The role of the fibroblast in wound contraction and healing

Although wound contraction appears to be organised by fibroblast cells within granulation tissue, the interaction between fibroblasts and the extracellular matrix is not fully understood. There are two dichotomous theories: the cell contraction theory and the fibroblast (cell traction) theory. This article discusses each theory and suggests that both may be correct but may occur at different times along the healing pathway. Further investigation into the mechanism of foetal scarless healing may uncover a way of manipulating wound contraction leading to faster healing, reduced scarring and elimination of fibrotic diseases.

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Healing takes place in four well-defined but overlapping stages: haemostasis; inflammation; proliferation; and remodelling. Each stage forms a continuum which relies upon a delicate balance of molecular signs involving an intricate series of ordered, inter-related events that include chemotaxis, mitosis, neo-vascular synthesis of new extracellular matrix and the formation of a scar. Remodelling continues for up to 18 months (Majno and Joris, 1996).

Most repairs are distinct from the surrounding tissues and never achieve more than 80% of the tensile strength of unwounded skin (Waldrop and Doughty, 2000). However, some wounds are capable of regenerating tissue without contraction and scar formation, leading to a perfect repair that matches the surrounding tissue (Martin, 1997), e.g a first-trimester foetus will heal cutaneous wounds without scarring and intra-oral wounds can also contract and form scars which are considerably smaller than the original wound.

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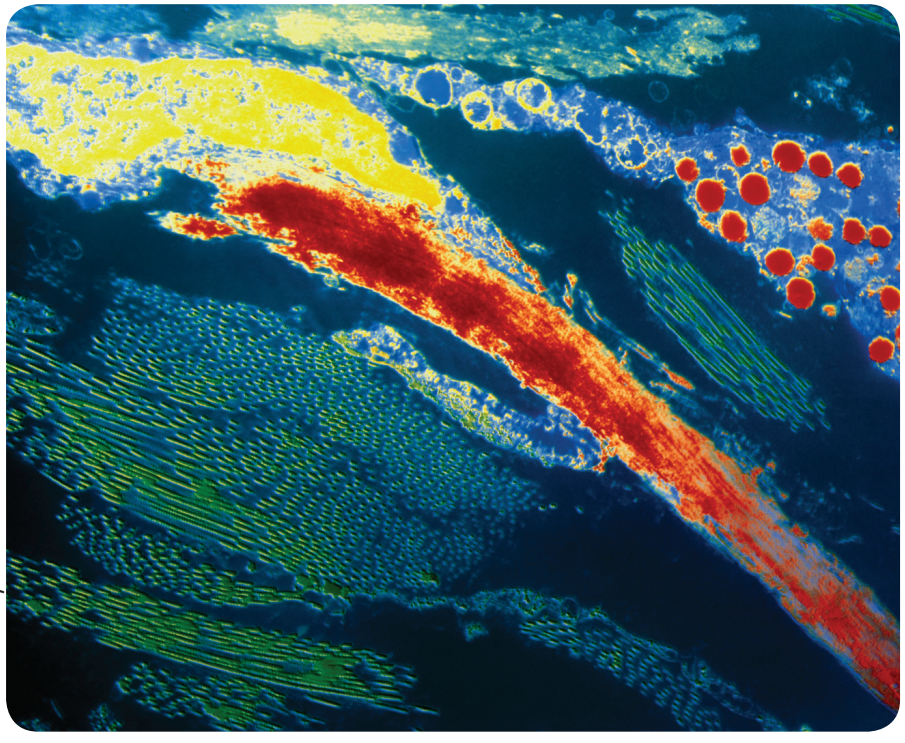
Proliferation is characterised by angiogenesis, formation of a matrix of elastin fibronectin, collagen synthesis, proteoglycans, glycosaminoglycans and other proteins, epithelialisation and contraction (Martin, 1997). The way that a wound contracts has been subject to debate. This paper focuses on the important role of the fibroblast and the debate surrounding its role in wound contraction and closure.

Wound contraction: an overview

According to a review by Tejero-Trujeque (2001) contraction occurs when the wound edges move towards each other in a centripetal fashion thus reducing the wound's dimensions. Contraction involves a dynamic process where cells organise their surrounding tissue matrix to reduce normal healing time by shrinking the amount of extracellular matrix (ECM) that needs to be produced (Jones et al, 2004). In many respects, wound contraction is beneficial as it can significantly reduce healing time because less granulation tissue needs to be produced to replace tissue loss (Calvin, 1988). The result, however, is scarring, which is less desirable as it diminishes function and appearance. In an animal model, contraction has been shown to begin in an acute skin wound about 4–5 days after wounding, peaking at 14 days, and progressing at roughly 0.6–0.75mm per day, depending on the shape of the wound and tissue type (Lawrence, 1998). In humans, sacral wounds contract rapidly because the skin is loose and subcutaneous fat is present. In contrast, pre-tibial wounds that are stretched with the dermis closely tethered to underlying tissue heal slowly, mostly by epithelialisation (Butterworth, 1993). Wound contraction stops usually by 4–6 months (Rudolph et al, 1977).

Fibroblasts are cells that are responsible for most collagen and elastin synthesis and organisation of the ECM components. They are indispensable in determining how well the wound will ultimately heal. Fibroblasts synthesise a fibronectin-rich ECM as soon as they enter a wound haematoma (Mehendale and Martin, 2001). Together with abundant amounts of the glycosaminoglycan hyaluronic acid, fibronectin — a glycoprotein — may serve as a channel to guide fibroblasts into the provisional matrix of the fibrin clot (Greiling and Clark, 1997). Fibronectin appears in the body in several isoforms. Soluble fibronectin in plasma enhances blood clotting, wound healing and phagocytosis, while all other forms are attached to the cell



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Coloured transmission electron micrograph of an elastin fibre (diagonal red band) in human connective tissue. The elastin fibre is in contact with a fibroblast cell (upper left, yellow/blue), which is probably synthesising the fibre. Nearby are collagen fibres (speckled green).

surface and are secreted into the ECM as highly insoluble fibronectin fibrils (Alberts et al, 2002).

In fibroblasts, fibronectin fibrils are linked to integrin $\alpha 2\beta 1$ transmembrane adhesion proteins in areas called fibrillar adhesions that keep the cell attached to the matrix. On the cell surface the fibronectin fibrils are elongated and are under tension and they usually coordinate with actin stress fibres within the cell. ECM signals can alter the intracellular actin cytoskeleton and thus the tension on the fibrils. The migrating fibroblasts change their phenotype to a profibrotic type, influenced by transforming growth factor-$\beta 1$ in order to synthesise and then rework a new collagen-rich matrix (Welch et al, 1990).

Collagen is the predominant extracellular component of the dermis. Wound contraction was initially thought to be caused by newly deposited collagen fibres. Abercrombie et al (1956) devised a method to test the theory using guinea pigs that were given a diet deficient in ascorbic acid for 15 days. Wound contraction occurred despite the formation of new collagen being severely compromised. Therefore contraction was considered to be the result of forces generated by cells in the central part of the granulation tissue. However, the authors admitted that half the guinea pigs were on the deficient diet for only a short time; therefore it was possible that their body reserves of vitamin C were not depleted and this could affect the outcome. (Guinea pigs have a 5–7 year lifespan, are unable to store ascorbic acid and will develop symptoms of scurvy in 3–4 weeks on a deficient diet.)

Currently, there are two leading, but contradictory paradigms which suggest a possible explanation of how fibroblasts generate and spread the force required to contract a wound. Gabbiani et al (1971) postulated that a specialised fibroblast which behaves similarly to a smooth muscle cell is responsible for ECM contraction. This is the myofibroblast theory. In contrast, Harris et al (1981) proposed that fibroblast cells exert traction as they move across the ECM. Cell locomotion forces realign the collagen fibrils associated with them. This is the fibroblast theory.

Myofibroblast theory

It has been proposed that myofibroblasts exhibit morphological traits of both fibroblasts and smooth muscle cells (Gabbiani et al, 1971; Majno et al, 1971). The differentiated cells are presumed to be responsible for the process of wound contraction via the movement of parallel actin smooth muscle contracting the cell in the same way as muscle would. Myofibroblasts are rich in cytoplasmic microfilaments (actin-rich stress fibres) and the expression of alpha smooth muscle actin (αSMA), also termed stress fibres, contract the myfibroblast in a muscle-type action. Additionally, myofibroblasts exhibit cell-to-cell and cell-to-matrix (fibronexus) links joined together by gap junctions and desmosomes which are thought to be necessary for fibroblasts to contract in a synchronised fashion (Darby et al, 1990). These contacts provide the force that pulls collagen I and III fibrils in the ECM towards the body of the cell, resulting in a reduction in the area of granulation tissue. The force that makes the entire wound contract is caused by many synchronised myofibroblast cells 'pulling' up tissue and 'fixing' it in place with collagen (Ryan et al, 1974). Myofibroblasts then undergo programmed cell death (apoptosis) as the wound closes (Desmouliere et al, 1995).

Myofibroblasts are thought to arise from the conversion of the wound fibroblast phenotype rather than from the smooth muscle cell (Darby et al, 1990). How quickly they change their phenotype depends upon the degree of mechanical tension and forces resisting the contraction. This was demonstrated by Grinnell (1994) in his studies of fibroblasts on floating or anchored collagen matrices. He noted that fibroblasts changed their morphology by developing fibronexus junctions and prominent stress fibres when added into a tethered matrix. Conversely, fibroblasts seeded into a free-floating collagen matrix retained a similar phenotype to a wound fibroblast.

Although the myofibroblast theory forms the basis of current teaching it remains speculative and controversial. According to Ehrlich and Rajaratnam (1990) the idea has many histological studies to support it, but there is little physiological evidence that shows that myofibroblast cells contract or that the cell reduces granulation tissue as it contracts or that it organises collagen fibres.

Fibroblast theory

In contrast to the myofibroblast theory, Harris et al (1981) proposed that wound contraction is achieved by many individual fibroblasts exerting traction forces across the centre of the matrix. Fibroblasts exert a traction force like a treadmill on the collagen fibrils in the ECM which they rearrange and compact, thus reducing its size. During cell elongation and spread across the ECM, shearing forces exert traction and thus cause the wound to contract. According to this theory, fibroblasts neither shorten in length, nor synchronise their efforts, they are randomly orientated and cell-to-cell contact is rare. The theory gained support from Ehrlich (1988) when he found no difference in contraction in a fibroblast-populated collagen lattice (FPCL) when myofibroblasts were added or removed from the lattice. He concluded that collagen fibre reorganisation is a result of fibroblastic locomotion (Ehrlich and Rajaratnam, 1990).

Both theories have many studies to support them, but they are often in vitro and involve animal models, implants and collagen lattices. Therefore considerable caution needs to be exercised when drawing conclusions. Using an acute wound model, Berry et al (1998) postulated that differences may exist between animal and human wound contraction because the behaviour of fibroblasts and myofibroblasts differ in their connection with collagen. Human skin heals predominantly by formation of new dermis and re-epithelialisation, with wound contraction representing a small fraction of the healing process in humans compared with other animals (Moulin et al, 2000).

The ability of the foetus to heal cutaneous wounds without scarring has attracted significant interest. However, evidence exists that not all foetal wounds heal scarlessly and the underlying mechanisms remain incompletely understood (Ferguson et al, 1996).

Tomasek et al (1992) proposed that FPCL contraction was mediated by myofibroblasts, however, the FPCL, which was first developed by Bell et al (1981), can closely mimic the biochemical and cellular aspects of normal skin but does not contain blood vessels or react the same, for instance it will not reproduce the inflammatory response. FPCLs also lack the full complement of signals and relationship variables between the complete range of all possible reactions in vivo that may influence cell behaviour (Kuhn et al, 2000). Conversely, for some types of experimental questions it is useful to isolate a single variable of interest and FPCLs continue to add to our understanding of cell biology.

McGee et al (1992) advises caution as transdifferentiation of cells including morphology and gene expression can be induced in culture. Therefore, it can be argued that myofibroblasts may be a phenomena inadvertently created by the culture or the scientist. Ehrlich (1988) has demonstrated that collagen matrices will contract without myofibroblasts and will also contract when keratinocytes are substituted for fibroblasts. Hembry et al (1986) used mice with tight skin to demonstrate that wound contraction occurred without myofibroblasts and proposed that their function is to apply tension or maintain the new balance that has been created. The results of animal studies are difficult to compare as a variety of animals with dissimilar body weights have been used. The size and shape of wound in ratio to the animal's size is important as is the technique of positioning the animal and measuring the area of the wound (Cross et al, 1995).

Fibroblasts appear in the wound after 2–3 days, yet myofibroblasts predominate at day 12 when wound contraction is almost 80% complete (Darby et al, 1990; Desmouliere et al, 1995). Darby et al used a rat model to show an 85.7% wound reduction detected by electron micrograph, however, it should be noted that high resolution imaging can pick up sub-cellular artefacts in addition to meaningful phenomena. Hence, McGrath and Hundahl (1982) noted some years earlier that a myofibroblast phenotype may be due to particular culture conditions and appear as a consequence of contraction rather than its cause.

Ehrlich et al (1994) studied αSMA in keloid and hypertrophic scars and initially found that only the nodules in hypertrophic scars were positive for myofibroblasts expressing αSMA. When placed in culture, both hypertrophic and keloid scars produced similar amounts of αSMA suggesting the cultured environment influences the expression of this protein. This may demonstrate the changes when cells are removed from their normal environment highlighting the importance of studying ECM and cell-to-cell interactions in vivo (McGee et al, 1992).

Scarless wound healing

The ability of the foetus to heal cutaneous wounds without scarring has attracted significant interest. However, evidence exists that not all foetal wounds heal scarlessly and the underlying mechanisms remain incompletely understood (Ferguson et al, 1996). There are a number of factors that influence certain foetal wounds to heal with little or no scarring and fibroblasts appear to have a crucial role in this. In common with intra-oral fibroblasts, foetal fibroblasts are phenotypically distinct, migrate at a faster rate and are more efficient at organising the ECM environment (Stephens et al, 1996). Unlike adult fibroblasts, foetal fibroblasts produce hyalonuric acid in consistent amounts that does not decrease with greater cell density thus allowing for faster migration, faster repair and cross-linking of collagen. Estes et al (1994) discovered that myofibroblasts in foetal wounds in sheep were absent at 75 days gestation when cutaneous wounds are expected to heal without a scar; but present in increasing amounts at 100 and 120 days when scarring escalated.

Gestational age appears crucial; Ferguson et al (1996) described the gradual transition or continuum from the ability of the early foetus to regenerate all of the dermal elements leading to invisible repair, to later gestation where a wound which is barely visible, to a small mark and finally an obvious scar as the foetus nears full term. Wound size and the amount of tissue damaged affects the extent of inflammation and cytokine response and thus scarless repair. A splinter or injection site usually heals without a scar but a full thickness injury will result in a clumsy repair with disorganised collagen and a dense mesenchymal scar (Ferguson et al, 1996). However, cases are documented where infants are born with hypodermic needle scars following prenatal diagnostic sampling (Paller and Mancini, 2005). The ability of the early gestation foetus to heal cutaneous injury rapidly and scarlessly appears to be intrinsic to foetal cells, ECM, cytokine expression and ultimately, genetic control. Current intensive research is focused upon understanding the factors involved in an attempt to benefit the quality of wound healing.

The relationship between the ECM and fibroblasts and how they interact to effect contraction of granulation tissue is still little understood... The study of adult tissues that retain the ability to heal quickly with minimal scarring, such as oral mucosa, and the study of scarless wound contraction is developing in exciting directions.

Conclusion

There is a stumbling block to understanding wound contraction because a suitably accurate model of human wound healing does not exist. Animal models, FPCLs and implants all offer useful insights into targeted areas of wound contraction but each has their own particular weaknesses. The meaning of experimental results is difficult to transfer to complex human wounds in vivo. The answer may lie somewhere between the two theories in that both influence wound contraction in different ways and at different times along the healing continuum. It seems clear that the fibroblast is not the only cell to produce contraction in vitro, however, the movement of fibroblasts through all levels of the ECM during the first days of wound healing before αSMA expression seems to be an essential requirement for the wound contraction process. Myofibroblasts that appear in the wound as contraction progresses may be responsible for tensile strength and scar formation. Moderating the rate of apoptosis during wound closure may be important in controlling scar formation and ultimately ameliorating pathological scars. It is evident that

Key Points

- Repair and healing can be broadly described in four well-defined but overlapping stages of haemostasis, inflammation, proliferation and remodelling.
- The repair is distinct from the surrounding tissues and never achieves more than 80% of the tensile strength of unwounded skin.
- Contraction involves a dynamic process where cells organise their surrounding tissue matrix to effect a reduction on normal healing time by shrinking the amount of extracellular matrix.
- In many respects, wound contraction is desirable as it can significantly reduce healing time by reducing the amount of granulation tissue that must be produced to replace tissue loss, but does result in scarring.
- Two theories about the mechanism of wound contraction exist: the myofibroblast theory and the fibroblast theory. There have been no definitive conclusions, because a suitably accurate experimental model of human wound healing does not exist.

gene expression and proliferation of cells is controlled by a mechanical force exerted on the tissue, but the suggested signalling pathways remain guesswork.

The relationship between the ECM and fibroblasts and how they interact to effect contraction of granulation tissue is still little understood and the role of cytokines and growth factors in the regulation of chemical processes involved in wound healing is not clear. The study of adult tissues that retain the ability to heal quickly with minimal scarring such as oral mucosa and the study of scarless wound contraction is developing in exciting directions. When the transition from foetal scarless phenotype to imperfect adult wound contraction is fully understood then future possibilities will exist whereby wound contraction can be manipulated to enhance faster healing, superior tissue regeneration and less scarring, and pathologies such as abnormal scar formation, contractures and fibrotic disease may be ameliorated. WUK

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