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Review

Connexins in wound healing; perspectives in diabetic patients ☆

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ABSTRACT

Skin lesions are common events and we have evolved to rapidly heal them in order to maintain homeostasis and prevent infection and sepsis. Most acute wounds heal without issue, but as we get older our bodies become compromised by poor blood circulation and conditions such as diabetes, leading to slower healing. This can result in stalled or hard-to-heal chronic wounds. Currently about 2% of the Western population develop a chronic wound and this figure will rise as the population ages and diabetes becomes more prevalent [1]. Patient morbidity and quality of life are profoundly altered by chronic wounds [2]. Unfortunately a significant proportion of these chronic wounds fail to respond to conventional treatment and can result in amputation of the lower limb. Life quality and expectancy following amputation is severely reduced. These hard to heal wounds also represent a growing economic burden on Western society with published estimates of costs to healthcare services in the region of $25B annually [3]. There exists a growing need for specific and effective therapeutic agents to improve healing in these wounds. In recent years the gap junction protein Cx43 has been shown to play a pivotal role early on in the acute wound healing process at a number of different levels [4–7]. Conversely, abnormal expression of Cx43 in wound edge keratinocytes was shown to underlie the poor rate of healing in diabetic rats, and targeting its expression with an antisense gel restored normal healing rates [8]. The presence of Cx43 in the wound edge keratinocytes of human chronic wounds has also been reported [9]. Abnormal Cx43 biology may underlie the poor healing of human chronic wounds and be amenable therapeutic intervention [7]. This article is part of a Special Issue entitled: The Communicating junctions, composition, structure and characteristics.

Contents

1. Gap junctions in skin

Our skin is the largest organ of the body and its maintenance is essential for survival. In the avascular epidermis gap junctions are essential for the passage of nutrients between cells as well as gaseous exchange. There are a wide variety of different connexin types that have been reported to be expressed at different levels in mammalian skin, including Cx26, 30, 30.3, 31, 31.1, 32, 37, 40, 43 and 45 [10–19]. The reasons for the widespread expression and the relative importance of the different connexin types remains obscure but the requirement for the normal functioning of several different connexins has been made clear by the diseases that arise following point mutations in certain types of connexins, *e.g.*, hystrix-like ichthyosis with

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deafness (HID), keratitis-ichthyosis-deafness syndrome (KID), Vohwinkel syndrome and erythrokeratoderma variabilis (EKV), [16,17,19–21].

Whilst many different types of connexin have been detected in the skin, most are expressed at very low levels. However, connexins 43, 30 and 26 are found to dominate. In rodent skin Cx43 is by far the most ubiquitous and is found in dermal fibroblasts, blood vessels, and appendages such as sweat glands, sebaceous glands, hair follicles, and mast cells and activated leukocytes as well as epidermal keratinocytes [4,17,22]. Connexins 26 and 30 are not found in the dermal fibroblasts but are reportedly expressed in the epidermal spinous layers of rodent skin. Cx26 is more strongly expressed in the basal layers of rodent epidermis, whereas Cx30 is expressed more strongly in suprabasal layers of human and porcine skin, as well as hair follicle keratinocytes [18]. Cx31.1 can be detected in skin granular layer cells undergoing death, similar to the expression seen in cells about to die in the atretic follicles of the ovary and it might be possible that this connexin serves to coordinate cell death in these situations [4,14,23].

2. Wounding brings about rapid changes in connexin expression in the injured epidermis

Any wound to the skin sets off a series of four interrelated and overlapping processes (haemostasis, inflammation, proliferation and migration, followed by scar tissue remodelling) designed to prevent infection and bring about wound closure [24]. Haemostasis processes take place in the first few hours and are followed by inflammation that rapidly increases and lasts for several days. As these events progress there are concomitant changes in the expression of different connexins (Table 1). The first changes in epidermal connexins can be observed about 6 h after injury in rodent skin wounds, at which time downregulation of both Cx43 and 31.1 can be observed in wound edge keratinocytes. The downregulation continues so that virtually none is detectable within the first 1–2 days after wounding [4,25,26]. This downregulation is correlated with keratinocytes adopting a migratory phenotype as they start to crawl across the wound bed to close the epidermal breach. Conversely whilst Cx43 and 31.1 are turned off in wound edge keratinocytes, Cx30 and 26, which are normally expressed at low levels, are greatly upregulated, and remain so in the leading edge keratinocytes during the process of migration, only returning to normal when the wound is closed [4]. Although Cx43 is greatly reduced in actively migrating leading edge keratinocytes, it is found at relatively high levels a few cell rows back where cells are actively proliferating [4,15,25]. Very similar connexin dynamics are seen the healing response in explants of human skin despite the subtle different connexin distributions between the laminae. A downregulation of Cx43 occurs in wound edge keratinocytes as early as 5 h after wounding and by 24 h Cx43 is hard to detect whilst Cx26 and Cx30 have greatly increased in its place [9,27].

Interestingly, whilst Cx30 and 26 can interact and form gap junctions with each other, they do not do so with Cx43 and have very different coupling and dye transfer properties [28]. It is therefore possible that migrating keratinocytes require different communication properties and may even form a communication compartment exclusive to the leading edge cells which does not communicate with the cells following a few rows back. Such communication compartments have previously been proposed to coordinate the morphogenic movements of tissues in embryonic development and also wound healing [24,27,29,30]. A role for protein kinase C has also been proposed in phosphorylating Cx43 serine 368 at the wound edge in order to decrease its communication, a hypothesis which is supported by dye transfer studies which show restriction in communication between wound edge cells [27,31–34]. However, these changes are all transient and as soon as the migration is complete and the wound edges have joined up, connexin levels start to return to their normal expression pattern and distribution [4,8]. The reason for these dynamic changes in connexin expression patterns and the precise communication requirements during the healing process still remain to be fully determined. However, it appears that it is not a complete absence of communication that is required for keratinocyte migration but an absence of Cx43 [4,9].

Table 1
Summary of connexin dynamics in acute wound healing of normal and diabetic rodent skin.

	Cx43	Cx31.1	Cx26	Cx30
Connexin dynamics in rodent skin acute wound healing				
Migratory phase				
Epidermis	↘	↘	↗	↗
Leading edge keratinocytes				
Dermis migrating fibroblasts	↘			
Inflammatory phase				
Blood vessels	↗			
Mast cells	↗			
Neutrophils	↗			
Macrophages	↗			
Granulation tissue phase				
Fibroblasts	↗			
Epidermal revision phase				
Keratinocytes	↗	↗	↘	↘
STZ diabetic rat wounds				
Non-Migratory phase 1 day after wounding				
Non-migrating Epidermis	↗		↗over a wide zone	↗over a wide zone
Leading edge keratinocytes				
Dermis non- migrating fibroblasts	↗			
Migratory phase 2 days after wounding				
Epidermis	↘		↗over a wide zone	↗over a wide zone
Leading edge keratinocytes				
Dermis migrating fibroblasts	↘			

Why might an absence of Cx43 from migrating cells be desirable in order to promote migration? There are several potential explanations for this. The cytoplasmic tail of Cx43 can bind to an array of other junctional and cytoskeletal proteins, either directly via a PDZ domain or indirectly via other proteins such as membranous Cadherins, Zonular Occludin-1 (ZO-1), α- and β-catenin, as well as cytoskeletal microtubules and actin [35–42]. Such interactions may affect both cell adhesion and cytoskeletal dynamics and therefore cell migration and wound healing. However, some of the interactions are with transcription factors such as β-catenin, and binding to it may keep β-catenin at the cell membrane, preventing it from influencing gene expression. Indeed, it has been proposed that Cx43 forms the centre of a protein complex or "nexus" acting as a master gene that can influence expression of other over 300 other genes [43,44]. If so, the influence from Cx43 may not just be at the level of protein–protein interactions but may also have influence at the transcriptional level. This would involve a role that is potentially independent from its functions of cell–cell communication as a gap junction channel. In sum, Cx43 may influence wound healing in different ways including affecting the expression of other genes and proteins that can influence wound healing.

3. Dermal connexin responses to injury

Similar to keratinocytes, wound edge dermal fibroblasts downregulate Cx43 in the first 24–48 h after wounding as they start to migrate into the wound bed and form new granulation tissue [6]. However, whilst Cx43 is downregulated in fibroblasts it is upregulated in the endothelial cells of blood vessels as they become inflamed

and leaky. The upregulation of vascular Cx43 is rapid and can be detected in the first few hours after injury and is maintained at a high level throughout the inflammatory period in a similar way to that seen following central nervous system injury [4,6,45]. The upregulation of Cx43 is likely to be brought about by the proinflammatory signals being released at the wound site and this correlates with the blood vessels becoming leaky to leukocytes, proteins and fluids which in turn cause the dermis to swell.

The inflammatory response has long been thought to be central to the tissue repair process though the positive nature of this role is now being questioned [46]. Inflammatory signals come from a variety of sources starting with mast cells and degranulating platelets that are forming a fibrin clot to plug the wound. These release platelet derived growth factor (PDGF) which in turn attracts neutrophils (which, interestingly, also express Cx43) into the wound bed and surrounding tissues where they release more inflammatory signals which in turn recruits monocytes that transform into macrophages [46–49]. The recruitment of the neutrophils serves a key role in the immediate defence of any bacterial infection and prevents potential death by sepsis. Macrophages in this setting serve to clear up spent neutrophils and any other debris in and around the wound site whilst releasing additional inflammatory signals such as transforming growth factor beta 1 (TGF-β1) and fibroblast growth factor 2 (FGF-2) [50].

The production of a strong inflammatory response is thought to provide the signals to stimulate wound repair by transforming wound edge fibroblasts and keratinocytes into a migratory phenotype that will crawl forward to close the wound and fill the wound bed with granulation tissue [50]. Research in recent years, however, has produced evidence that may call this concept into question. For instance, Smad3 knockout mice which have a lower than normal inflammatory response to wounding, with significantly less neutrophils, are reported to show enhanced rates of epidermal wound closure [51]. Similarly in the PU-1 knockout mouse, which lacks mast cells, neutrophils and macrophages, wounds will heal at a normal rate, again suggesting that an inflammatory response driven by these cells is not essential for healing [52]. Indeed active depletion of neutrophils from a wound site has also been reported to result in faster re-epithelialization [53]. In a related manner, evidence from embryonic wound healing studies on both animals and humans shows that during the time before an embryo has developed the ability to mount an inflammatory response to injury, it will heal (by purse string contraction rather than cells crawling forward) and it does so without forming a scar [54–56]. This suggests that far from being essential for triggering the onset of wound healing the inflammatory response, it may in fact be contributing to fibrosis and scar formation [57]. Indeed large numbers of neutrophils at a wound site may do more than just kill bacteria; the release of free radicals designed to kill bacteria may also kill healthy cells in the intact surrounding tissues [46].

However, the inflammation story is not so simple and there is considerable evidence (though some of it contradictory) that shows beneficial effects from application of certain proinflammatory growth factors, *e.g.*, TGFβ-1, FGFs and PDGF, in promoting different aspects of wound healing such as cell proliferation, migration and granulation tissue formation [58,59]. Indeed, PDGF is the active ingredient the FDA approved drug Regranex® (or becaplermin) and, although it now has a "black box" warning due to cancer concerns, has been reported to increase the rate of closure of diabetic ulcers by 10% [59]. Conversely, by far the vast majority of the growth factors that have entered clinical trials aimed at healing chronic ulcers have failed to promote healing [59,60]. Getting the balance of inflammatory signalling right in both acute and chronic wound healing is clearly important. In terms of diabetic ulcers, it is unlikely that the addition of any one growth factor, at a particular concentration and time is going to be the answer to triggering and sustaining the healing of these or other chronic wounds.

4. The effects of targeting Cx43 expression at a site of skin injury with antisense

Cx43 is by far the most ubiquitous connexin, being expressed in very many cell types in both healthy and disease conditions, where its presence is not always beneficial. As mentioned earlier, in the skin Cx43 is primarily expressed in keratinocytes, fibroblasts, hair follicles and blood vessels and, on wounding, it naturally slowly downregulates, being noticeable in 2–6 h but reaching a maximum in 24–48 h in wound edge cells as they become migratory [4,25,27,61]. Conversely Cx43 increases in blood vessels in and around the site of injury in the first few hours as they become inflamed and leaky [4,45]. Cx43 has a relatively short half-life of about 1.5 h [62,63], and its expression in skin has been targeted using a Cx43 specific antisense oligodeoxynucleotide (Cx43asODN) in a thermo-reversible, slow release, Pluronic gel. On topical application to a fresh wound in rodent skin or cornea, it rapidly downregulates Cx43 protein levels in the wound edge keratinocytes and fibroblasts whilst reducing the inflammatory upregulation of Cx43 in the blood vessels [5–7,64–68]. The accelerated downregulation of Cx43 takes place in 2 h rather than 24–48 h and has the effect of speeding the migration of keratinocytes and fibroblasts, which results in closing the wound and forming the granulation tissue considerably faster than untreated wounds [5,6] (Fig. 1). Concomitantly, Cx43asODN has the effect of reducing Cx43 upregulation that normally takes place in the blood vessels around the site of injury, reducing their leakiness and the swelling and oedema at the wound site of skin or in other sites such as central nervous system lesions [5,45,69,70].

Macroscopically the effects of topical application of Cx43asODN gel to acute wounds is pronounced and rapid; within 6 h treated wounds are significantly less red and swollen than control wounds, release less exudate and stimulate less inflammatory leukocytes [5]. The beneficial effects continue beyond the lifetime of the ODN itself and both re-epithelialization and granulation tissue formation are significantly enhanced over the ensuing days, resulting in a thinner flatter scar [5,6]. Within the scar itself the collagen bundles appear to be more basket-weave in appearance rather than the typical aligned parallel bundles seen in most scars. Similarly the healing of tail skin wounds in the inducible Cx43 knockout mouse show comparable enhancements of healing [71,72]. Both the lack of Cx43 in Cx43 inducible knockout mice and treatment with Cx43asODN gel appear to kick-start the acute wound healing process by targeting some of its earliest events (Fig. 1). This results in accelerating some normal aspects of the healing response such as keratinocyte and fibroblast migration, whilst reducing negative aspects such as inflammation, enabling Cx43asODN to have beneficial effects on healing long beyond the lifetime of the antisense itself.

In conjunction with reduced inflammation, the numbers of neutrophils both in and around the wound sites on days 1 and 2 after injury [5,6] are reduced following application of Cx43asODN. This reduction in neutrophil number may seem counterintuitive as their role is to help eliminate bacterial infections in the wound and prevent sepsis. However, the action of neutrophils can also be damaging to the host tissues and reducing their numbers can in fact promote re-epithelialization [53], which is very similar to our findings [5]. Reduced numbers of neutrophils are in turn followed by reduced numbers of macrophages [5,6] and the pro-inflammatory chemokines and cytokines that they release such as chemokine ligand 2 (Ccl2) and later on the cytokine tumour necrosis factor alpha (TNF-α). As both of these cytokines normally attract even more neutrophils and macrophages [73], their reduced expression helps explain the dampened inflammatory response after Cx43asODN treatment of wounds. In addition, Cx43asODN treatment is likely to reduce Cx43 levels in neutrophils and activated leukocytes where its expression is involved in cytokine and immunoglobulin release and further inflammatory cell recruitment [74–76].

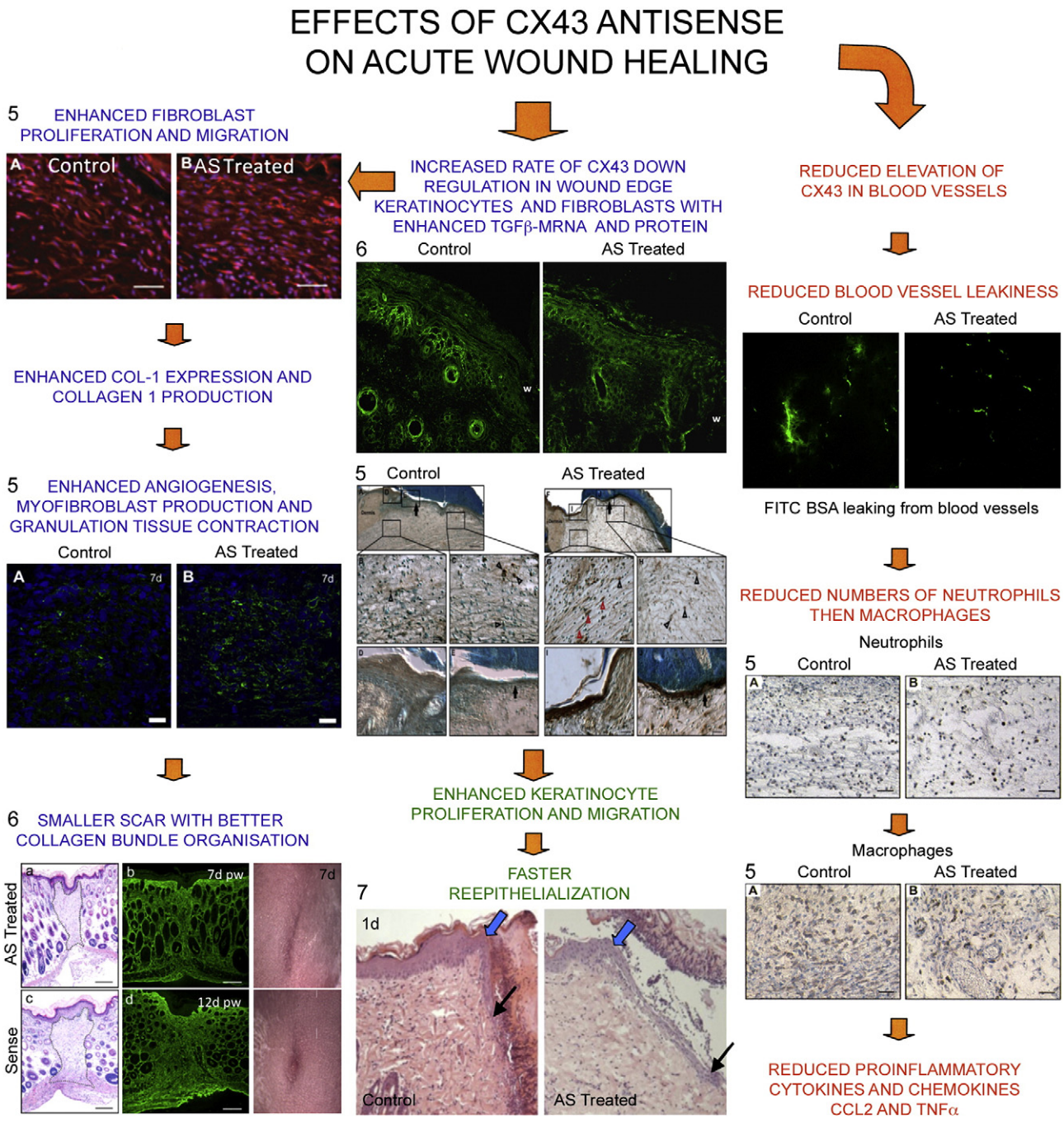

Fig. 1. Flow diagram of the effects of Cx43 antisense treatment on acute wound healing of normal rodent skin [5–7].

Treatment with Cx43asODN was also found to increase the expression of transforming growth factor beta-1 (TGFß-1) mRNA 2 days after wounding with elevated protein levels being found in the nascent dermis and epidermis. This fits well with the known effects of TGFß-1 on enhanced migration and proliferation [77] seen in the epidermis and dermis [6]. Other effects of enhanced TGFß-1 may relate to enhanced collagen production as both TGFß-1 and decreased Cx43 are known to promote Col1 a1 expression [78,79], which was found in treated wounds on days 2 and 7 after injury. Enhanced collagen production results in the enhanced granulation tissue maturation and angiogenesis [58] that is also seen in treated wounds. Very fine blood vessels are found throughout the granulation tissue of treated wounds as early as 7 days after injury when blood vessels are just beginning to enter the edges of the granulation tissue of control wounds. Myofibroblasts are also found to appear and then clear 2–3 days earlier in the granulation tissue of treated wounds thereby contributing to the faster maturation and smaller scar [6].

5. Connexins and diabetic wound healing

The onset of diabetes with associated hyperglycemia and oxidative stress has deleterious effects on many organs of the body, but particularly the vasculature, resulting in reduced delivery of oxygen and nutrients to peripheral tissues. There have been a variety of *in vivo* and *in vitro* studies relating to connexins and the diabetic vasculature that have produced mixed and often contradictory findings [80–84]. It has been reported that in diabetic endothelial cells, Cx37, Cx40 and Cx43 either decrease or remain at the same level (though not necessarily at the same time). However, there is a consensus that there is a general decrease in blood vessel Cx43 and gap

junctional communication, which is often associated with a breakdown of the blood vessels and cell death [80–84]. Studies of optic nerve head blood flow in diabetic rabbits was reported to show significantly decreased blood flow under conditions of raised intraocular pressure and a lack of normal blood vessel function, which appears to be due to gap junctional dysfunction in the diabetic vessels [85]. In relation to this, in a variety of vascular cells, both in diabetic animals and when cultured in high glucose, a decrease gap junctional communication whilst increasing Cx43 phosphorylation, potentially by the action of PKC 9, has been reported [82,86–88]. Renal tissues are also adversely affected by the onset of diabetes and again reports on the effects of diabetes or *in vitro* high glucose culture conditions are mixed and vary with cell type. For example, Cx43 has been reported to both increase and decrease in diabetic kidney tubules [89–91]. Collecting duct epithelial cells cultured in high glucose were reported to have significantly increased Cx43 protein [92]. The mechanisms behind these different effects on connexin expression, in different cell types, remain unknown.

We have found that in the skin of Streptozotocin (STZ) diabetic rats there is a significant alteration in connexin protein levels within 2 weeks of the onset of diabetes and long before peripheral neuropathy sets in [7]. In the epidermis Cx43 and Cx26 protein and gap junctional communication were significantly decreased but in the dermis Cx43 protein and communication increased. The differential effect on connexin expression in different cell types in diabetes clearly varies with the cell type and is not just a global downregulation of connexins, as in some instances diabetes appears to result in enhanced connexin expression [7].

Wound healing in patients with diabetes is perturbed and in humans can often result in diabetic ulcers that are very difficult to heal [59,93,94]. Given the reduced levels of Cx43 in the epidermis of STZ rats it might be assumed that re-epithelialization would be faster as there is less Cx43 to downregulate. However, the Cx43 dynamics following wounding of STZ skin are dramatically different to normal; instead of decreasing in the leading edge keratinocytes in the first 24 h, Cx43 is massively increased as a non-migratory bulb of keratinocytes forms at the wound edge [7]. In these STZ diabetic rats keratinocyte migration does not begin until Cx43 begins to downregulate some 48 h later. Cx26 expression, on the other hand, does still elevate in the diabetic rat leading edge cells but does so over a wider area much further back from the wound edge than normal. It is possible that both of these abnormal expression patterns contribute to differences in communication, or communication compartments in the leading edge and thereby prevent or alter keratinocyte migration.

High levels of Cx26 in keratinocytes have also been associated with hyperproliferative conditions [95]. Indeed, it was found that driving the expression of Cx26 in Involucrin INV-Cx26 heterozygous mouse keratinocytes delayed re-epithelialization and kept the epidermis in a hyperproliferative state, delaying remodelling and recovery of barrier function [96]. Clearly the appropriate levels of connexin expression are crucial for normal healing to take place. Attempts to normalize the expression of Cx43 in STZ diabetic wound edge keratinocytes were made by application of Cx43asODN gel to the wound at the time of injury. This treatment prevented the abnormal Cx43 post wounding upregulation and the formation of a bulb of non-migratory cells, thereby rescuing the rate of migration and returning healing rates to normal and above [7]. It remains to be determined whether or not diabetic humans show the same abnormal Cx43 response.

Today, throughout the world there is a rapidly increasing prevalence of diabetes and obesity in the population, resulting in rising levels of the incidence in diabetic ulcers [59]. These ulcers have a major impact on both the quality of life and patient morbidity. They are also extremely costly to healthcare services. Various non-biological treatments are used to improve the healing of diabetic ulcers, such as off-loading of pressure, but patient compliance can be difficult and treatments are not always effective. As a result a large number of these diabetic ulcers fail to heal or healing takes place over a very protracted period of time. This manifests not only in increased health care costs, but in a larger number of lower limb amputations and a significantly shortened life expectancy of the patient. The STZ diabetic rat studies [7] support the idea of Cx43 as a potential therapeutic target in human diabetic wound healing. One group has also reported a persistence of Cx43 protein in wound edge keratinocytes from biopsies of human diabetic ulcers [9]. However, whilst there have been reports of disrupted cell proliferation and elevated communication in cultures of human diabetic fibroblasts [97,98], examination of Cx43 and Cx26 mRNA and protein in keratinocytes from human diabetic and non-diabetic skin biopsies revealed no differences in expression [99]. Subsequently the localization and levels of Cx43 were shown to be the same in cultures of keratinocytes and fibroblasts derived from groups of control and diabetic human biopsies, as shown by immunostaining and Western blot analysis [34]. If Cx43 expression is indeed abnormal in human diabetic wounds it is possible that targeting its expression may improve the healing process in these diabetic wounds.

6. Hemichannels and mimetic peptides in wound healing

Intercellular gap junction channels are formed by the docking of two connexons, or "hemichannels," one provided by each cell. Gap junctional communication has been studied for many years and we are gaining an increasing understanding of its roles in embryonic development, homeostasis and disease conditions. Further, roles for undocked connexons in signalling are becoming increasingly accepted [100]. Transient opening of a hemichannel may allow the extracellular release of signalling molecules such as ATP into the extracellular space when cells or tissues are under stress [101]. However, if the connexons open for too long the cells will be compromised and die, and excessive hemichannel opening may be a reason for cell death in some forms of tissue injury.

Preventing or reducing the opening of connexons in different stress or injury conditions may be beneficial. Connexon opening has been reported to be rapidly modified by application of short stretches of peptide that mimetically match the extracellular loops of connexins [102–105], which in the short term will block connexon opening but in the longer term will prevent connexon docking to form gap junctions [106]. Because the extracellular loops are so highly conserved between different connexin isoforms the most commonly used peptides, GAP26 and GAP27, from Cx43 and Cx32 are effective on many different connexins [44,102,107,108]. However, it is not just connexon opening and docking that has been reported to be affected by application of mimetic peptides. Other studies have suggested that the peptides can block the communication properties of docked gap junctions without affecting their structure [109]. As such application of these peptides may affect connexon opening, docking and gap junctional communication in a variety of different types of connexin [110].

Recently mimetic peptides have been applied to a variety of culture models of wound healing with some intriguing findings. Application of both GAP26 and Gap27 to cultures of human fibroblasts or keratinocytes reduced gap junction communication within 1.5 h, which returned within 8 h. In scratch wound assays of these cell cultures, Cx43 was found to naturally downregulate in the wound edges, and adding the peptide every 12 h over a 5 day period increased the rate of migration [110]. Extensions of this work to 3D cultures models of human and porcine cell wound healing have been used to compare effects of the peptides on cells from donors of different ages and diabetic backgrounds [34,111]. It was found that the peptides accelerated the migration of both infant and adult fibroblasts and keratinocytes in scratch wound assays [34,112]. Interestingly the peptides were unable to enhance the migration of diabetic fibroblasts or keratinocytes in early passages of culture but did have an effect on very late passages [34]. It is not clear why the early passages of diabetic cells did not respond to the peptides as the authors reported

no differences in the levels of Cx43 between diabetic cells and control cells as shown by immunostaining and Western blot [34]. However, they also reported that the GAP27 peptide induced an elevation in the phosphorylation of Cx43 at serine 368, which is known to alter Cx43 conductance, and it has been suggested that this change in conductance may be required for normal wound healing. These findings do not entirely fit with those from the STZ diabetic rat where Cx43 was found to be abnormally over-expressed in non-migratory wound edge keratinocytes and needed to be eliminated in order for them to migrate [7]. Normal levels of Cx43 in cultures of human diabetic fibroblasts and keratinocytes does not help to explain their failure to respond to mimetic peptide treatments. There is an evident need for more research in order to better understand the roles of connexins in diabetic wound healing.

An alternative peptide approach has been to target the interaction between the C-terminal tail of Cx43 with proteins such as Zonular Occludin-1 (ZO-1) by utilizing the internalization protein antennapedia linked to the last 9 amino acids, RPRPDDLEI, of the Cx43 tail (named ACT1) [113]. The peptide will bind to the PDZ2 domain on ZO-1 and compete out its binding with the Cx43 C-tail. In addition it can compete out alternative Cx43 C-tail interactions with the matricellular protein CCN3 (associated with gliomas and tissue response to injury) [114,115] 14–3–3 proteins [116] and protein kinases [117]. The resulting effect on Cx43 of ACT1 treatment appears is reported to stabilize Cx43 in the cell membrane resulting in the formation of larger plaques [113] whilst isolating it from cytoplasmic tail interactions [118]. When applied to skin lesions in animal models ACT1 is said to have very similar affects to the Cx43 antisense in terms of speeding up the rate of healing whilst reducing scar formation [118–120]. ACT1 applied to cryo-injuries of the ventricle of the heart reportedly resulted in reduced association of Cx43 with ZO-1 in the injury border zone and a subsequent reduction in arrhythmias [121] with similarities in repair being compared to that of cutaneous healing [122].

7. Conclusions

Connexins play a pivotal role in a variety of aspects of the acute wound healing process. By manipulating them in different ways it is possible to accelerate and improve the normal healing process by speeding up the phenotypic change of cells to a migratory phenotype whilst at the same time dampening down some of the potentially negative aspects of the normal inflammatory response to injury resulting in faster healing and reduced scar formation.

Accelerating acute healing is not however, where the main challenge lies. Initiating healing when it has stalled in various types of chronic wounds is the real challenge. Diabetic wound healing offers a substantial unmet clinical need [1–3]. Trying to understand why these chronic wounds fail to heal and the potential involvement of connexins is intriguing, although the story in diabetic tissues is not yet clear. Animal models of diabetes suggest that connexin function can be manipulated by antisense to augment healing, thus offering promise that a variety of connexin modulation therapies currently in development may have a role in treating the human disease.

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