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Discuss aspects of healing in skin surgery

Scarless healing: lessons from the embryo

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Introduction

Following any form of skin surgery, a scar remains as a permanent visible reminder of the procedure that has been performed and the abnormality that has been corrected. Every year in the developed world, 80 million people acquire new surgical scars, resulting from 55 million elective operations and 25 million operations following trauma [1]. Scars can be of great concern to the patient: as well as being visually undesirable, they may be itchy and painful and lead to anxiety, depression and disruption of daily activities [1].

The ideal endpoint of injury to the skin would be complete restoration of the structural and functional attributes of the original uninjured skin. Following such cursory deliberation, there appears to be little advantage in having a scar, a permanent visual reminder of previous trauma, which has at best 70-80% of the tensile strength of normal skin [2].

This essay will first explore teleological theories of why adult vertebrates heal with scars rather than without signs of scarring as in embryos. Following a description and comparison of the mechanisms of scarring in normal adults and embryos, we will explore whether any differences can be exploited to allow adults to heal without scars following dermatological surgery.

Why do we scar?

The wound-healing mechanisms which produce scars may have evolved in response to the widespread, dirty wounds, such as those following bites or severe blows, which were commonly faced by our ancestors [3]. In nature, following non-fatal wounds, a selective pressure may have existed to minimise wound infection, by rapidly replacing damaged tissue and avoiding wound breakdown, even if this was at the expense of restoring perfect functionality. In contrast, in today's world, wounds are mostly created in relatively clean conditions by sharp instruments such as the surgeon's scalpel.

Alternatively, throughout time, scars may have simply served an aesthetic function as a marker of individuality and a means of attracting mates and warding off rivals: scarred individuals can be assumed to be hardier and braver, as they will have survived battles and injuries in sustaining those scars. Individuals with the scar-forming genotype may have been more likely to reproduce, and thus the scar-forming characteristic has survived for such

cosmetic reasons. However, our increasing obsession with youth and perfection may mean that scars, now associated with old age and a lack of self-care, may no longer have the desired effect of attracting mates.

Wound healing in adults

Following injury to the skin, the main stages of wound healing can be divided into haemostasis, inflammation, proliferation and maturation [4] [5] [6] [7].

Macroscopically, haemostasis is evidenced by tissue blanching, clot formation and the cessation of bleeding. Following tissue injury, endothelial cells lining the blood vessels retract to expose subendothelial collagen, the medium to which platelets attach and form the primary platelet plug. On attachment, platelets are activated, that is increase their expression of surface receptors, aggregate and degranulate, releasing a host of factors which aid clot and matrix formation.

The inflammatory phase is characterised by *rubor*, *calor*, *dolor*, *tumor*: that is redness, warmth, pain and swelling. This is brought about by a combination of a vasodilatation and increased capillary permeability, induced by platelet degranulation, and the concomitant release of agents such as histamine and bradykinin twenty minutes post-injury. During this phase, white blood cells are recruited, predominantly neutrophils at first, later replaced by macrophages, which phagocytose foreign material, exterminate bacteria through the release of free radicals and degrade damaged tissue using enzymes, including elastases and collagenases. T-helper cells also enter the wound and secrete cytokines causing further T cell division and macrophage recruitment and enhancing vasodilatation and vascular permeability.

Macrophages are stimulated by the hypoxic wound environment to release platelet derived growth factor, which promotes chemotaxis and proliferation of fibroblasts (2-3 days post injury), and agents which promote angiogenesis, the formation of new blood vessels. Granulation tissue comprises this mixture of new blood vessels, fibroblasts, inflammatory cells and new components of the extracellular matrix. Epithelial cells can then migrate across this new tissue, either from the wound margins inwards, or where the basement membrane remains intact from the stratum basale upwards (as in normal skin). The migration continues until cells from the newly formed edges meet, at which point contact inhibition prevents any further migration.

Seven days after the wound has occurred, the wound begins to contract: this process is driven by myofibroblasts, may take several weeks, and in extremis can cause disfigurement and loss of function. The contraction phase ceases when the myofibroblasts stop shortening and undergo apoptosis.

After contraction finishes, and the rates of collagen production and breakdown equalise, the maturation phase of wound repair begins. This may last for over a year, and involves the reduction of cellular content and blood vessels at the wound site and the replacement of disorganised bundles of type III collagen, present during proliferation, with stronger type I collagen aligned along tension lines [4], augmenting the wound tensile strength and leaving a scar.

Wound healing in mammalian embryos

Mammalian fetuses of early gestational age demonstrate the remarkable ability to sustain skin wounds which heal with complete restitution of the normal skin architecture, with no signs of scarring and no impairment of function [8]. This finding has been repeated *in vivo* across all mammalian species investigated thus far, from mice to monkeys [3] [8] [9] [10], and which we hope to be able to extrapolate to humans.

After the first third to half of gestation, there is a gradual progression from the scarless healed wounds of early embryos to the scar-forming wounds of adult mammals. In the early embryo, following repair of a wound, the extracellular matrix is noted to be deposited in an orderly criss-cross fashion, recapitulating the structure of normal skin. In the adult mammal, smaller, parallel bundles of extracellular matrix are deposited. It is this failure to replicate the structure of normal skin, rather than biochemical differences in the composition of the healed wound, which is believed to underlie the phenomenon of scarring.

Extensive research into the molecular, cellular and environmental differences between early embryonic, late embryonic and adult wounds has been performed. Ferguson and O'Kane [3] stipulate two conditions which must be satisfied before inferring that any measured difference actually contributes to the scar-forming phenotype. Firstly, manipulating a proposed variable in one direction should cause an alteration from the scar-forming phenotype to the scarless phenotype. Secondly, manipulation of the same variable in the opposite direction should induce the opposite phenotypic shift. When these preconditions are applied, very few of the surfeit of proposed differences between the young fetal and adult healing processes survive as potential therapeutic targets.

One promising molecule is transforming growth factor beta (TGF-β), a skin morphogenetic factor released by degranulating platelets and monocytes in adults and by infiltrating keratinocytes and fibroblasts in early embryos. Fetal wounds contain a high concentration of TGF-β3 than adult wounds, and lower concentrations of TGF-β1 and TGF-β2 [3].

Manipulation of adult wound healing

The aforementioned differences have been investigated in animal models to see whether adult mammals may have the potential to heal without scars. Application of antibodies directed against all three isoforms of TGF-β does not improve scarring [11]. The addition of exogenous TGF-β3 to healing wounds results in improved scarring with more rapid healing [11], a result also seen in transgenic murine embryos homozygous negative for TGF-β3 [12].

Shah *et al.* [11] [13] found that application of antibodies against TGF-β1 and TGF-β2 to a healing adult rodent wound results in reduced scarring, as does preventing their activation using mannose-6-phosphate, a competitive inhibitor [3]. Likewise, when gene transfection is used to downregulate TGF-β1 expression in adult incisional wounds, there is reduced inflammatory infiltrate, faster healing and reduced scarring in the recipients of the gene therapy compared to the controls [14].

Early application of such agents, within 48 hours of injury, is suggested to produce optimal healing because earlier after injury there are fewer signalling molecules, thus the autoinduction of the rapid cascade effect of cytokines and cellular responses leading to scar formation is avoided. Also, the earlier application of therapeutic agents may cause a very different repertoire of cells to be activated and recruited [3].

Together, these results suggest that simulating the embryonic wound environment early during the healing process in adult wounds may improve scarring, and that TGF-β isotypes are important molecules in achieving this end. Human trials with pharmaceutical agents aiming to simulate this embryonic wound environment have now commenced: initial results whilst reported as being promising remain to be published [3].

Conclusions

The evolutionary reasons why mammals heal with scars are not applicable to patients undergoing dermatological surgery today. The evidence considered indicates that it may be possible, by manipulating the cocktail of cytokines surrounding an incisional wound to mimic the wound healing environment of the embryo, thus permitting adults to heal with reduced scar formation. In future, novel therapeutic agents may become available to prevent scarring from being an inevitable consequence of wound healing, thus improving the functional and cosmetic outcomes of skin surgery.

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