Wound healing is a well-orchestrated highly dynamic reparative process that occurs after all surgical procedures or traumatic injuries\(^1\). In order to achieve healing, three overlapping yet distinct phases are involved: inflammation, tissue formation and tissue repair\(^2\). For tissue to repair, various factors are involved, including cell-cell interactions involving various cell types, growth factors and cytokines\(^3\). Foetal wound healing differs from adult wound healing in a number of areas including the inflammatory response, the components of extracellular matrix (ECM), growth factor expression and responses and the profile of gene expression\(^4\). A number of animal models have been used to study foetal wound healing in vivo with monkeys\(^5\), sheep\(^6\) and mice\(^7\). In addition, a number of in vitro studies have used cells derived from human foetuses\(^8,9,10\).

An early study by Gerstein\(^11\) suggested that there was no difference between foetal epidermal proliferation and adult proliferation but later research by Khorramizadeh et al\(^12\) discovered that foetal fibroblasts proliferate more rapidly than adult fibroblasts\(^12\). The foetal regenerative phenotype has shown a difference in many processes involved in wound healing which can be manipulated to reduce or even prevent scarring\(^3\).

Lorenz et al\(^13\) observed that in human foetuses, wounds healed without scarring up to week 24 and scars formed after this time. A further observation in incisional and excisional wounds in lamb foetuses was that incisional foetal lamb wounds healed faster and without scarring at an early gestational age and for excisional wounds the size of the wound was strongly associated with an increase in frequency of scarring as the gestational age increased. This study was supported by Cass et al\(^14\) who used a foetal sheep model to find that gestational age was an important factor in the transition from scarless foetal wound healing to postnatal wound healing, which is characterised by an excess of collagen in ECM, loss of dermal appendages (such as hair follicles, sweat glands and sebaceous glands) and a flattened epidermis.

This article will focus on the literature about scarless foetal wound healing and contrast it with adult wound healing with scar formation.

Examining the evidence

In examining the amputated limb of a human foetus caused by amniotic constriction bands, Rowlatt\(^14\) found that early-stage foetuses had the capacity to heal dermal wounds without scar formation. This was supported by an experimental study by Lorentz et al\(^15\) who looked at human foetus skin grafts of various gestational age that were placed cutaneously and subcutaneously on athymic mice. The subcutaneous group healed scarlessly, while the cutaneous group healed with scar formation and this was found to be independent of the intrauterine environment and appeared to be linked to foetal skin itself.

In contrast, a later experimental study by Longaker et al\(^16\), using adult sheep and full-thickness skin grafts transplanted onto a 60-day-old lamb foetus, showed that wounds on the adult skin graft healed with scar formation, while foetal incisional wounds healed without a scar. They concluded that the unique quality of foetal wound healing is intrinsic to foetal skin and is primarily the result of the foetal environment. Further observations were made by Armstrong et al\(^17\) who studied a marsupial embryo.
and found that foetal regeneration was independent of the moist, sterile environment of the uterus.

The use of different species in wound healing studies can make direct comparisons either difficult or impossible as different species demonstrate variations in a number of wound healing processes[3]. Further complications in comparing foetal wound healing are in the wound itself with some studies using incisional wounds and some using excisional wounds or even burn wounds. Interestingly, the ability of the foetus to heal excisional wounds with perfect regeneration has been shown to be species-dependent[7]. Furthermore, some foetal excisional wounds, such as those in sheep, undergo contraction[17] while others show no contraction in closing excisional wounds (i.e. rabbits and monkeys) [5, 17].

Foetal wound healing and inflammation

In wound healing in adults, inflammatory cells including neutrophils, macrophages and lymphocytes are recruited at the site of injury to defend the body against invading substances, to dispose of dead and dying tissue and set the stage for new tissue formation[2, 18]. In contrast, experimental work on rabbit foetuses of variable ages by Adzick et al[19] found that although neutrophils were completely lacking, macrophages appeared earlier than fibroblasts but were lower in numbers when compared with adults. Later studies showed that foetal cells were capable of responding in the same way as non-foetal cells to irritants such as turpentine or carrageenan or by avirulent bacteria through the influx and action of neutrophils, macrophages and lymphocytes[20, 21]. However, Jennings et al[22] demonstrated that neutrophils and foetal serum were unable to phagocytose opsonised Staphylococcus aureus until the third trimester. This evidence suggests that in foetal wound healing neutrophils are not involved in the inflammatory response while macrophages are more evident.

Monocytes migrate along a chemotactic gradient into tissue where they differentiate into macrophages, which play a pivotal role in adult wound healing by removing cellular debris and by producing cytokines and growth factors[23]. In an experimental study, Hopkinson-Wooley et al[24] investigated the role of macrophages in apoptosis in a developing embryo and in wound healing in foetal mice by using the monocye/macrophage-specific monoclonal antibody. The authors found that monocyte-derived macrophages were responsible for phagocytozing and clearing areas of inter-digital apoptosis while in foetal wound healing the findings revealed that macrophages were not playing an active role in the healing of excisional wounds made in the mouse embryo until a certain age. Beyond this transition stage, significant recruitment of macrophages within 12 hours of wounding were seen. Hence macrophages can be attracted to wounds in earlier embryos if the wound results in significant cell death — for instance after a burn injury.

Later research by Cowin et al[25] found complex differences between the inflammatory response elicited in adult and foetal dermal wounds. In this experimental study on wounds in foetal mice using antibodies to monocytes and macrophages, the authors identified that although cells were recruited at the site of both adult and foetal wounds the number and persistence of these cells were lower in the foetal model, suggesting that manipulation of either the numbers or the activation states of inflammatory cells at the adult wound site may be an approach to the control of scarring during adulthood.

Cytokines and interleukins

Cytokines are a group of proteins produced by multiple cell lines consisting of growth factor, interleukins (IL), tumour necrosis factors and interferon[26]. They play an important role as mediators in cell proliferation, migration, matrix synthesis, deposition and degradation and the repair process[27]. IL-6 and IL-8 are pro-inflammatory cytokines and are essential for stimulating inflammation in postnatal wound healing. However, their response is decreased during foetal wound healing[28, 29]. Liechty et al undertook two different studies[28, 29] in which human foetal and adult dermal fibroblasts were incubated with platelet-derived growth factor (PDGF) and were then placed in a severe combined immunodeficiency (SCID) mouse. IL-8 and IL-6 mRNA were detected in unstimulated adult fibroblasts but not in the foetal fibroblasts with lower levels of IL-8 and IL-6 mRNA found in stimulated foetal fibroblasts than adult fibroblasts. The authors concluded from both studies that a diminished inflammatory
cytokine response by foetal tissues may be responsible for the lack of cellular recruitment and inflammation seen in foetal wound healing and may contribute to scarless wound repair. Conversely IL-10 is an anti-inflammatory cytokine and inhibits not only IL-6 and IL-8 but also the recruitment of inflammatory cells to the site of injury\[36, 37\]. Fortunato et al\[30\] carried out experimental studies in which amniochorionic membranes were collected from women undergoing elective caesarean sections and were then treated with recombinant IL-10. The authors concluded that the addition of IL-10 leads to a transcriptional regulation of interleukin-6, which results in decreased production of mRNA by the human amniochorionic membrane. In other studies conducted by the same authors in 1998, it was found that IL-10 in the presence of lipopolysaccharide showed a down-regulation of interleukin-8 mRNA.

In contrast, Peranteau et al\[32\] studied scar formation in a murine model that had been genetically manipulated to over express IL-10. They showed a decreased inflammatory response, a decrease in abnormal collagen deposition and restored normal tissue architecture. Overall, various animal studies were done to determine the role of cytokines in foetal wound healing. Peranteau et al\[32\] concluded that overexpression of IL-10 decreases the inflammatory response to injury, creating an environment conducive to regenerative adult wound healing.

Cyclooxygenase-2 (COX-2), a part of the arachidonic acid cascade, is up-regulated in response to an injury and it controls various aspects of inflammation by producing prostaglandins\[31\]. Wilgus et al\[36\] studied the COX-2 enzyme by blocking it using celecoxib in adult wounds and showed a reduction in scar formation without disrupting reepithelialisation or decreasing tensile strength. In a later study by Wilgus et al\[31\], a mouse model of scarless healing, demonstrated low levels of COX-2 and prostaglandin E2 (PGE2) while, conversely, the addition of exogenous PGE2 induced scar formation. More recently, Sandulache et al\[30\] proposed that PGE2 modulates both inflammatory and fibrotic processes during wound healing by inhibiting foetal fibroblasts' and adult fibroblasts' migration though foetal fibroblasts appeared to be refractory to migration. This effect may have significant and specific relevance to scarless foetal wound healing.

Foetal wound healing and ECM
Dermal fibroblasts are stimulated and recruited by platelets and macrophages and they play a pivotal role in wound healing, ranging from the synthesis of ECM to mediating its remodelling\[32, 37\]. ECM is important in regulating growth factors and cytokines and altering cell behaviour and hence requires a balance between biosynthesis and degradation for the final outcome of wound healing\[36\]. The ECM consists of fibrous glycoproteins (collagen and elastin), structural glycoproteins (fibronectin, laminin, tenascin C and vitronectin), proteoglycans (decorin) and glycosaminoglycans (hyaluronic acid and chondroitin sulphate)\[35\].

Collagen
Collagen, a fibrous glycoprotein, makes up the majority of the proteins in ECM, with 70% being type I and about 10% type III. In early wound healing, type III is laid down first, with the proportion of type I increasing as the wound progresses and remodelled\[36\]. A number of differences exist between the synthesis of foetal and adult collagen including the rate of deposition, collagen ratios and the quantity of collagen\[36\].

A study by Lovvorn et al\[41\] suggested that the collagen deposited by foetuses is less mature with less cross-linking which reduces rigidity but does not affect tensile strength. The reduced cross-linking shown by Lovvorn et al\[41\] was later linked to a lower expression of lysyl oxidase by Colwell et al\[42\]. However, studies by Goldberg et al\[43\] and Carter et al\[43\] suggested that foetal fibroblasts not only showed increased collagen III expression, but the new collagen was deposited in fine reticular or basket weave patterns similar to that seen in uninjured tissue. All these findings may help to design future treatment strategies that induce a foetal-like repair of adult wounds.

Structural glycoproteins
Structural glycoproteins such as fibronectin, laminin, tenascin C and vitronectin are involved in cell-surface interactions, particularly in the capacities of adhesion and cell migration\[44\]. Fibronectin consists of up to 20 splice variants and is involved in migration of endothelial cells, fibroblasts and keratinocytes in human foetal wound healing\[40\]. Early expression of fibronectin in foetal wounds was demonstrated in several studies that examined scarless wound

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healing. Other studies in both foetal and adult sheep and mice showed similar temporal and spatial expressions of fibronectin and early deposition of tenascin C in foetal wounds that enhanced reepithelialisation. Cass et al showed an increased expression of laminin and collagen receptors and neoexpression of fibronectin and tenascin C receptors in wounded foetal skin. This may further explain the foetuses’ ability to reepithelialise a wound rapidly with a reduced presence of inflammatory cells.

Hyaluronic acid
HA is the only glycosaminoglycan not covalently conjugated to a protein and also acknowledged as a key component throughout the wound healing process. In a study by Longaker et al, HA in adult wound fluid was found to increase rapidly, peaking in three days and returning to zero within seven days. In contrast, the HA level in foetal wound fluid increased rapidly and remained significantly elevated for three weeks. Longaker et al suggested that the prolonged presence of HA promoted foetal fibroblasts’ movement and proliferation and inhibited cellular differentiation, thus promoting healing by regeneration rather than by scarring.

Subsequently, West et al found a reduced activity of hyaluronidase in foetal wound fluid compared with adult wound fluid in wounds of foetal and adult lambs. These data suggest that lower hyaluronidase levels may underlie the different pattern of HA deposition seen in foetal wounds. Further evidence that HA affects the function of foetal fibroblasts came from Toole et al, who demonstrated increased levels of HA in foetal wound healing; HA promotes both the proliferation and migration of a number of cell types by binding to growth factors and cytokines which can result in temporal and spatial differences of these factors.

Earlier studies also signify the role of HA in foetal wound healing. For example, Mast et al showed that by reducing HA expression, adult type healing was observed in foetal rabbits; also interaction of HA with reparative cells through HA receptors in foetal wound healing was observed by Alaish et al who found high levels of HA receptor in foetal fibroblasts when compared with adult fibroblasts. Further studies by Locono et al and Hu et al found that an exogenous addition of HA reduces the scar formation in adults by reducing transforming growth factor (TGF-β1 which is known to be elevated in scar tissue.

While a reduced or delayed expression of decorin was shown to be associated with scarring in a number of adult models, a later study by Beanes et al showed that decorin was present in 95% of the proteoglycans in the dermis, and confirmed the reduced expression of decorin in foetal fibroblasts and foetal skin. It is thought to alter the biological activity of TGF-β.

Foetal wound healing and myofibroblasts
Wound contraction is an active cellular phenomenon and the contractile activity of fibroblasts and myofibroblasts provides the force for wound contraction. Myofibroblasts, which are differentiated fibroblasts, were detected earlier in foetal wound healing than post-natal wound healing. Furthermore, an in-vitro study by Rolfe et al showed earlier differentiation of human foetal fibroblasts to myofibroblasts when stimulated with exogenous TGF-β1. Martin and Lewis linked foetal wound closure to actin cables, which act like a purse string. These findings were opposed by Estes et al and McCluskey and Martin who showed the absence of alpha smooth muscle actin in murine and sheep foetal wounds. However, Martin and Lewis were supported by Brocks who studied chick embryos and found actin cables were assembling within minutes of an injury requiring guanosine triphosphate and Rho factor to reepithelialise foetal wounds; myosin cables may also be seen which act like a zip to close incisional wounds. Adult wound closure requires active movements of connective tissue and epidermis to allow the epidermis to migrate and cover the exposed connective tissue.

Foetal wound healing and TGF-β
A variety of expression of growth factors and their receptors play a vital role in wound healing with a number of aberrations including scarring. During the inflammatory phase, platelet activation and degranulation lead to the release of three inactive isoforms of TGF-β that become active by binding to receptor complexes and then exert their important biological actions in both tissue repair and scarring. While TGF-β1 mRNA when compared with adult wound healing, is rapidly induced and cleared in foetal wound healing, TGF-β3 is highly expressed in foetal
wound healing [10, 44, 68], and TGF-β2 was found to be expressed in higher levels in foetal wound healing [10]. Further, Shah et al. [99, 70, 71] have shown that while blocking TGFβ1 and TGF-β2 may reduce scar formation, exogenous TGF-β3 showed improved scar formation [11]. Thus early manipulation of the concentrations of selected cytokines could be a new approach to the control of scarring [11].

Conclusion
The precise mechanism of foetal regeneration remains unclear but a number of differences have been identified between foetal and adult wound healing. Various studies have suggested a role for foetal cells in difficult-to-heal wounds, for their ability to promote adhesion, proliferation and migration of existing cells. Further clinical work is required to understand how foetal cells promote regeneration and wound healing and the possibility of using this for adult wound repair. Work will also be needed to study the role that stem cells play in both adult and foetal wound healing. Understanding foetal wound healing and regeneration will have an impact on adult wound repair in the future and may lead to the reduction or even prevention of the formation of scar tissue in a number of organs.

References
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