grafting directly onto fascia results in less contraction than grafting onto more mobile tissues [11]. It has also been noted that skin grafts placed on granulation tissue contract more than those on tangentially excised burn wounds [13] and that the likelihood of contraction increases with burn size [4,8,14]. In addition, severity of contraction is higher among paediatric patients than among adults [2,15,16]. This may be due to the changes in TGF-β profile seen with age [17]. These are discussed further below.

A graft on one patient may contract more than on another, suggesting inter-individual variation. However, grafts placed at different sites on the same patient may contract to different extents. Graft contracture is most marked in the neck, axilla, hand and knee [4,8,15], which are all highly mobile areas, and this is further highlighted by the different factors that contribute to this in the different regions.

| Table 1 – Relative risk of contracture formation following skin grafting for burns |
|---------|-----------------|-----------------|
| Factor          | Low risk             | High risk             |
| Age              | Adult               | Child               |
| Burn area        | Small               | Extensive            |
| Site of burn     | Trunk, limb sites remote from joints | Neck, axilla, hand, knee |
| Wound bed preparation | Tangentially excised wound | Granulating wound bed |
| Mobility of the wound bed | Low               | High                |
| Thickness of graft | Thick SSG          | Thin SSG            |
| Donor site       | Anterior trunk       | Posterior trunk      |
strongly suggesting that lack of mechanical resistance may be relevant. The donor site from which the graft is harvested also appears to affect contraction with grafts harvested from the back contracting more than those from the anterior chest and abdomen [11]. The reason for this is uncertain but may be due to site-specific mechanical properties of the skin. The factors that predispose to graft contraction are summarised in Table 1. Thus, at the clinical level it is possible to predict which patients are most at risk of graft contracture and this can be used to influence the application of pressure garments and splints post-operatively.

3. Clinical approaches to prevent graft contraction

Attempts to prevent the contraction of skin grafts are largely designed to counteract the mechanical forces generated by the contracting graft. The main methods in current usage involve splinting joints [18,19] and wearing pressure garments [20,21] for several months after grafting. These approaches have changed little in the last 40 years and, as recently highlighted by Richard and Ward, properly constructed studies to develop guidelines for application and duration of use are lacking [19].

Wounds healing under tension exhibit increased scarring [22,23] and the application of external pressure garments or use of splints to dissipate the intrinsic tension in the wound is thought to reduce the amount of scar tissue generated [20,24]. The pathogenesis of scarring and of contraction appears intimately related, and therapies designed to counteract one of these often form effective treatments for the other.

It is encouraging that pressure therapy is effective in causing regression of hypertrophic scarring in 60–85% of patients [25]. Constant pressure in excess of 25 mmHg induces rearrangement of collagen bundles and decreases scar oedema, vascularity, mucopolysaccharide production, mast cell degranulation, oxygen saturation and myofibroblast numbers [25–27]. Comparison of scar maturation in patients receiving “normal” versus “low” pressure therapy following burn injury indicate that it is pressure rather than simple occlusion of the scar by the garment that is necessary for reduction of scar hypertrophy [28]. Pressure therapy is only effective while the scar is immature [29] and has been reported to become less effective after 6 months of treatment [30]. It is generally recommended that pressure be maintained between 24 and 30 mmHg for 6–12 months [31,32]. Pressure of greater than 24 mmHg is required to exceed the inherent capillary pressure [33,34] but the applied pressure should be below 30 mmHg, to prevent reduction in peripheral blood circulation [33]. It is therefore probable that pressure garments create a hypoxic environment that reduces fibroblast proliferation and collagen synthesis [34]. Early release of the garments tends to be followed by rebound hypertrophy [35].

It is widely recognised that splinting and pressure therapy are maximally effective in the early stages of graft and scar contracture and are usually continued for 6 months to a year post-operatively [18,19,36]. They are cumbersome and unpleasant to wear [21] and also prolong the period of medical treatment after the injury and therefore delay return to normal daily activity. The evidence base for use of pressure garments in clinical practice needs to be strengthened. In particular, we need to know when pressure garments should be applied and for how long pressure therapy should be continued. Any biochemical or physical markers of the process of contraction which could aid clinical judgement in these areas would be valuable.

Once contractures have developed, excision and grafting of the area is usually undertaken. There is however, a strong likelihood of recurrence of the contraction. Donelan describes two approaches to treat contraction [37]. Release can be performed either by incising or excising the contracture. Incisional releases create a smaller defect and the surrounding hypertrophic scar tissue tends to soften and flatten once the tension has been relieved [37]. Donelan proposes that releases should be performed to over-correct the contracture deformity and grafts should be sutured with a bolster dressing. On removal of the bolster at the first graft check, he then packs the contour defect with semi-rigid foam-rubber conformers to minimise subsequent contraction [37]. Hence, surgical release is combined with mechanical splinting and pressure in an attempt to minimise recurrent contracture. In order for treatment and prevention to progress from the use of simple mechanical devices such as pressure garments and splints, with surgical release as a salvage procedure, greater understanding of the cellular and molecular mechanisms of graft contraction are needed in the hope that this can lead to improved therapies to prevent contraction.

4. Contraction at the cellular level—the role of the fibroblast

In attempting to identify biochemical markers of contraction, consideration must be given to the cellular activity underlying it. The theory that has received the most research emphasis to date is that contraction occurs secondary to the differentiation of fibroblasts to form myofibroblasts with expression of a-actin filament bundles [38–40]. These myofibroblasts possess intrinsic contractile properties similar to smooth muscle cells and organise their actin cytoskeleton along the lines of greatest skin tension [41]. The actin filaments become more ‘compact’ due to a sliding-filament mechanism involving actin and myosin filaments, similar to that observed in muscle sarcomeres [42]. As the myofibroblasts are adherent both to one another [43] and to the fibronectin-rich wound bed [39,44], the entire mass of granulation tissue contracts.

The majority of studies of fibroblast and myofibroblast behaviour in contraction have been carried out using fibroblast-impregnated collagen gels. Since the original publication of this model by Elsdale and Bard in 1972 [45], there have been in excess of 300 publications that employ this model to study fibroblast-mediated contraction, 31 of which were published within 2006 alone. These three-dimensional gels induce morphological changes in fibroblasts that at least partially mimic those seen in vivo [45]. Free-floating collagen gels are used in vitro to model relaxed tissue such as non-injured human dermis [46,47], and anchored collagen gels are used to model the stressed granulation tissue of a healing wound bed. In relaxed collagen gels the fibroblasts maintain their normal phenotype, whereas in anchored gels,
myofibroblast differentiation occurs [48] and the cells become bipolar, orienting themselves along lines of tension [49,50]. These studies show without doubt that fibroblasts are capable of exerting considerable contractile force on collagen gels. Fibroblasts in anchored collagen gels display a ‘fibrogenic’ phenotype with increased expression of collagens I, III, VI, XII, tenasin-C, procollagen C-proteinase, β-actin, TGF-β, connective tissue growth factor (CTGF) and other cytokines. Synthesis of α2 integrins and MMP-1 is decreased [51–53].

The role played by the fibroblast and myofibroblast has also been studied extensively in vivo. The reduction in contraction seen when a skin graft is applied to a wound correlates with a reduction in the inflammatory infiltrate [7,54,55] and myofibroblast differentiation [56]. Application of a skin graft also reduces the established contracture of a granulating wound [57], transforming the granulations into dense avascular fibrous tissue [58]. Similarly, use of a vascularised skin flap to obtain wound closure results in myofibroblast apoptosis [59,60]. Full-thickness grafts are more effective than split-thickness grafts in suppressing myofibroblast differentiation [56] and reducing the degree of contraction [11] and it is dermal rather than epidermal or total thickness that appears to be the important factor. The application of a skin graft also inhibits prolyl hydroxylase activity, so collagen synthesis decreases in the graft bed [61].

5. A role for the keratinocyte in contraction? Whilst the majority of research into cell-mediated contraction has concentrated on the fibroblast and myofibroblast, keratinocytes are also capable of contracting collagen gels in vitro [62–65] and their role is being increasingly recognised in vivo. In animal studies, experimental wounds may undergo a reduction in surface area of up to 25% during the initial 2–3 days after wounding, before significant fibroblast influx and myofibroblast differentiation have occurred [66]. Actin-filament organisation within fusiform keratinocytes at the

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Fig. 1 – $5 \times 10^4$ passage 1 keratinocytes were incorporated in 360 μl of a 2.7 mg/ml collagen gel and photographed at (a) day 0 and (b) day 7. Contraction of the gels was observed over the 7-day period. Scale bar = 5 mm. (c) Contraction of cell-seeded collagen gels was measured over 5 days (n = 2 experiments, three replicates per experiment). (■) Cell-free collagen gel; (●) $5 \times 10^4$ fibroblasts (passage 3–6) incorporated in the gel; (▲) $5 \times 10^4$ keratinocytes in the gel; (▼) $1.25 \times 10^4$ fibroblasts in the gel plus $3.75 \times 10^4$ keratinocytes seeded on top of the gel. A trend towards increased contraction with the combination of fibroblasts and keratinocytes is seen compared with keratinocytes or fibroblasts alone, although this does not achieve statistical significance (ANOVA + Bonferroni correction, P > 0.05). (d) Histology of collagen gel seeded with fibroblasts in the gel and keratinocytes on top of the gel and cultured for 7 days demonstrates that keratinocytes migrate to the central area of the gel and form a well-differentiated epidermis during contraction. H&E stain. Scale bar = 100 μm (Data and images are courtesy of Miss L.E. Smith, University of Sheffield).
wound margin appears to be responsible for this epidermal "purse-string phenomenon" [66,67], which has been demonstrated in both embryonic [68] and adult [69] mammalian wound healing.

Keratinocytes possess strong intercellular adhesions, with cultured confluent sheets of keratinocytes rapidly contracting to 70% of their original area following detachment from tissue-culture plastic in vitro [70]. Keratinocytes are more effective at contracting collagen gels when they are seeded on the top of the gel, rather than when they are dispersed through it, unlike fibroblasts [64]. This mimics the in vivo situation, in which the keratinocytes migrate across the wound surface during re-epithelialisation. At relatively low densities of surface-seeded keratinocytes, the contraction is equivalent to that seen with much higher densities of gel-incorporated human dermal fibroblasts [64]. In contrast with fibroblasts which become bipolar, the keratinocytes maintain their polyhedral shape but migrate towards the centre of the gel leaving the edges devoid of cells by 24 h after seeding [64]. Lillie et al., performed histological sectioning of keratinocyte-contracted collagen gels and found that the basal layer of keratinocytes was highly corrugated but a smooth stratum corneum was present [63]. Lillie therefore postulated that the adjustment took place within the stratum spinosum. Our own results using keratinocyte and fibroblast-seeded collagen gels are summarised in Fig. 1.

### 6. Development of tissue-engineered models for the study of contraction

Collagen gel models are well established and widely utilised for studying contraction. However, these collagen gels are relatively amorphous dilute collagen matrices and do not replicate the complex cell–matrix interactions seen in normal human skin where cells interact with mature cross-linked dermal collagen. As previously discussed, animal models are generally unsuitable for contraction studies, due to the dermal collagen. As previously discussed, animal models are needed which more closely mimic the in vivo situation, in which the keratinocytes migrate across the wound surface during re-epithelialisation. At relatively low densities of surface-seeded keratinocytes, the contraction is equivalent to that seen with much higher densities of gel-incorporated human dermal fibroblasts [64]. In contrast with fibroblasts which become bipolar, the keratinocytes maintain their polyhedral shape but migrate towards the centre of the gel leaving the edges devoid of cells by 24 h after seeding [64]. Lillie et al., performed histological sectioning of keratinocyte-contracted collagen gels and found that the basal layer of keratinocytes was highly corrugated but a smooth stratum corneum was present [63]. Lillie therefore postulated that the adjustment took place within the stratum spinosum. Our own results using keratinocyte and fibroblast-seeded collagen gels are summarised in Fig. 1.

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The MacNeil group has developed an in vitro tissue-engineered model of human skin, based on sterilised human dermis, obtained following routine plastic surgery procedures, sterilised with glycerol and ethylene-oxide and rendered acellular. This is seeded with laboratory-expanded human keratinocytes and fibroblasts and cultured at an air-liquid interface [72–74]. The tissue-engineered skin model is based on normal mature human crosslinked collagen. It retains a basement membrane [75], to which keratinocytes attach firmly and form a stratified epithelium, whereas fibroblasts penetrate and migrate through the dermis. The tissue-engineered skin developed by the MacNeil group contracts by 25–40% during 10 days culture in vitro [76,77] and by up to 60% over 30 days of culture [74]. In addition, these autologous tissue-engineered skin constructs have been grafted onto patients for the release of burn scar contractures in vivo, where they contract to a similar extent as the split-thickness grafts previously used in the same site [78].

Tissue-engineered models such as this, which provide a mature dermal matrix, are likely to form more physiologically relevant in vitro models for the study of graft contracture than the much simpler collagen gel model. For example, Medalie et al., compared culture of keratinocytes and melanocytes on acellular human dermis, sterilised using a combination of glycerol and cryopreservation, with culture on fibroblast-contracted collagen gels [79]. They reported enhanced epithelial organisation and enhanced rete ridge formation when human dermis was used as the matrix. When the composites were transferred onto athymic mice, pigmentation was increased when dermal matrix was used and epithelial hyperproliferation was reduced when compared with composites constructed on fibroblast-contracted collagen gels. After an initial phase of hyperkeratosis [80], the epithelium matured to resemble that of normal human skin [81] and blood vessels could be identified within tissue-engineered skin within 8 weeks of grafting [80].

Use of tissue-engineered skin also confers significant theoretical advantages in vivo. These materials may be constructed with a thicker dermal component than conventional skin grafts, and hence obtain a better cosmetic outcome in vivo. The fact that the number of keratinocytes and fibroblasts can be hugely expanded by culture from relatively small skin biopsies also offers the possibility of resurfacing large areas of the body in patients with major burns who lack sufficient donor sites for conventional split skin grafts. Despite the lack of preformed vasculature in tissue-engineered skin equivalents, the kinetics of inflammation, fibroplasia and remodelling, parallel those seen with autologous skin grafting [82].

Several research groups have developed tissue-engineered skin models for both in vitro [74,79,80,83] and in vivo use [78,84,85] and have studied the contraction of these models. Hull et al., synthesised skin equivalents from human cadaver fibroblasts, rat-tail collagen gels and autologous keratinocytes. They grafted these composites onto six patients and reported no contracture or hypertrophic scarring 18 months after grafting [86]. However, in a study of full-thickness burns in mice, wounds left to heal by secondary intention contracted to 4.5% of their original surface area by 6 months, whereas wounds grafted with these skin equivalents exhibited less contraction, reaching 34% of their original size [84]. Interestingly, Hull et al., found that omission of fibroblasts from their composites markedly increased contraction of these skin equivalents in vivo [87], consistent with the ability of keratinocytes alone to contract collagen gels. This also highlights the complex interplay between fibroblasts and keratinocytes within the contracting skin as the omission of fibroblasts from the gel increased the ability of the keratinocytes to cause contraction.

Kremer et al., centrifuged a disaggregated suspension of human keratinocytes onto sheets of Integra™ prior to engraftment and compared this approach with Integra™ allowed to re-epithelialise from the wound margins (a process which took 5–6 weeks) [85]. At 8 weeks after grafting, no difference in the degree of contraction was noted between the seeded Integra™ (21.6% of original area) and that allowed to
re-epithelialise (23.1%). These tissue-engineered models are gel based and hence lack the advantages of employing a mature dermal matrix with retained basement membrane proteins such as those developed by Ghosh et al. [72], Chakrabarty et al. [73] and Bechettoille et al. [83].

Using a three-dimensional model based on mature human dermis, Ralston et al. [76] and Chakrabarty et al. [77] noted that fibroblasts alone were unable to contract this mature dermal matrix, whereas keratinocytes caused marked contraction. The degree of contraction achieved with keratinocytes and fibroblasts did not differ significantly from that achieved with keratinocytes alone [76]. However, both cells are necessary for the formation of a stable basement membrane [73,75]. These results are summarised in Fig. 2. As with the collagen gels of Hull et al. described above [87], keratinocytes appear to play a major role in contraction of this mature human dermis, with fibroblasts having little or no effect. This is somewhat surprising given the volume of studies on fibroblast and myofibroblast contractile activity in collagen gels discussed above and highlights the influence of cell–matrix interaction on cellular activity.

Other research groups used a human dermal matrix and also found that the keratinocyte appears to play a major role in contraction. Erdag and Sheridan cultured fibroblasts and keratinocytes on cryopreserved acellular human dermis and measured contraction in the nude mouse model [88]. Their composites were cultured for 7 days in vitro at an air–liquid interface and subsequently grafted onto nude mice. Epidermal thickness and vascularisation were enhanced by inclusion of fibroblasts in the composites. Contraction was studied over a period of 4 weeks in vivo. Composites cultured with fibroblasts and keratinocytes showed negligible (2%) contraction, whereas composites cultured with keratinocytes but no fibroblasts contracted by 29% of their original surface area.

Interestingly Guo and Grinnell found that the ability of keratinocytes to contract dermis was increased by enzymatic removal of the basement membrane [89], although their use of

Fig. 2 – $3 \times 10^5$ passage 1 keratinocytes and $1 \times 10^5$ passage 3–6 fibroblasts were seeded onto de-epidermised acellular human dermis (DED) and cultured at air–liquid interface. Surface area was measured at (a) day 0 and (b) day 28 and a reduction of up to 60% of the original surface area was observed. (c) Contraction of cell-seeded DED was measured over 8 days ($n = 3$ experiments, three replicates per experiment). (■) Unseeded DED; (●) DED + $1 \times 10^5$ fibroblasts; (▲) DED + $3 \times 10^5$ keratinocytes; (▼) DED + $3 \times 10^5$ keratinocytes + $1 \times 10^5$ fibroblasts. Contraction by approximately 50% of the original surface area was seen with DED seeded with keratinocytes alone and keratinocytes + fibroblasts ($P < 0.01$). No significant difference was seen between keratinocytes alone and keratinocytes + fibroblasts (ANOVA + Bonferroni correction *$P < 0.05$, **$P < 0.01$). (d and e) Histology of tissue-engineered skin cultured with keratinocytes and fibroblasts for (d) 10 days and (e) 28 days at air–liquid interface. Keratinocyte differentiation occurs during the contraction process with a reduction in proliferating basal cells and an increase in keratin (Image is courtesy of Miss C.A. Hernon). H&E stain. Scale bar = 50 μm.
trypsin to remove the basement membrane may have altered the pliability of the dermis demanding less force generation by the keratinocytes in order to cause contraction. The ability of keratinocytes to contract collagen gels or dermis is widely known to be closely correlated with collagen gel concentration [63] or dermal pliability [74,77].

7. Factors affecting contraction of tissue-engineered skin based on human dermis in vitro

Looking in some detail at the nature of keratinocyte-mediated contraction of normal dermis, the work of the MacNeil group suggests that keratinocytes contract the dermis as they differentiate. Increasing keratinocyte differentiation with Vitamin C provokes premature differentiation and hyperkeratinosis with a marked increase in keratinocyte-driven contraction of the tissue-engineered skin [77]. In contrast, reduction in differentiation by using low-calcium medium reduces contraction (Thornton et al., submitted for publication). The relationship between keratinocyte differentiation and contraction is perhaps easy to understand in that a reduction of differentiation leads to a reduction in the formation of intercellular desmosomes [90] and this failure of intercellular adhesion may be at least partly responsible for the reduction in contraction seen with poorly differentiated cells. It may be the ability of keratinocytes to adhere tightly to each other that allows them to exert contractile force on the underlying dermal collagen.

Boyce et al., reported that in grafted wounds, wound surface area tends to stabilise by 4–5 weeks after grafting whereas in ungrafted wounds it proceeds for a variable duration beyond this [91]. The in vitro data of Thornton et al., indicates that mechanical splinting of tissue-engineered skin for a period of 8 days results in a reduction in the rate and extent of subsequent contraction over 32 days after the restraints are removed (Thornton et al., submitted for publication). This may explain how the early splinting of grafted wounds with foam conformers described by Donelan, results in a reduction in the subsequent graft contraction [37].

Also, it appears that keratinocyte-mediated contraction is initially reversible [77] as the epidermis can be peeled off the tissue-engineered skin within the first 10 days to leave behind the basal layer of keratinocytes, following which the dermis rapidly relaxes back to its original surface area (Thornton et al., submitted for publication). This is consistent with the research of Lillie et al. discussed above, where surface area adjustment is seen within the stratum spinosum of the epidermis [63]. Although keratinocyte-mediated contraction of human dermis is initially reversible, it subsequently becomes irreversible. This change appears to be mediated by covalent crosslinking of adjacent collagen fibrils. Culture of dermis-based tissue-engineered skin with β-aminopropionitrile (β-APN), an inhibitor of the lysyl oxidase crosslinking enzyme, leads to a reduction in contraction in vitro [74]. These findings have also been seen in the fibroblast-impregnated collagen gel model where lysyl oxidase-catalysed collagen crosslinking is observed during the contraction process [92] and again this contraction can be inhibited by β-aminopropionitrile [93].

8. Role of growth factors in contraction

While a skin graft sutured in place bears a superficial resemblance to normal skin, in practice it is a large wound environment in which keratinocytes, fibroblasts and other cells become activated and angiogenesis must take place. Within this environment a complex cellular interaction takes place through production of cytokines and growth factors, and direct interaction occurs between different cell types and between cells and the extracellular matrix. The complex interaction between cells is dynamic throughout the processes of wound healing and contraction as different cells take over dominant roles at different times. Whilst both the in vitro and in vivo studies of tissue-engineered skin models described above demonstrate a dominant role for the keratinocyte in the early phase of graft contraction, it is still possible that the fibroblast and myofibroblast play a dominant role in the later stages. Also complex interactions between keratinocytes and fibroblasts take place, with fibroblasts initially appearing to either have no effect on keratinocyte-mediated contraction [76,77,87], or to inhibit the contraction process [88]. As the keratinocytes and fibroblasts are separated by a basement membrane, such interaction is likely to take place via the production of cytokines and growth factors.

Oshita et al., studied the effect of keratinocyte-conditioned medium (KCM) and fibroblast-conditioned medium (FCM) on contraction of fibroblast-impregnated collagen gels [94]. Both KCM and co-culture-conditioned medium stimulated contraction significantly, whereas FCM did not. These results indicate that cytokines released by the keratinocytes are capable of stimulating fibroblast-mediated contraction in the simple collagen gel model. Oshita et al., then added exogenous endothelin-1 (ET-1) to this model and found a dose-dependent increase in contraction. They concluded that keratinocytes released ET-1 into the medium and this stimulated fibroblast-mediated gel contraction. However, keratinocytes alone are also capable of contracting collagen gels so the process is more complex than this finding initially suggests.

The role of growth factors in contraction of skin grafts is poorly understood. It is known that skin graft contraction is more severe in children than in adults [2,15,16], although the reason for this is uncertain. Ashcroft et al., studied the changes in transforming growth factor-β (TGF-β) expression with age and found a reduction in expression of TGF-β1 and TGF-β2 expression with aging, accompanied by an increase in TGF-β3 [17]. TGF-β1, TGF-β2 and TGF-β3 expression occur in suprabasal human keratinocytes [95–97]. TGF-β3 upregulates both its own expression and the expression of the other TGF-β isoforms, creating an amplification loop [98–101].

TGF-β1 and TGF-β2 stimulate fibroplasia and collagen deposition, inhibiting matrix-degrading metalloproteinases and upregulating the synthesis of proteinase inhibitors. The addition of TGF-β3 to wounds has been shown to down-regulate the production of TGF-β1 and TGF-β2 [302,103]. Uprogulation of TGF-β1 and TGF-β2 have been proposed as a primary mechanism of hypertrophic and keloid scarring [104]. TGF-β1 increases expression of α-smooth muscle actin by fibroblasts, hence promoting myofibroblast differentiation [105], and can increase the ability of fibroblasts to contract collagen gels [106]. In addition, TGF-β1 upregulates platelet-
derived growth factor (PDGF) synthesis [107]. PDGF accelerates granulation tissue formation [108] and stimulates collagen synthesis during the later stages of wound healing [109,110]. The role of TGF-β in contraction of tissue-engineered skin in vitro has been investigated using mannos-6-phosphate as a competitive inhibitor of TGF-β at the IGF-2 receptor [77]. Mannose-6-phosphate was noted to have a marked anti-tissue scarring effect in rodent, pig and human wounds [111]. However, it had no effect on contraction of tissue-engineered skin seeded with keratinocytes and fibroblasts indicating that TGF-β induced stimulation of myofibroblast differentiation is not likely to be primarily responsible for contraction in the tissue-engineered model [77].

The role of other cytokines and growth factors have also been studied in vitro using tissue-engineered skin. Insulin-like growth factor-1 (IGF-1) plays a major role in wound healing [112]. It is produced by fibroblasts but not keratinocytes in monoculture [113]. Synthesis of IGF-1 leads to increased collagen synthesis and deposition [114]. It shares many fibrogenic characteristics with TGF-β1, and is found in elevated levels in hypertrophic scar tissue when compared with patient-matched normal skin [115]. Exogenous application of IGF-1 has been shown to accelerate wound healing [112,116]. In addition to stimulation of keratinocyte proliferation [117], IGF-1 enhances keratinocyte migration [118]. In dermal fibroblast cultures obtained from normal skin, IGF-1 treatment was found to result in increased TGF-β1 gene transcription and a doubling of TGF-β1 levels in the conditioned medium [119]. Using a tissue-engineered model of skin in vitro, IGF-1 was found to have no significant effect on contraction [74]. Similarly addition of exogenous basic fibroblast growth factor (bFGF), tumour necrosis factor-α (TNFα) and prostaglandin-E2 (PGE2) had no effect on contraction [74]. Interestingly, culture with estrone or estradiol resulted in a marked increase in contraction, although the reason for this is unclear. It may be related to an increase in lysyl oxidase-catalysed collagen crosslinking in response to estrogens [120–122]. As described above, addition of β-APN, a lysyl oxidase inhibitor, effectively inhibits contraction in vitro.

9. Conclusion

We suggest that in vitro tissue-engineered skin models offer an interesting perspective on what may be happening in vivo following skin grafting. Although fibroblasts and myofibroblasts are very effective at contracting collagen gels, they appear ineffective at contracting more complex dermal architecture in these in vitro tissue-engineered models. Keratinocytes however are effective at contracting both collagen gels and the complex dermal architecture of tissue-engineered skin both in vitro and in vivo. The influence of cytokines and growth factors on the contraction process is still poorly understood, although it appears to differ from that of hypertrophic scarring, which is predominantly TGF-β based.

The knowledge that keratinocyte differentiation seems to be inextricably bound up with contraction of tissue-engineered skin (irrespective of any role that the fibroblast may or may not play) offers an easily accessible target for topical therapy to reduce skin graft contraction. Topical delivery (e.g. by hydrogels) of agents capable of reducing keratinocyte differentiation such as calcium-chelating agents, or agents that prevent collagen crosslink formation such as β-APN may reduce graft contraction. Also, the finding that early mechanical restraint of tissue-engineered skin leads to a reduction in the rate and extent of contraction indicates that use of splints and semi-rigid conformers immediately following grafting is likely to be more effective in reducing contraction than at a later stage, when the contraction process is established.

In summary we suggest a combination of early mechanical restraint and hydrogel delivery of topical agents to either delay keratinocyte differentiation or prevent crosslink formation should be explored in the hope that this may significantly improve quality of life for patients after burn by improving cosmesis and function and reducing the need for subsequent surgical contracture release and grafting.

Ethical approval

Ethical approval was obtained from the Sheffield Teaching Hospitals NHS Foundation Trust for any unpublished data in this manuscript.

Conflict of interest

None.

References


