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REVIEW ARTICLE

Cutaneous wound healing: Significance of proteoglycans in scar formation

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Proteoglycans (PGs), components of extracellular matrix (ECM), play an important role in modulating the structure and regulating the functions of the skin. The timely turnover of PGs influence the development and differentiation of cells. Wound healing also depends on the level of PGs which if not adequate leads to abnormal scars. The role of PGs in different phases of wound healing and their implication in the formation of abnormal scars and several other skin disorders are discussed in the present review.

PROTEOGLYCANS (PGs) comprise a part of the extracellular matrix (ECM) which participates in the molecular events that regulate cell proliferation, migration and adhesion. These processes are regulated by the interaction of PGs with other components which are mediated through the glycosaminoglycan (GAG) chains or through protein–protein interactions within the core proteins of the PGs. The protein core functions as a scaffold for immobilization and spacing of GAG chains. GAGs are linear polysaccharides where the inherent structural feature is a repeating disaccharide unit composed of uronic acid and hexosamine. There are four main types of GAGs, heparin/heparan sulphate, chondroitin/dermatan sulphate, keratan sulphate and hyaluronic acid. While chondroitin and dermatan sulphates consist of *N*-acetyl galactosamine and uronic acid, the keratan sulphate consists of *N*-acetyl glucosamine and galactose. The sugars in GAGs are sulphated either at the 4th or 6th position to varying degrees; an exception is non-sulphated hyaluronic acid which exists as a free glycosaminoglycan. Due to the water absorbing capacity, PGs occupy a large space and may fill most of the intercellular spaces. PGs play a critical role as shock absorbents in the umbilical chord in the embryonic stage and at every stage of development in different ways throughout the life span. They also play a vital role in cell proliferation, migration and adhesion[1]. Thus PGs are found to be prominent molecules during wound healing through their influential role in cell–cell and cell–matrix interactions (Figure 1).

An attempt has been made in the present review to explore the role of PGs during wound healing. Their role in tumour invasion, aging, etc. has also been discussed.

Distribution of PGs in different layers of skin

The skin is the most affected organ following an injury. To understand the role played by PGs during wound

healing, it is essential to analyse the distribution of PGs in different layers of the skin. The skin as such consists of three layers namely dermis, basement membrane and epidermis. These layers put together act as a barrier between the organism and the environment. Recent studies have shown that PGs are synthesized by all types of mammalian cells[2]. Distribution of different types of PGs in different layers of the skin is given in Table 1. Specific PGs may be responsible for specific functions of these layers because PGs give a particular structural identity to the layers by sorting out specific cell types by migrating and retaining at the specific layers[2].

Wound healing

Wound, the damage caused by environmental insults such as mechanical and chemical injuries, may extend from the epidermis deep into the muscles depending on the severity of damage. Wound thus caused can be healed by a spontaneous process in the organism through a cascade of events, which starts by switching on various chemical signals in the body; this facilitates the restoration of anatomical continuity and function. While partial thickness wound heals by mere epithelialization, the healing of full thickness wound which extends through the entire dermis involves more complex well-regulated biological events[3] (Figure 2). However, in certain predisposed individuals, these events go awry resulting in the formation of hypertrophic scars or keloids[4,5] (Figure 3).

The healing process begins with the clotting of blood and is completed with remodelling of the cellular layers of the skin. The whole process can broadly be classified into 5 overlapping phases namely inflammation, granular tissue formation, re-epithelialization, matrix production and remodelling[6]. The PGs are reported to play an essential role at every stage of wound healing.

Role of PGs in different phases of wound healing process

Most of the growth factors and cytokines that are involved in wound healing are immobilized at the cell surface and in ECM through PG binding[7].

Hyaluronic acid (HA) is one of the major members of GAG present in the skin. During the inflammatory phase intact HA in the blood clot of wound helps in the physical stabilization of the matrix. It also stimulates cell infiltration and migration, and controls the degradation of fibrin. The degradation products of HA–fibrin matrix act as regulator molecules of the wound healing process. Small HA fragments stimulate both angiogenesis and phagocytic activity of macrophages[8]. Several studies have reported an increased production of hyaluronan during inflammation in wound repair[9].

Basic fibroblast growth factor (bFGF) is another substance mainly involved in angiogenesis. It is sequestrated and protected by binding with heparan sulphate which gives stability to bFGF rather than free bFGF[10]. This binding also gives the necessary conformation for optimal interaction with the cell-surface receptors[11]. The binding of FGFs to heparin appears to protect the growth factor from degradation[7]. The activities of some proteases and anti-proteases found in inflammatory fluids can be modified *in vitro* by heparin[12]. The inflammatory phase serves as a scaffold for the next phase, the granulation phase[13–16].

One of the major events in granulation tissue formation is the deposition of a loose ECM. HA is a major component of early granulation tissue[17] and creates an environment for cell movement by expanding the extracellular space. It has been reported that in the wounds of both foetal and adult sheep, the HA content of the granulation tissue increases until three days after injury. The higher level of HA persists in the foetus[18], but falls quickly back to normal in the adult[19]. Hence it has been suggested that the prolonged presence of HA in the wound may account for the scarless repair in the foetus[20]. This clearly indicates that HA helps in scarless healing and if suitable levels are maintained in adults during wound healing, scar formation can be prevented.

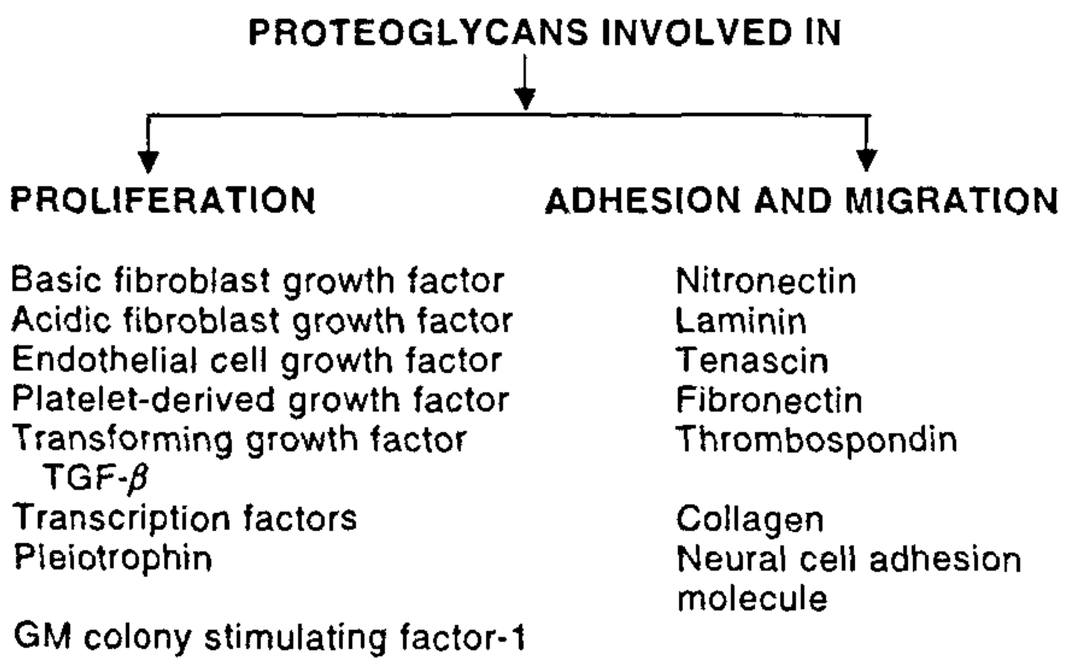

Figure 1. Type of function performed by specific proteoglycans.

Table 1. Distribution of PGs in layers of the skin.

Layers of the skin	Types of PGs
Epidermis	Heparan sulphate, keratan sulphate, chondroitin sulphate, PG-100, CD-44, syndecan
Basement membrane	Heparan sulphate, chondroitin sulphate
Dermis	Heparan sulphate, chondroitin sulphate, Dermatan sulphate, decorin, biglycan, fibroglycan, versican, glypican

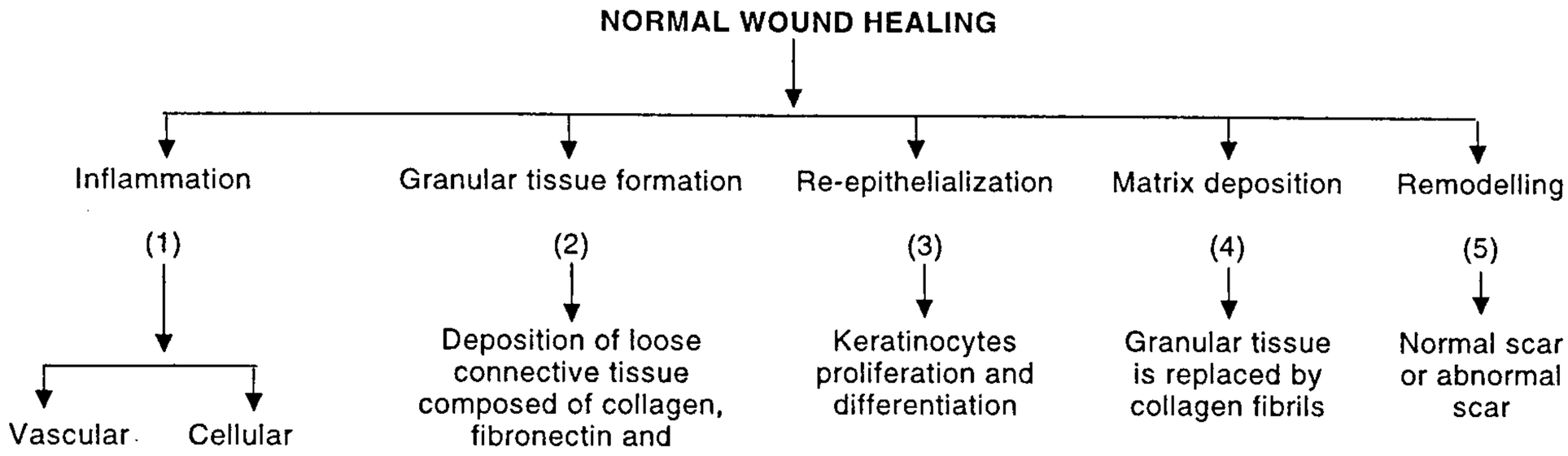

Figure 2. Normal wound healing process divided into 5 different steps. Proteoglycans play their role from step (2). In step (5) it is decided whether the healing would be normal or abnormal. The role played by proteoglycans in steps 2–4 would be the determining factor.

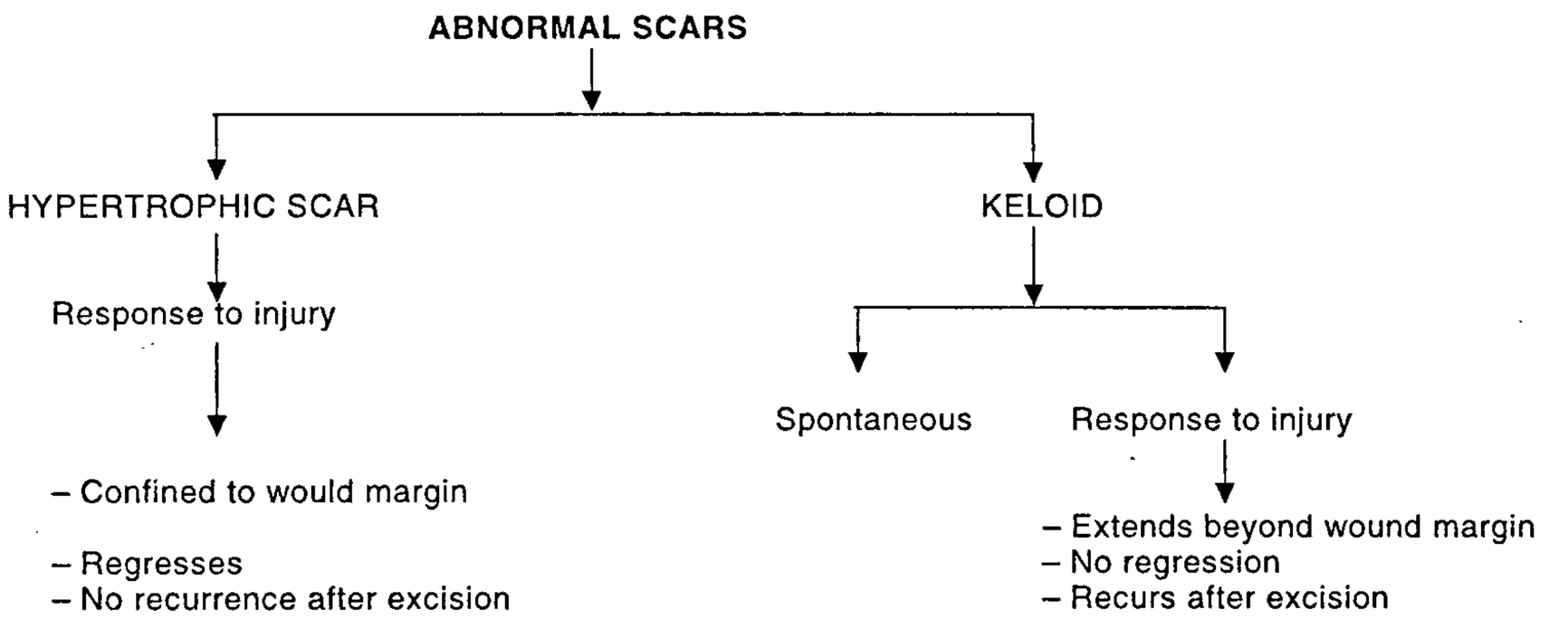

Figure 3. Types and response of abnormal scars.

During maturation of the wound, the HA content of the connective tissue tends to decrease quite rapidly while the chondroitin sulphate and dermatan sulphate contents tend to increase[21]. These PGs are involved in collagen fibre formation and chondroitin sulphate-4 (CS-4) has been shown to accelerate polymerization of type I collagen. *In vitro* CS-4 is involved in initial polymerization of collagen molecules while dermatan sulphate may modify the formation of collagen fibres and bundles. The small dermatan sulphate proteoglycan, decorin, is thought to be involved in regulating collagen fibre formation[22]. Syndecan, a cell surface heparan sulphate proteoglycan was found to be involved during this phase of healing. Syndecan 1 ecto is a strong inhibitor of heparin-mediated FGF2 division of cells. However, on removal of syndecan 1 from the system by degradation, cells start proliferating again. Syndecan 1 also binds to the collagens and fibronectin deposited during repair, due to which the tissue reverts to a quiescent state[23].

Restoration of the basement membrane (BM) is an essential event during wound healing as it appears to play a profound role in the organization of cells into functional units. Keratinocytes, the cells of the epidermis, migrate laterally only on an intact BM, if the BM is destroyed by injury the cells migrate across a provisional matrix of fibrin and fibronectin[24]. The wound keratinocytes themselves are capable of synthesizing the BM components such as laminin and type IV collagen and regenerate the BM. An important group of representative macromolecules of the BM are PGs (ref. 25). The principle PG in BM is a large heparan sulphate proteoglycan–syndecan which contains multiple domains with homology to adhesive molecules and is also involved in regulatory functions such as cell proliferation, differentiation and migration. Several studies have reported that basement membrane heparan sulphate proteoglycans (BMHSPGs) are capable of binding a variety of biologically active proteins including growth factors such as bFGF (ref. 7). They play an essential role in the assembly and integrity of the BM by interacting with other BM components such as fibronectin, laminin and collagen type IV (refs 26, 27). The expression levels and the quality of heparan sulphate proteoglycans, could affect several phases of wound healing such as re-epithelialization, epidermal growth, etc. BMHSPGs have been reported to alter the growth behaviour of the basal keratinocytes during re-epithelialization[28].

Syndecan-1 is a major component of the epidermis and its expression is strongly induced in migrating and proliferating keratinocytes during wound healing[29]. Syndecan is also known to bind a variety of ECM ligands while structural modifications in this PG may regulate the adhesion of cells to different ECMs. In the remodelling phase the PG comes to its normal level thus providing a suitable environment for the collagen bundles to align in a proper orientation.

In general, GAGs are over-expressed during the early stage of wound healing and come to their normal level in the remodelling phase. The cross linking between collagen and GAG provides adequate strength to the tissue and it becomes resistant to collagenase digestion[30]. HA which allows the migration of cells to the sites of connective tissue development, is the predominant GAG during the early phase of healing[31]. The role of GAGs in the remodelling phase is crucial; by blocking the cleavage sites of collagen they may inhibit the action of collagenase. Due to lack of timely signals, the PGs are over-expressed in hypertrophic scar (HS) or keloids[4]. The possible occurrence of a collagen–GAG complex could result in continuous synthesis of collagen matrix in keloids causing a delay in remodelling of the tissue.

Role of PG in formation of scars

Post-burn HSs have been reported to contain 2.4 times more uronic acid compared to the normal skin[32]. Immunohistochemical localization of decorin, biglycan and versican in HS revealed marked reduction in decorin and significant increase in large chondrotin sulphate proteoglycan (biglycan) and versican[33]. Quantitative analysis showed that the decorin present in HS is only 25% that of normal skin, while versican and biglycan were reported to be six fold higher[32]. While HA-staining of the papillary dermis in keloid was minimal when compared to HS, granular and spinous layers of the keloid epidermis exhibited an intense HA-staining[34]. We have shown in an ultrastructural study that cells and extracellular materials migrate from the dermal region to the epidermal region of the keloid skin through the gaps in BM which results in keloids[35]. Hence, by controlling the levels of HA, HS and keloids can be controlled; however more information in this direction is required to draw such conclusions. Even though extensive studies on HS and keloids have been carried out on the aspects of collagen synthesis and PG synthesis, the detailed mechanism involved in the collagen–GAG complex formation has not been addressed so far. Unlike the normal skin, the collagen bundles in keloids are arranged in a haphazard manner. The improper orientation of collagen bundles in keloids may be attributed to changes in the GAG levels, which have certain regulatory functions influencing collagen fibre formation and the three-dimensional organization of collagen[36].

A variety of conditions such as interaction of collagen with proteoglycans[37,38] have been postulated to be important in the regulation of fibrillar architecture. Studies on the effects of GAG on collagen fibre formation *in vitro* demonstrated that HA and chondroitin sulphate accelerated the fibre formation at the nucleation phase[39]. Formation of collagen fibres is considered to be an entropy-driven process where the exclusion of water molecules takes place resulting in an increase in entropy. The elevated level of GAGs in keloid collagen, by preventing the removal of water molecules may result in the decreased lateral growth (unpublished). This was further confirmed by forming segment-long-spacing crystallites of collagen where the lateral growth of fibrils was observed to be reduced compared to normal skin collagen. Due to the water retaining or absorbing capacity, GAGs swell in solution and occupy a large volume.

Our recent study on viscosity measurements supports the above fact, where hydrated specific volume is found to be higher in keloid collagen when compared to normal skin collagen. Viscosity measurements further show an increase in the denaturation temperature of keloid collagen by ~5°C when compared to normal skin collagen. This was further confirmed by differential scanning calorimetric studies[40]. Increase in the transition temperature of the helix to coil transition of the keloid collagen could account for the increase in the GAG content in keloid[41]. Aberrant PG metabolism could be a significant factor contributing to the altered physical properties of keloids.

Normalization of epidermal proliferation plays an essential role during wound healing. It is also proposed that complete BM maturation following skin wounding is a slow process and may account for the epidermal abnormalities that persist after wound healing[28].

Recent studies showed that there is a persistent epidermal hyperproliferation in keloids as indicated by the enhanced expression of proliferative specific keratins K5/K14 (ref. 42). The normalization of epidermal hyperproliferation follows the kinetics of normalization of the BM. It has been reported that BMHSPG is absent in migrating epithelial cells. The mechanism of hyperproliferation is unknown, however, it is reasonable to speculate that persistent epidermal hyperproliferation could be due to the absence of the O-sulphated HSPG epitopes which change the action of the growth factor bFGF (refs 7, 43). Growth behaviour of basal keratinocytes is altered due to abnormal regeneration of BM. The ultrastructural observations made in the keloid tissues are in good agreement with the above facts where discontinuity in the basement membrane is seen[35].

PGs in aging and skin disorders

Besides wound healing, GAGs also play an essential role during aging and in several other skin disorders.

Aging

Hyaluronate, the predominant GAG in the early developing dermis, decreases rapidly with age while the amount of dermatan sulphate increases[2]. The decrease in the HA content as mentioned earlier in adults has been suggested as one of the causes for scar formation and therefore the aging skin is more prone to scars if there is a wound.

Tumour and other skin disorders

Hyaluronate and urinary sulphated GAGs (ref. 2) levels are elevated in patients with systemic scleroderma. Another disorder characterized by excessive synthesis of GAGs is pretibial myxoedema. HA chondroitin/dermatan sulphate is also more abundant in and around solid tumours. The altered GAG composition of ECM in tumour stroma may stimulate cell migration and also facilitate cell growth. Tumour cell lines synthesize more HA *in vitro* than their normal counterparts. Syndican-1 is diminished or lost in invasive squamous cell carcinoma[29].

Conclusions

PGs are known to be important in several physiological aspects. Besides playing a structural role by providing mechanical strength, with their capacity to absorb water and fill the space between the collagen and elastin fibres, they also have regulatory functions such as influencing collagen fibre formation, cell proliferation, cell migration and cell adhesion during wound healing and in several skin disorders. An in-depth study on the mechanism of GAG during these processes will give a better understanding and pave the way to therapeutic methods for the treatment of several skin disorders.

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