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Review

Skin innervation: important roles during normal and pathological cutaneous repair

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Summary. The skin is a highly sensitive organ. It is densely innervated with different types of sensory nerve endings, which discriminate between pain, temperature and touch. Autonomic nerve fibres which completely derive from sympathetic (cholinergic) neurons are also present. During all the phases of skin wound healing (inflammatory, proliferative and remodelling phases), neuromediators are involved. Several clinical observations indicate that damage to the peripheral nervous system influences wound healing, resulting in chronic wounds within the affected area. Patients with cutaneous sensory defects due to lepromatous leprosy, spinal cord injury and diabetic neuropathy develop ulcers that fail to heal. In addition, numerous experimental observations suggest that neurogenic stimuli profoundly affect wound repair after injury and that delayed wound healing is observed in animal models after surgical resection of cutaneous nerves. All these observations clearly suggest that innervation and neuromediators play a major role in wound healing. Interactions between neuromediators and different skin cells are certainly crucial in the healing process and ultimately the restoration of pain, temperature, and touch perceptions is a major challenge to solve in order to improve patients' quality of life.

Key words: Skin healing, Cutaneous nerve fibres, Neuromediators, Peripheral neuropathy, Diabetes

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Skin innervation

Sensory as well as autonomic (essentially sympathetic) nerves are present within the skin and influence a variety of physiological and pathophysiological cutaneous functions. In unstimulated nerves, neuromediators are barely detectable within the skin tissues. Upon direct stimulation by physical or chemical means, or during pathological situations such as inflammation or trauma, a significant increase of neuromediators is observed. Thus, mediators derived from sensory or autonomic nerves may play an important regulatory role in the skin under many physiological and pathophysiological conditions, including particularly wound healing. It is important to underline that the perception of skin sensitivity is a basic need in daily life. Human beings can live while being deprived of other sensorial systems (blindness, deafness, anosmia or ageusia); in contrast, deprivation of skin sensory stimulation can cause major and irreversible disorders. For example, body thermoregulation involves the stimulation of temperature-sensitive nerve endings within the skin, and a rise of central body temperature, resulting in the reflex release of sympathetic vasoconstrictor/vasodilator tone in the skin of the extremities, is an essential process for the maintenance of body homeostasis.

In addition, as underlined by Roosterman et al. (2006), beside the peripheral nerves, a subtle complex communication network exists between the spinal cord, the central nervous system, and the immunoendocrine system. More precisely, an important brain-skin connection with local neuroimmunoendocrine circuitry exists, illustrated in the context of stress by Paus et al.

(2006). A modern concept of cutaneous neurobiology in which the central and peripheral nervous system, the endocrine and immune system, with the participation of numerous neuromediators (neuropeptides, neurotransmitters, neurotrophins, and neurohormones), and almost all skin cells are involved is now admitted (Steinhoff et al., 2003).

Cutaneous sensory nerve fibres are endings of dorsal root ganglia (DRG or spinal ganglia) neurons that carry signals from sensory organs toward the appropriate integration centre of the brain via the spinal cord (Fig. 1). In the skin, autonomic nerve fibres almost completely derive from sympathetic (cholinergic) neurons (in the face, in addition, rare autonomic nerve fibres derive from parasympathetic, also cholinergic, neurons) (Fig. 1). When deep skin damage occurs, cutaneous nerves (sensory and sympathetic nerves) and sensory receptors are destroyed, while the sensory and sympathetic neuron cell bodies persist in the ganglia along the spinal cord (respectively, dorsal root ganglia and paravertebral

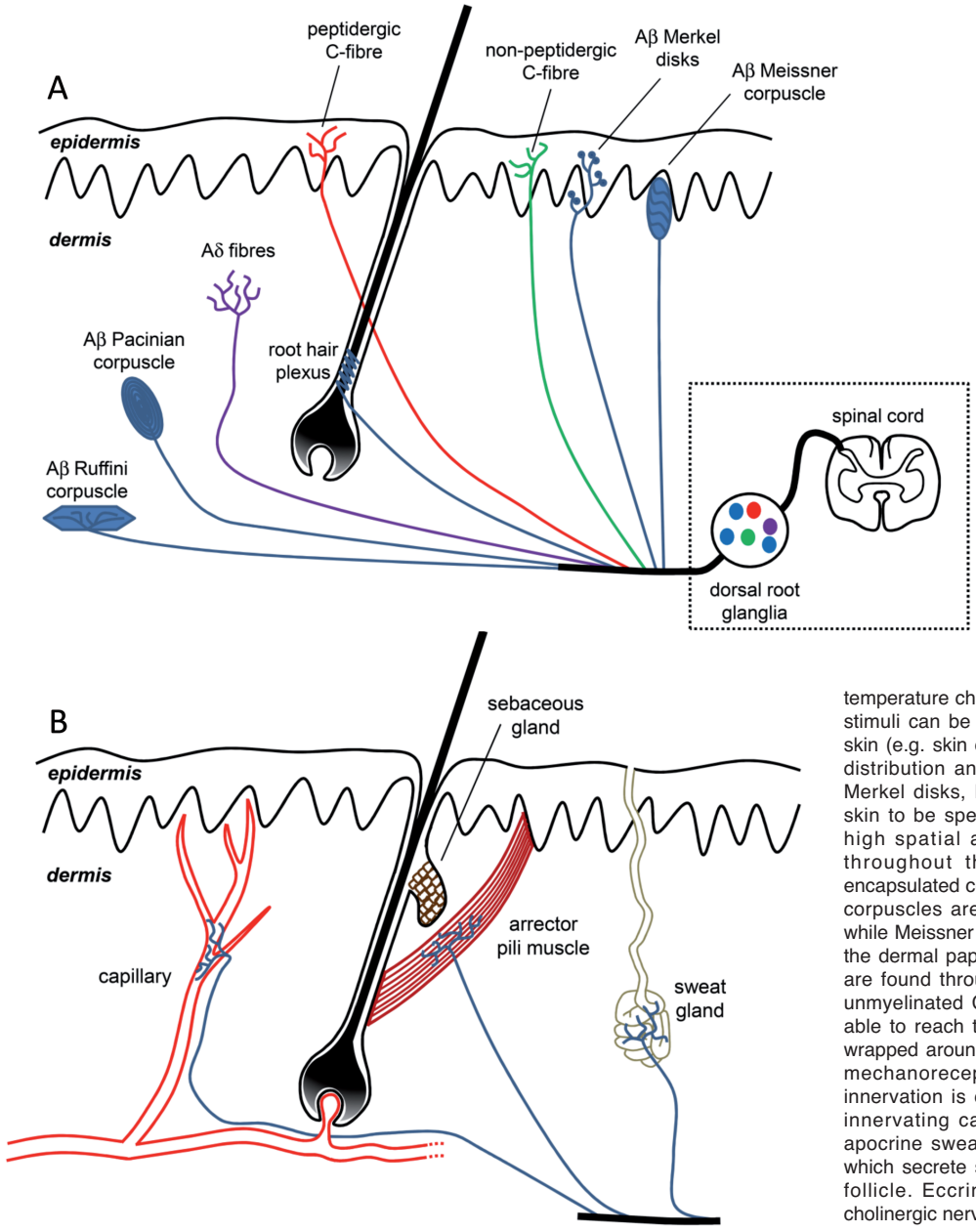

Fig. 1. Schematic diagram of skin innervation. A: Cutaneous sensory nerves arise from cell bodies located in the dorsal root ganglia and form different subtypes of nerve endings. These nerve endings mediate our sense of touch by detecting and encoding stimuli such as pain, itch, temperature changes, pressure or vibrations. Sensory stimuli can be elicited from both hairy and glabrous skin (e.g. skin of the palm); however, changes in the distribution and density of mechanoreceptors (e.g Merkel disks, Meissner corpuscles) allow glabrous skin to be specialized for discriminative touch with high spatial acuity. Nerve endings are located throughout the dermis and epidermis. Large encapsulated corpuscles such as Pacinian and Ruffini corpuscles are usually located deep in the dermis while Meissner corpuscles are preferably found within the dermal papillae. Moderately myelinated Aδ fibres are found throughout the dermis whereas only free unmyelinated C-fibre endings and Merkel disks are able to reach the epidermis. In hairy skin, plexuses wrapped around the hair follicles are also involved in mechanoreception. **B.** The cutaneous autonomic innervation is composed of adrenergic nerve fibres innervating capillaries, arrector pili muscles and apocrine sweat glands (not shown in the schema) which secrete sweat into the pilary canal of the hair follicle. Eccrine sweat glands are innervated by cholinergic nerve fibres.

sympathetic ganglia).

Sensory skin innervation

The skin is a highly sensitive organ which is densely innervated with different types of nerve endings, associated or not with specific receptors, which discriminate between pain, thermal and tactile sensations.

The different kinds of sensory stimuli that are picked up by sensory neurons are grouped into two categories: epicritic and protopathic. Epicritic neurons detect gentle touch such as caresses, light vibrations, the ability to recognize the shape of an object being held, and two-point discrimination which is the spacing of two points being touched simultaneously. Protopathic neurons are responsible for detecting pain, itch, tickle, and temperature. The different types of stimuli that are detected by a given receptor allow for a relative specificity between stimuli and receptor.

Sensory neurons arise from the dorsal root ganglia synapse in the dorsal horn of the spinal cord and transfer information about touch sensations (epicritic), or pain and temperature (protopathic). While both types of sensory neurons must first synapse in the dorsal horn of the spinal cord, the area of the dorsal horn where they synapse is different. Their pathways to reach the thalamus are also different (for more information, see Kandle et al., 2013).

Sensory cutaneous receptors are found in the dermis and the epidermis. They are part of the somatosensory system. Sensory receptors in the skin are mechanoreceptors (Ruffini, Meissner and Pacinian corpuscles, Merkel disk and free nerve endings), thermoreceptors and nociceptors (Paré et al., 2002; Lewin and Moshourab, 2004; McGlone and Reilly, 2010) (Fig. 2).

In both the epidermis and the dermis, thermoreceptors and nociceptors are found. Mechanoreceptors observed in the epidermis are Merkel disk and free nerve endings. Mechanoreceptors present in the dermis are Ruffini, Meissner, and Pacinian corpuscles, and free nerve endings which are slight axon expansions. In mucous membranes (as in the conjunctiva or genitals), bulboid corpuscles (or end-bulbs of Krause) functioning as sensory cold receptors are also found. In non-glabrous skin, root hair plexus are nerve endings which form a network around hair follicles and serve as receptors for touch sensation.

Merkel disks and Meissner corpuscles (also known as tactile corpuscles or type I mechanoreceptors) are the most superficial and are closely affiliated with the pattern of fingerprints in monkeys and humans. Merkel disks are nerve endings flattened against specialized Merkel cells clustered in the basal lamina at the base of the thickened intermediate epidermal ridges that underlie the raised fingerprints (Smith, 1970). Meissner corpuscles are located in dermal papillae that bud off dermal papillary ridges between the intermediate epidermal ridges. They are supplied by several axons that terminate between lamellar Schwann cells (Cauna, 1956). Pacinian and Ruffini corpuscles (also known as type II mechanoreceptors) are located deeper in the dermis. Pacinian corpuscles (also known as lamellar corpuscles) are ellipsoid structures consisting of an axon terminal wrapped by several layers of lamellar cells (Chouchkov, 1971). Ruffini corpuscles (also known as bulbous corpuscles) have been described as long

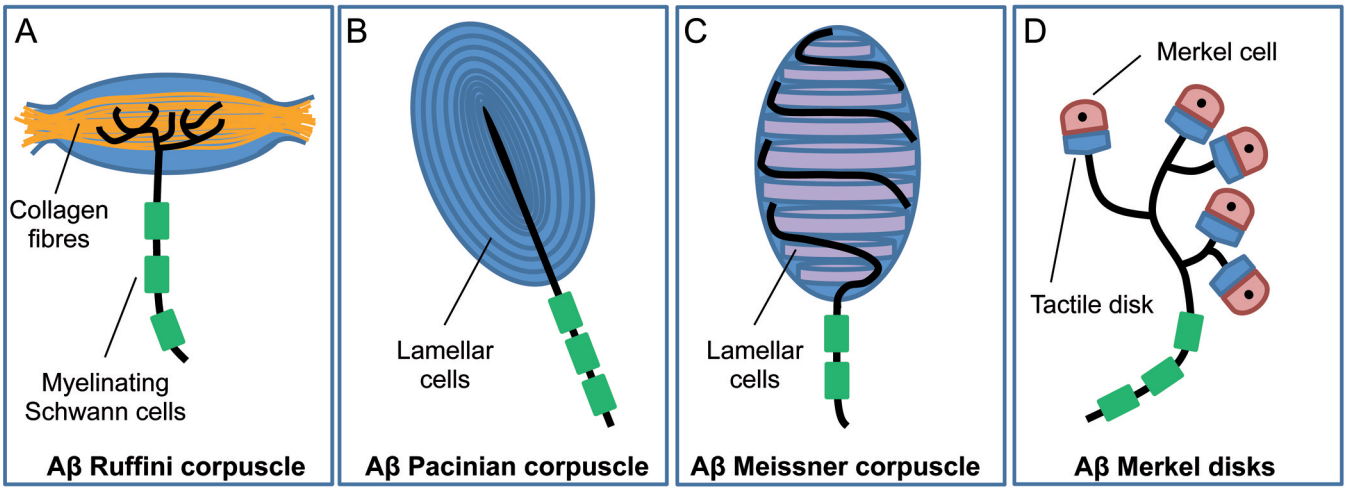

Fig. 2. Schematic diagrams of main sensory cutaneous receptors. **A.** The Ruffini corpuscle is a slowly adapting spindle-shaped receptor and is sensitive to skin stretch, pressure and distorsion. **B.** The Pacinian corpuscle is a rapidly adapting oval-shaped receptor constituted of layered lamellar cells. It can detect deep pressure and high-frequency vibration. **C.** The Meissner corpuscle is a rapidly adapting ellipsoid receptor constituted of stacked lamellar cells. It is involved in fine touch and detects pressure and low frequency vibration. **D.** Merkel receptors are slowly adapting receptors composed of tactile disks and Merkel cells involved in fine touch.

fusiform encapsulated structures within which a large-calibre axon terminates as numerous branches intertwined among collagen bundles (Chambers et al., 1972).

With the above mentioned receptor types, the skin can sense touch, pressure, vibration, temperature and pain modalities. These modalities and their receptors are partly overlapping, and are innervated by different types of fibres (Table 1).

Cutaneous sensory nerves are broadly classified according to their diameter and speed of impulse as Aβ, Aδ, and C nerve fibres, from the biggest and fastest to the smallest and slowest, respectively (Table 2). All kinds of fibres are accompanied by Schwann cells in the dermis but not in the epidermis. The larger nerve fibres, Aβ and free nerve endings Aδ, are associated with Schwann cells, which secrete a basal lamina around them and also produce myelin sheaths. C free nerve endings are the termination of unmyelinated fibres and are associated in the dermis with Schwann cells which do not produce myelin (Fig. 3).

Mechanical stimuli are detected via mechano-receptors associated with sensory corpuscles through Aβ fibres or with Aδ free nerve endings, temperature via the thermo-receptors through Aδ and C fibres, and pain via the nociceptors through Aδ and C fibres (Table 1) (for more details, see the review by Roosterman et al., 2006). Aδ fibres constitute 80% of primary sensory nerves sprouting from dorsal root ganglia, whereas C fibres make up 20% of the primary afferents (Alvarez and Fyffe, 2000; Lawson, 2002). Moreover, the activation threshold of Aδ fibres is higher than C fibres. In addition to mechanical, thermal and pain stimuli, both C and Aδ free nerve endings respond to a variable range of stimuli such as physical (trauma, heat, osmotic changes, ultraviolet light) as well as chemical (toxic agents, allergens, proteases, microbes) agents (reviewed in Steinhoff et al., 2003).

Table 1. Overview of different types of fibres and receptors activated according to stimulus modalities.

Modality	Type	Fibre type
Touch	Rapidly adapting cutaneous mechanoreceptors (Meissner and Pacinian corpuscles, hair follicle receptors, some free nerve endings)	Aβ fibres
Touch & pressure	Slowly adapting cutaneous mechanoreceptors (Merkel disk and Ruffini corpuscles, some free nerve endings)	Aβ fibres (Merkel disk and Ruffini corpuscles), Aδ fibres (free nerve endings)
Vibration	Meissner and Pacinian corpuscles	Aβ fibres
Temperature	Free nerve ending thermoreceptors	Aδ fibres (cold receptors) C fibres (warmth receptors)
Pain	Free nerve ending nociceptors	Aδ fibres (cold receptors) C fibres (warmth receptors)

Nociceptors are particular receptors generally divided into 4 classes: mechanoreceptors, thermal receptors, chemoreceptors, and polymodal receptors (these respond to all 3 stimuli). Nociceptors are similar to other receptor types but generally respond to higher levels of stimulus. e.g. general thermal receptors respond to temperatures <45°C, whereas nociceptive thermal receptors respond to temperatures >45°C. Nociceptors have either free nerve endings or non-encapsulated end organs.

In human peripheral nerves, 45% of the cutaneous afferent nerves belong to a subtype of sensory nerves that are mechano-heat responsive C fibres. However, only 13% of these nerves were found to be only mechano-sensitive, 6% were heat sensitive, 24% were neither heat nor mechano-responsive, and 12% were of sympathetic origin (Schmidt et al., 1995).

Autonomic skin innervation

Although very effective, autonomic (essentially

Table 2. Description of different types of cutaneous nerve fibres.

Type of fibres	Myelinated	Axonal diameter (μm)	Conduction velocity (m/s)
Aβ	moderately	6-12	80
Aδ	thin myelin sheath	1-5	4-30
C	unmyelinated	0.2-1.5	0.5-2

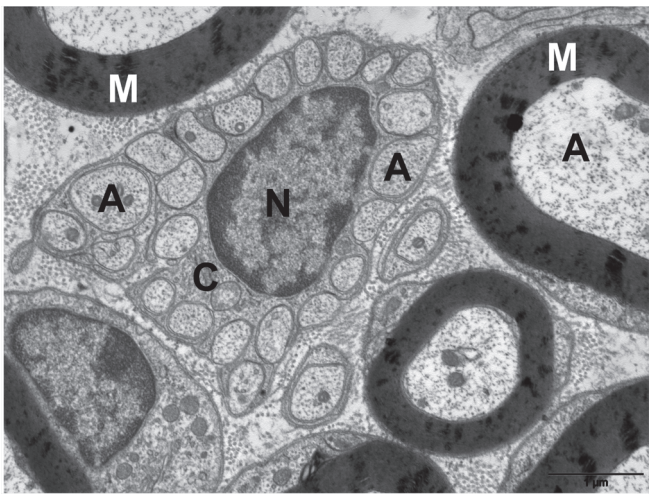

Fig. 3. Transmission electron microscopy of a rat sciatic nerve cross section. Aβ fibres are surrounded by a thick layer of myelin while C fibres are unmyelinated. Unmyelinated axons are surrounded by cytoplasmic invagination of non-myelinating Schwann cells, forming Remak bundles. Aδ fibres (not visible on this image) like Aβ fibres are myelinated but the layer of myelin is thinner and the diameter of the fibres is smaller. C: cytoplasm of a Schwann cell; N: nucleus of a Schwann cell; A: axoplasm; M: myelin sheath.

sympathetic) nerve fibres constitute only a minority of cutaneous nerve fibres compared with sensory nerves. They are restricted to the dermis, innervating blood vessels, arteriovenous anastomoses, lymphatic vessels, erector pili muscles, eccrine glands, apocrine glands, and hair follicles (Vetrugno et al., 2003). The cutaneous autonomic nervous system plays a crucial part in regulating sweat gland function, vasomotricity, skin blood flow and thereby body temperature homeostasis. Acetylcholine is an important regulator of sweating but adult human sweat gland innervation also co-expresses all of the proteins required for full noradrenergic function.

Various neuropeptides are produced and released by a subpopulation of unmyelinated afferent neurons (C fibres) defined as C-polymodal nociceptors, which, as mentioned above, represent 70% of all cutaneous C fibres in the skin. To a lesser extent, small myelinated Aδ fibres and autonomic nerve fibres are also capable of releasing a number of neuropeptides that also act on neuronal and non-neuronal target cells. In addition, recently, cutaneous cells themselves such as keratinocytes, microvascular endothelial cells, Merkel cells, fibroblasts, or leukocytes were found to be capable of releasing neuropeptides under physiological circumstances.

General mechanisms involved in skin repair and neuromediators associated with the different phases of healing

In our daily life, the skin may be damaged by chemical, physical or mechanical injuries. A rapid and efficient repair process is essential to restore skin integrity and re-establish its main function as protective barrier for the body against the environment.

Immediately after wounding, the healing process begins and consists of a coordinated succession of cellular and biochemical events that can be classified in three time-dependent phases: the inflammatory phase, the proliferative phase and finally the remodelling phase. Each phase can be distinguished by specific morphological changes ultimately leading to the formation of a scar (Martin, 1997; Gurtner et al., 2008; Profyris et al., 2012). Neuropeptides, short chains of amino acids secreted by neurons, are implicated in various phenomena involved in skin wound healing, such as induction of vasodilatation and angiogenesis, promotion of cell chemotaxis by mast cell degranulation, modulation of immune response and stimulation of migration and proliferation of keratinocytes and fibroblasts (Brain, 1997; Toda et al., 2008; Amadesi et al., 2012).

Inflammatory phase

The first stage of wound healing is immediately initiated after injury and is completed after several hours or days depending on the extent of the wound. The damage of blood vessels triggers the extravasation of blood constituents leading to the formation of a clot and of a provisional wound matrix mainly composed of fibrin and fibronectin (Robson et al., 2001). In the meantime, platelets involved in the blood clot and the matrix formation release cytokines, growth and differentiation factors contributing to the recruitment of leukocytes and the initiation of the inflammatory response. Neutrophils quickly migrate to the wound followed by monocytes and lymphocytes (24 hours after injury). These additional immune cells secrete growth factors and cytokines attracting more cells implicated in wound healing and stimulating their proliferation and survival. Immune cells secrete antibacterial products (reactive oxygen species, proteinases) and cells with phagocytic activities such as macrophages help to prevent infection. Platelets also contribute to the recruitment of fibroblasts and endothelial cells via the release of chemokines (Steed, 1997; Gawaz and Vogel, 2013). Mast cells are also involved in the inflammatory phase via the secretion of histamine and pro-inflammatory mediators acting on vascular permeability and cell recruitment (Wulff and Wilgus, 2013). Due to the injury, nerve endings may be crushed or sectioned; however cutaneous nerve fibres are important actors in the wound healing process. It has been shown that sensory nerves and to a lesser extent autonomic nerves can produce and release neuropeptides involved in the inflammatory, proliferative and remodelling phases which allow cross-talks with different cell populations participating in tissue repair (see Table 3). Neuropeptides such as tachykinins (SP, neurokinin A, neuropeptide Y), calcitonin gene related-protein (CGRP), vasoactive intestinal peptide (VIP), play a crucial role in the inflammatory phase (Lotti et al., 1995; Scholzen et al., 1998; Roosterman et al., 2006; Chéret et al., 2013). SP released on the site of injury allows vasodilatation and vascular permeability promoting plasma extravasation (Hughes et al., 1990; Holzer, 1998). Nitric oxide (NO) and histamine mediated-vasodilatation is induced by SP via the neurokinin (NK)-1 receptor present on both endothelial cells and mast cells (Ansel et al., 1993; Columbo et al., 1996). SP also stimulate the amplification of the inflammatory response by inducing the degranulation of mast cells and release of tumour necrosis factor (TNF)-α and histamine, the synthesis and release of interleukin (IL)-1β and transforming growth factor (TGF)-β by keratinocytes, the production of TNF-α, IL-2, IL-6 and IL-8 by leukocytes (Ansel et al., 1993; Delgado et al., 2003; Wei et al., 2012). CGRP is implicated in vasodilatation and the formation of an inflammatory oedema (Brain et al., 1986). CGRP also stimulates mast cells and keratinocytes to secrete respectively TNF-α and IL-1α (Niizeki et al., 1997). VIP participates in the inflammatory phase by inducing histamine release by mast cells and acts on NO-mediated vasodilatation (Gonzalez et al., 1997).

Inflammatory mediators secreted by platelets, immune cells and sensory fibres elicit pain. Thus, bradykinin, serotonin (5-HT), prostaglandins, cytokines

and H^+ ions can directly or indirectly stimulate nociceptors present on unmyelinated C-nerve fibres (Julius and Basbaum, 2001; Ständer et al., 2003). Another well studied signal transducer is the transient receptor potential cation channel subfamily V member 1 (TRPV1) also known as the capsaicin receptor or the vanilloid receptor 1. Sensitization of TRPV1, especially after a burn injury, causes the depolarization of nociceptors and transduction of pain signals (O'Neill et al., 2012).

Proliferative phase

During the proliferative phase, leukocytes and cells from the connective tissue such as fibroblasts, mast cells, macrophages and endothelial cells are recruited to the injury site and create an inflammatory granuloma called granulation tissue. The cell composition of the granulation tissue evolves over time. Initially, neutrophil, the major immune cell population, is progressively replaced by monocytes and activated macrophages. Monocytes and macrophages secrete different factors, cytokines and chemokines to recruit B and T lymphocytes in order to eliminate necrotic tissue and apoptotic cells (Koh and DiPietro, 2011). Meanwhile, fibroblasts and endothelial cells migrate and proliferate within the granulation tissue and new blood vessels are formed. Angiogenesis is essential to restore vascular perfusion in the wound and to deliver nutrients and oxygen to the cells involved in tissue repair (Singer

Table 3. Neuropeptides derived from cutaneous sensory nerves involved in wound healing.

Neuropeptides					Involvement in wound healing phases				
Family	Name	Acronym	Receptor	Targets	Inflammatory	Proliferative	Remodelling	Actions	References
Tachykinin	Substance P	SP	NK-1R	mast cell, keratinocyte, endothelial cell, fibroblast	+	+	+	↗pro-inflammatory cytokines, histamine, prostaglandins, NGF and EGF. Vasodilatation, plasma extravasation, angiogenesis, neurite outgrowth, proliferation and migration of fibroblasts and keratinocytes	Paus et al., 1995; Ansel et al., 1996; Wiederman et al., 1996; Holzer, 1998; Altun et al., 2001; Burbach et al., 2001; Pennefather et al., 2004; Chéret et al., 2014
	Neurokinin A	NKA	NK-2R				ND*		
	Neuropeptide Y§	NPY	Y1 to Y6	endothelial cell				vasoconstriction, angiogenesis	Kellogg, 1985; Movafagh, 2006
Calcitonin	Calcitonin gene-related peptide§	CGRP	CL-R/RAMP1	mast cell, endothelial cell, keratinocyte, melanocyte, immune cell	+	+	+	vasodilatation, immunomodulation, ↗NGF, keratinocyte proliferation, angiogenesis	Sung et al., 1992; Brain and Grant, 2004; Dallos et al., 2006a; Chéret et al., 2014
Secretin	Vasoactive intestinal peptide§	VIP	VPAC1, VPAC2	keratinocyte, Merkel cell, immune cell, endothelial cell	+	+	+	vasodilatation, anti-inflammatory immunomodulation, ↗VEGF, NGF angiogenesis	Schulze et al., 1997; Dallos et al., 2006a; Kakurai et al., 2009; Yang et al., 2009; Chéret et al., 2014
	Pituitary adenylate cyclase-activating peptide	PACAP		endothelial cell, keratinocyte	+	+	ND	vasodilatation, plasma extravasation, keratinocyte proliferation	Granoth et al., 2000
	Peptide histidine methionine/ peptide histidine isoleucine	PHM/PHI		keratinocyte	ND	+	ND	keratinocyte proliferation	Takahashi et al., 1993
Somatostatin		SST	SSTR1 to R5	keratinocyte, fibroblast, Merkel cell, Langerhans cell, endothelial cell, immune cell	+	+	ND	anti-inflammatory, anti-proliferative effect on keratinocyte, inhibit neurotransmitter release	Gaudillère et al., 1997; Hagströmer et al., 2006; Wang et al., 2009; Vockel et al., 2011
Galanin		GAL	GALR1 to R3	endothelial cell, keratinocyte	+	+	ND	vasoconstriction, axonal regeneration,↗NGF, keratinocyte proliferation	Holmes et al., 2005; Dallos et al., 2006b; Schmidhuber et al., 2007; Hill et al., 2010

* Not determined. § Neuropeptides shown to be released by cutaneous autonomic nerves.

and Clark, 1999; Profyris et al., 2012). Fibroblasts produce a large amount of extracellular matrix (ECM), mainly composed of type III collagen, and are activated into myofibroblasts expressing α-smooth muscle actin (Darby et al., 1990). Myofibroblasts display contractile properties and induce the progressive contraction and maturation of the granulation tissue (Hinz et al., 2012; Vedrenne et al., 2012). Keratinocytes are also key players in the proliferative phase. They migrate from the edges of the damaged site and proliferate to promote the reepithelialisation of the wound (O'Toole, 2001; Gurtner et al., 2008). During the proliferation phase, growth factors such as transforming growth factors (TGF-α, β1, β2 and β3), fibroblast growth factors (FGF-2, 7 and 10), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) are released and amplify the cellular response and modulate granulation tissue morphology (Singer and Clark, 1999; Barrientos et al., 2008). EGF stimulates keratinocyte migration in the reepithelialisation process (Morris and Chan, 2007; Peplow and Chatterjee, 2013).

Neuropeptides secreted by sensory and autonomic nerves are implicated in the proliferative phase (see Table 3). They contribute to cell stimulation with CGRP participating in angiogenesis by enhancing endothelial cell proliferation (Haegerstrand et al., 1990). CGRP and VIP are also implicated in reepithelialisation by stimulating keratinocyte proliferation (Hara et al., 1996). NGF acts on B lymphocytes by increasing their proliferation and differentiation and promoting fibroblast migration in the granulation tissue (Church and Clough, 1999; Chen et al., 2014). Neurotrophins such as NGF, neurotophin-3 (NT-3), brain-derived neurotrophic factor (BDNF) and their receptors (high affinity TrkA, B, C and low affinity p75NTR) are expressed by keratinocytes, fibroblasts, myofibroblasts and melanocytes and promote their proliferation and differentiation (Marconi et al., 2003; Botchkarev et al., 2006; Palazzo et al., 2012). Neurotrophins also have an impact on sensory nerve fibres themselves by stimulating neurite outgrowth and promoting nerve regeneration (Lentz et al., 1999; Cao and Shoichet, 2003; Gölz et al., 2006) (see Table 4).

Remodelling phase

The third phase consists of the remodelling of the granulation tissue and in scar tissue formation. Matrix metalloproteases (MMPs) secreted by keratinocytes, (myo)fibroblasts, endothelial cells as well as immune cells play a major role in this process by modulating the ECM and promoting cell migration (Martins et al., 2013). Meanwhile, the synthesis of ECM by (myo)fibroblasts is reduced and progressively type III collagen is replaced by type I collagen which is the main component of intact uninjured dermis. As the remodelling process progresses, the major part of the cell population within the ECM undergo apoptosis and the skin tends to recover its normal composition with a type I collagen matrix forming the scar tissue (Desmoulière et al., 1995). Although little is known about the role of sensory and autonomic fibres in the remodelling phase, a recent study has shown that SP, CGRP and VIP can modulate MMP-2 and MMP-9 activities. These neuropeptides also affect collagen I and collagen III production during skin wound healing (Chéret et al., 2014).

Peripheral neuropathies and wound healing delay

Prominent medical factors contributing to the incidence of cutaneous wounds include multifactorial pathologies such as diabetes, obesity and aging. In these situations, patients tend to develop spontaneous skin injury (pressure sores, diabetic foot ulcers, chronic venous ulcer) and fail to heal properly. Wound healing deficiency can be attributable, in the case of these pathologies, to vascular, neuronal and/or immune factors. Impaired or delayed wound healing is also observed in neurologic disorders like spinal cord injury or hereditary sensory neuropathy, suggesting that cutaneous nerve terminals are crucial in skin repair. Increased incidence of skin injury in sensory small fibre neuropathy (SSFN) as in the rare syndrome of congenital insensitivity to pain may be explained by lack of sensation. Patients are not aware of pain, and lose protective withdrawal reflex to avoid tissue harm.

Table 4. Neurotrophins involved in cutaneous sensory nerve regeneration during wound healing.

Neurotrophins					Involvement in wound healing phases			
Name	Acronym	Receptor	Sources	Targets	Inflammatory	Proliferative	Remodelling	Actions
Nerve growth factor	NGF	p75NTR, TrkA	keratinocyte, fibroblast, myofibroblast, melanocyte	keratinocyte, fibroblast, myofibroblast, sensory fibre	ND*	+	ND	keratinocyte proliferation; myofibroblast differentiation, migration and contraction; neurite outgrowth, neuropeptides release and nerve regeneration
Neurotrophin-3	NT-3	p75NTR, TrkC						
Neurotrophin-4/5	NT-4/5	p75NTR, TrkB						
Brain-derived neurotrophic factor	BDNF	p75NTR, TrkB						

* Not determined.

However, several studies also demonstrate specific involvement of cutaneous innervation in pathological wound healing.

Diabetes mellitus

Diabetes is a particularly important risk factor for the development of chronic wounds because it is often associated with vasculopathy and neuropathy. Diabetic neuropathy (DN) is a common complication of diabetes mellitus. Approximately 60-70% of diabetic patients develop a DN (World Health Organization, 2011), most commonly seen as distal symmetrical sensorimotor polyneuropathy. DN impairs sensory, autonomic and motor nerves. Sensory nerve impairment diminishes the perception of pain that is protective when tissue injury has occurred. Autonomic nerve impairment causes the skin to become dry and susceptible to skin fissures, tearing and infection due to a loss of sweat and sebaceous gland function. Motor nerve impairment induces muscle atrophy particularly in the feet, resulting in altered biomechanical properties and thereby increased risks for the development of neuropathic foot ulcers in combination with other neuropathic complications. However, motor impairment when it occurs is only seen in the most advanced cases of DN. Polyneuropathy found in diabetes is a main risk factor for non-healing wound and lower-extremity amputation (Adler et al., 1999). Diabetic patients fail to heal because of cutaneous homeostasis, which is compromised not only by nerve dysfunctions, but also by vascular dysfunctions. These complications are intimately involved in skin repair and it is difficult to distinguish underlying nervous mechanisms that occur in chronic wounds of diabetic patients.

Diabetic neuropathy biology

Hyperglycemia is a leading causative factor for DN, but the pathogenesis of neuropathy remains a subject of debate. Several hypotheses have been proposed. The "vascular" concept proposes that microangiopathy is a key player in the development of DN, implying that endoneurium hypoxia induced by endothelium dysfunction induces nerve degeneration (Ogawa et al., 2006). Another concept is "metabolic" and based on the molecular alterations of the peripheral nervous system induced by hyperglycemia. Protein fixation and accumulation of advanced glycation end-products in nerves can produce structural and functional alterations of peripheral nerves (Singh et al., 2014). Peroxynitrite, a product of superoxide anion radical reaction with nitric oxide, is a major oxidant in pathological conditions associated with oxidative-nitrosative stress, including diabetes, and is involved in DN (Stavniichuk et al., 2014). Hyperglycemia activates the polyol pathway activity, leading to the production of sorbitol and depletion of *myo*-inositol. Intracellular accumulation of sorbitol in Schwann cells and nerve fibres results in osmotic stress and then in nerve degeneration; depletion of *myo*-inositol reduces ATPase Na^+/K^+ activity, necessary for nerve depolarization (Zychowska et al., 2013). Both vascular and metabolic disorders which occur in diabetes contribute to DN. It has been shown for example that in patients with mild to moderate diabetic neuropathy, elevated triglycerides correlated with myelinated fibre density loss independently of disease duration, age, diabetes control, or other variables, supporting the evolving concept that hyperlipidemia is instrumental in the progression of diabetic neuropathy (Wiggin et al., 2009).

Wound healing with diabetic neuropathy

Early symptoms of DN manifest by the symmetrical loss of distal skin innervation resulting from degeneration of small cutaneous nerve fibres. Multiple studies confirm a marked decrease in intra-epidermal nerve fibre (IENF) density in diabetic patients as well as in subjects with impaired glucose tolerance (Beiswenger et al., 2008). Sensory nerve impairment is marked by the degeneration of nerve fibres expressing SP and CGRP in skin of patients with early diabetic neuropathy (Lindberger et al., 1989). The content of neuropeptide Y, a marker of sympathetic neurons, is reduced in diabetic skin (Wallengren et al., 1995). Cutaneous autonomic nerve degeneration is highlighted by a significant loss of pilomotor nerve fibres (Nolano et al., 2010) and reduced sweat gland nerve fibre density (Gibbons et al., 2009), both correlated with sweating impairment. The reduction of Meissner corpuscle density and their myelinated afferent nerve fibres, associated with myelin abnormalities, was demonstrated in glabrous skin of diabetic patients (Peltier et al., 2013). In addition to skin denervation, a decrease of NGF level within the skin and in serum, usually correlated with nerve conduction velocity, is seen in diabetic patients (Blakytny and Jude, 2006). Finally, a reduced rate of IENF regeneration is found in diabetic patients (Polydefkis et al., 2004).

In healthy subjects, cutaneous wound repair progresses linearly through different phases of wound healing. In contrast, all phases of the wound healing process are affected in diabetic wounds. The impaired healing of cutaneous wounds in diabetic patients involves multiple pathophysiological mechanisms, including the presence of peripheral neuropathy. NGF, CGRP and SP levels, relevant to wound healing, decrease in the skin of patients with DN. The vasodilatation phase that occurs in response to injury is a neurovascular phenomenon and is impaired in diabetic patients (Schramm et al., 2006). Normal skin of diabetic patients displays an increase of inflammation and of blood vessel density (Tellechea et al., 2013). A study reports that pre-existing increased serum level of inflammatory cytokines and growth factors as well as an increase in pro-inflammatory factors at the skin level are associated with failed diabetic foot ulcer healing (Dinh et al., 2012). In contrast, Galkowska et al. (2006)

described low inflammatory cell accumulation in diabetic wound which can be correlated with a reduction of foot skin innervation and neurogenic factor expression, and they hypothesized that low inflammation may lead to chronicity of diabetic foot ulcer healing process. Diabetes affects leukocyte and neutrophil function, impairs host resistance and ultimately leads to wounds more prone to infection (Galkowska et al., 2006; Alavi et al., 2014). Moreover, mast cells play an important role in leukocyte recruitment, but this function is impaired in denervated skin (Siebenhaar et al., 2008). Angiogenesis decreases during wound healing in diabetes leading to a reduced entry of inflammatory cells into the wound, which could be associated with CGRP and SP content depletions (Galkowska et al., 2006). Thus, inflammation in diabetes is clearly impaired, with a chronic inflammatory state associated with hyperglycemia and an inflammatory response to injury in skin, which is disrupted and associated, in part, to the neuropathy. Another fundamental aspect of tissue repair is reinnervation. Polydefkis et al. (2004) show that the presence of neuropathy is associated with a decreased rate of cutaneous nerve regeneration. Similarly, diabetic patients developing or not a neuropathy, exhibit reduced regenerative rate. In a mouse model, diabetic animals exhibited a failure to send axons into newly reconstituted wound tissue (Cheng et al., 2013). Although diabetes is a complex disease with diverse consequences, much evidence highlights the specific and crucial involvement of nerve during cutaneous wound healing in this pathology.

Obesity and metabolic syndrome

Obesity is characterized by abnormal or excessive fat accumulation that may impair health. The World Health Organization considers that a body mass index (BMI, kg/m^2) greater than or equal to 30 signs a situation of obesity. In 2008, more than 10% of the world's adult population was obese. The prevalence of obesity continues to increase with more than 40 million children under the age of 5 who were overweight or obese in 2012. The common consequences of obesity are increased risks of coronary disease, hypertension, dyslipidemia, type 2 diabetes, musculoskeletal disorders, cancers, stroke and sleep apnea. The metabolic syndrome represents a cluster of metabolic abnormalities that occur together and increase the risk for coronary disease, stroke and type 2 diabetes. All risk factors for metabolic syndrome are related to obesity and include abdominal obesity, dyslipidemia (high levels of total and low-density lipoprotein cholesterol and triglycerides and low levels of high-density lipoprotein cholesterol), high blood pressure and elevated fasting plasma glucose (FPG) (Alberti et al., 2009). Obesity and metabolic syndrome have a major detrimental impact on the wound healing process. Obesity and metabolic syndrome greatly increase the risk of wound infections, pressure and venous ulcers and delayed wound healing after major surgeries (Guo and Dipietro, 2010; Pierpont et al., 2014). Growing evidence suggests that metabolic syndrome is associated with increased risk for the development of microvascular complications (retinopathy, nephropathy) and, most commonly, peripheral painful neuropathy, both crucial for the wound healing process (Smith and Singleton, 2013). Studies on humans and animals demonstrate that obesity and metabolic syndrome with or without hyperinsulinemia or hyperglycemia are associated with somatic small nerve fibre neuropathy (Pittenger et al., 2005; Herman et al., 2007). SSFN implicates impairment of small diameter cutaneous nerve fibres composed of both Aδ- and C-nociceptors and is a risk for wound occurrence and impaired wound healing (Illigens and Gibbons, 2013). SSFN in obese patients without evidence of hyperglycemia was confirmed by measure of pain and flare response to topical capsaicin application (Herman et al., 2007) or by assessment of IENF density in skin biopsy (Pittenger et al., 2005; Zhou et al., 2011; Smith and Singleton, 2013).

Metabolically-induced nerve injury

A 2008 study showed that the prevalence of dyslipidemia, but not hypertension, was higher in patients with neuropathy than in diabetic patients without neuropathy (Smith et al., 2008). Diabetic sensory polyneuropathy is often diagnosed after years of hyperglycemia in type 1 diabetes. In type 2 diabetes, SSFN is often detected before diagnosis, when the patient is in a pre-diabetic state (metabolic syndrome). Several clinical studies showed that glycemic control prevents development of clinical neuropathy in type 1 diabetes whereas it is not enough in type 2 diabetes (Callaghan et al., 2012). This observation allows to hypothesize that sensory neuropathy could appear in the absence of outright diabetes and could be a consequence of other factors than prolonged hyperglycemia. The aetiology of neuropathy developing prior to overt hyperglycemia is not well understood, and a number of clinical and experimental studies implicate obesity, elevated triglyceride, cholesterol, and non-esterified fatty acids, as well as oxidative-nitrosative stress (Obrosova et al., 2007; Lupachyk et al., 2012; Smith and Singleton, 2013). In a recent review, Callaghan and Feldman (2013) have recently provided a broad overview of the mechanisms underlying metabolically induced nerve injury and therapeutics. The authors describe a feed-forward cycle of cellular damage caused by hyperlipidemia that induces nerve injury. To simplify, hyperlipidemia causes fatty deposition in the nerve and extracellular protein glycation and oxidation. Excess nutrients lead to cellular damage with production of reactive oxygen species and mitochondrial dysfunction. Mitochondrial dysfunction leads to oxidative and endoplasmic reticulum stress which was associated with pre-diabetic neuropathy (Lupachyk et al., 2013). Moreover, chronic metabolic inflammation encountered

in obesity and metabolic syndrome (Kalupahana et al., 2012) maintains a vicious cycle intensifying nerve injury, with the recruitment of macrophages to stressed nerve and hypertension resulting in nerve ischemia.

Wound healing with obesity and metabolic syndrome

Wound healing impairment occurring in obesity and metabolic syndrome resulted from numerous factors which are summarized in Table 5. In addition, impairment of cutaneous wound healing in obese patients is an important factor of complication after various bariatric surgeries such as gastric bypass.

Aging

The term aging refers to the biological process of growing older in a deleterious sense, what several authors call "senescence". Aging is one of the most complex biological processes. Briefly, aging leads to a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death (López-Otín et al., 2013). Age-related diseases include arthritis, osteoporosis, cardiovascular disease, cancer, diabetes, and various neurodegenerative diseases such as dementia and Alzheimer's disease. According to the World Health Organization, the proportion of the world population over 60 years will double between 2000 and 2050. This population is expected to increase from 605 million to 2 billion over the same period. As the aging population is growing, age-related diseases including chronic and non-healing wound are increasing. A correlation between aging and wound healing impairment was first described in 1916 (Du Noüy, 1916), and since then many studies have examined age-related impairment of wound healing in human, in animal and in *in vitro* models. The conclusions of these studies are that aging (>60 years) is an independent risk factor for delayed wound healing but does not impair the quality of wound healing (Gosain and DiPietro, 2004). Among age-related skin changes that could be involved in delayed wound healing, a reduction in nerve endings can be taken into account (Sgonc and Gruber, 2013). Aging markedly influences morphological and functional features of the peripheral nervous system, leading to peripheral neuropathy. An assessment performed in subjects with different ages demonstrated that aging was inversely correlated with nerve conduction velocity and amplitudes of both sensory and motor responses (Rivner et al., 2001). Structural changes include loss of myelinated and unmyelinated fibres and decreased production of the major myelin proteins with subsequent myelin deterioration. This loss is not compensated because of the decrease in regenerative and re-innervating capacities of nerve fibres in the elderly (Verdú et al., 2000). The prevalence of idiopathic peripheral neuropathy in the elderly that is not associated with some underlying disease process such as diabetes, has been reported to 26% in persons aged 65-74 years and to 54% of people aged 85 and older (Mold et al., 2004). Loss of lower extremity sensory input is associated with impaired balance, falls and unintentional injury in the elderly (Richardson, 2002). As in diabetes, aging is associated with multiple pathophysiological pathways that impair the immune system, vascular functions, and central and peripheral nervous system. Alterations in the aging skin have been described and can be associated with a deleterious impact on wound healing. Peripheral neuropathy occurring in the elderly could be involved in these changes and in age-related delayed wound healing.

A review of the age-related impairments of wound healing reveals that all phases of cutaneous healing undergo characteristic age-related changes, including enhanced platelet aggregation, increased secretion of inflammatory mediators, delayed infiltration of macrophages and lymphocytes, impaired macrophage function, decreased secretion of growth factors, delayed reepithelialisation, delayed angiogenesis and collagen deposition, reduced collagen remodelling and decreased muscle strength (Gosain and DiPietro, 2004). Some of these changes could be attributed, in part, to age-related peripheral neuropathy. Sensory denervation in skin results in delayed protein extravasation and cell migration during the inflammatory phase, delayed wound contraction and epithelialisation, delayed angiogenesis and re-innervation into the wound (Chéret

Table 5. Summary of factors related to wound impairment in obesity (from Guo and Dipietro, 2010).

Local wound conditions	Associated diseases and conditions	Factors altering immune and inflammatory response
1. Decreased vascularity in adipose tissue	1. Hard to reposition	1. Adipokines: leptin, adiponectin, resistin
2. Skin folds harbor micro-organisms	2. Coronary heart disease	2. Cytokines: TNF-α, IL-1, IL-6, IL-8, IL-10
3. Increased wound tension	3. Atherosclerosis	3. Chemokines: IL-8, MCP-1, IP-10
4. Increased tissue pressure	4. Type 2 diabetes	4. Impaired release of neuropeptide: CGRP, SP, neuropeptide Y
5. Hematoma and seroma formation	5. Cancer	
6. Venous hypertension	6. Hypertension	
7. Decreased innervation in skin	7. Dyslipidemia	
	8. Stroke	
	9. Respiratory problem	
	10. Small fibre neuropathy	

et al., 2013; Ishikawa et al., 2014).

Spinal cord injury

Spinal cord injury (SCI) designates damage to any level of the spinal cord resulting from a trauma (e.g. road accident) or a disease (e.g. tumour, spinal cord infarction, ankylosing spondylitis). According to the World Health Organization (2011), the global annual incidence is estimated from 40 to 80 cases per million population and 90% of cases would be from traumatic origin. SCI causes permanent changes of body functions below the site of injury and the severity of these changes obviously depend on the extent of injury. SCI leads to blockage of nerve impulses resulting in paralysis and/or tactile disturbances in the body parts governed by the damaged nerves. The area of skin supplied by a single spinal nerve is called a dermatome, and similarly, a myotome is a group of muscles innervated by a single spinal nerve. The higher the injury on the spinal cord occurs, the more dysfunction will arise. Symptoms may include partial or complete loss of sensory function and/or motor control below the affected area. The severity of sensory, autonomic and motor loss also depends on the level of injury to the spinal cord, described as “complete” or “incomplete” lesion. Complete SCI at high cervical level causes paralysis of the four limbs (tetraplegia) and affects the systems that regulate bowel or bladder control, breathing, heart rate and blood pressure. Complete thoracic SCI commonly causes sensory and/or motor loss in trunk and legs (paraplegia). Incomplete injury defines a situation where sensory and/or motor functions are preserved below the lowest sacral segments. Chronic pain occurs in all forms of SCI (Kirshblum et al., 2011). A common occurrence in SCI is the frequency of skin breakdown, particularly on the sitting surface. Ulcers developed in patients with SCI are difficult to heal as in diabetic neuropathy. Paraplegic and tetraplegic patients present impairment of wound closure below the level of the spinal cord lesion (Basson and Burney, 1982). Tissue changes induced by denervation due to SCI impact cutaneous homeostasis and lead to chronic wounds (Rappl, 2008).

Pathophysiology of spinal cord injury

Traumatic SCI symptoms result from two separate mechanisms: primary injury and secondary injury. Primary injury corresponds to neurological damage in the spinal cord that begins after the initial mechanical damage (compression, laceration, contusion). The initial impact induces bleeding and cell death at the impact site leading to a cascade of biological events described as “secondary injury” (minutes or weeks after injury). Secondary injury leads to further neurological damages and is followed by the “chronic phase” (days to years after injury). Secondary impairment may lead to inflammation, neuronal cell death, loss of oligodendrocytes and activation of microglia and astrocytes, which results in glial scarring (Silva et al., 2014). The astroglial scar forms a barrier impeding axonal growth and regenerative processes. Functional neuronal connections between peripheral nerves and spinal cord are disrupted below the injury causing dormant body areas. Loss of peripheral nerve to spinal cord connection induces tissue changes in target areas.

Wound healing in spinal cord injury

Physiological changes occurring in trunk dermatomes contribute to the development of pressure ulcers and to the slow healing rate encountered in SCI patients. The consequences of denervation in skin are hypothesized to interrupt the natural wound healing cycle (Rappl, 2008). The skin of individuals with SCI is thinner and less distensible that in healthy subjects. Denervated tissues exhibit abnormal vascular reaction and impaired immune function (Rappl, 2008). Histopathology studies report that SCI patients present dermal fibrosis, progressive skin thickening and nail hypertrophy on lower limbs (Stover et al., 1994). This study shows that the severity of the phenotypes is progressive and correlates with the degree of injury, suggesting a role of the peripheral nervous system in the maintenance of skin and nail. Changes of skin associated with SCI result from deficient vascular reactivity, decreased fibroblast activity and higher collagen catabolism (Gefen, 2014). Metabolic changes associated with denervation tend to render the tissue insensate and thus increase the risk of developing ulcers and affecting the healing rate once an ulcer develops.

Hereditary sensory (and autonomic) neuropathy type 1

Hereditary sensory and autonomic neuropathy (HSAN) or hereditary sensory neuropathy (HSN), are clinically and genetically heterogeneous disorders of the peripheral nervous system that affect predominantly the sensory and autonomic neurons. HSN/HSANs are currently classified into five types (HSN/HSAN 1-5). Typically, HSN/HSANs are described as slowly progressive neurological disorders characterized by primarily prominent distal sensory loss, and variable autonomic and motor disturbances. HSN1 is an axonal form of hereditary sensory neuropathy distinguished by prominent early sensory loss and later positive sensory phenomena including dysesthesia and characteristic "lightning" or "shooting" pains. Negative and positive sensory symptoms are mainly distributed to the distal parts of the upper and lower limbs. HSN1 affects dorsal root ganglia neurons and motor neurons of the spinal cord. A study shows distal loss of unmyelinated and small myelinated nerve fibres before degeneration of large myelinated axons in nerve biopsy (Houlden et al., 2006). Loss of sensation can lead to painless injuries, which, if unrecognized, result in slow wound healing and subsequent osteomyelitis requiring distal amputations (Houlden et al., 2006; Auer-Grumbach,

2008). Ulcero-mutilating lesions developed in HSN1 are the most serious complication of this pathology. Although foot ulcerations in HSN1 are compared with "diabetic foot syndrome", no microvascular impairment is described in this disease. These observations suggest and reinforce the crucial role of innervation during wound healing.

Others cases of impaired or delayed wound healing

Leprosy

In 2009, the World Health Organization reported that leprosy is detected in 17 countries (including India and Brazil) with a prevalence of the disease less than 1 per 10,000 people (except for Brazil where prevalence is 2 per 10,000 people). Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, predominantly affecting peripheral nerves and skin. Leprosy causes a complex peripheral neuropathy of immunological origin that results in autonomic, sensory and motor nerve disorders. The presence of *Mycobacterium leprae* in peripheral nerves elicits an inflammatory response that is composed of epithelioid granuloma with lymphocytes or acid fast bacilli-loaded macrophages, leading to nerve degeneration. The most prevalent clinical presentations are mononeuropathy, multiple mononeuropathy and polyneuropathy (Cabalar et al., 2014). Leprosy is usually not recognized until a cutaneous manifestation occurs, and most patients first present numbness, sometimes years before the skin lesions appear. Temperature sensitivity by the skin is lost in the initial stages of the disease, followed by vision loss and pain. Sensory symptoms are especially focused on body extremities; hands and feet (Nascimento, 2013). Skin injury in patients with leprosy is defined as a chronic ulceration of the anaesthetic area, resistant to local or systemic therapy and characterized by recurrence (Price, 1959). Ulcers in patients with leprosy remain the most common consequence of leprosy. Leprosy-induced anaesthesia is the central factor in the pathogenesis of hand and foot ulcers. Un-protective behaviours and poor quality of scar, resulting from previous ulceration, are the main reasons for recurrence of neuropathic ulcer, and can lead to deformity and/or amputation of the affected limb.

Wound healing in patients with cancer

Payne et al. review physiological changes that patients with cancer undergo and how these changes impact on the wound healing process (Payne et al., 2008). Cancer therapy such as chemotherapy directly impairs acute wound healing. In animal studies, alkylating agents such as cisplatin have been demonstrated to reduce wound tensile strength, to decrease fibroblast proliferation, to reduce connective tissue proliferation and to inhibit neovascularisation. Vincristine, a plant alkaloid, appears to transiently decrease wound tensile strength during the first days after surgery, without inducing wound complication. The effect of cancer therapies on acute wound healing is multifactorial. Chemotherapy weakens the immune function of the patients, induces neutropenia, anemia and thrombocytopenia, making wounds more prone to infections. In addition to immune impairment, chemotherapy affects the nervous system. Chemotherapy-induced peripheral neuropathy (CIPN) is a common and potential dose-limiting complication of cancer therapy. CIPN is usually present at the point most distal from the trunk first, i.e. fingertips and toes. According to the substance used, CIPN may be in the form of purely sensory and painful neuropathy (cisplatin and carboplatin) or mixed sensory-motor neuropathy which may be accompanied by dysfunction of the autonomic nervous system (vincristine and taxanes) (Quasthoff and Hartung, 2002). Structural changes in peripheral nerves include loss of IENF (Han and Smith, 2013). The literature describes the involvement of different chemotherapy drugs in wound healing impairment and in nervous system damage but the relationship between innervation and wound healing delay is not however obvious in this context.

Proof of concept in animal models

Relationships between impaired wound healing and peripheral neuropathy are highlighted in several animal experiments, described in Tables 6 and 7. Two types of experiments have been performed in order to further study the link between sensory nerve dysfunction and wound healing; either using animal models of skin denervation (Table 6) or stimulating cutaneous nerve (Table 7) during wound healing.

Skin denervation can be induced surgically, chemically or genetically. Studies have shown that surgical denervation slows down skin wound healing with decreased wound contraction, delayed reepithelialisation and reduced inflammatory cell infiltration (Richards et al., 1999; Buckley et al., 2012). Capsaicin is a potent agonist of TRPV1, a cation channel expressed by both sensory Aδ- and C-fibres. Capsaicin induces the degeneration of TRPV1-expressing nerve fibres and leads to reduced and delayed wound healing, mostly associated with poor reepithelialisation (Smith and Liu, 2002; Toda et al., 2008; Martínez-Martínez et al., 2012). Oxidopamine (6-OHDA)-induced sympathectomy also causes impaired wound healing which was associated with a decrease of neurogenic inflammation (Kim et al., 1998; Souza et al., 2005). Streptozotocin (STZ) is a chemical compound particularly toxic for insulin-producing β cells located in the pancreas. Therefore, injection of STZ promotes diabetes in animal models and is used to study the impact of diabetic neuropathy on wound healing. Skin wounds of STZ-treated mice and of db/db mice (genetically-induced diabetes) show delayed healing compared to non-diabetic animals, and are associated with impaired re-innervation of the wound (Gibran et al.,

2002; Cheng et al., 2013). These experiments further confirm that the functional integrity of the cutaneous nervous system is necessary for optimal wound healing (Table 6).

Gürgen et al. (2014) have set up a daily transcutaneous electrical nerve stimulation (TENS) treatment on rats during cutaneous wound healing and have shown that nerve stimulation shortened the healing time and increased wound repair efficacy. TENS is a method based on electrical stimulation which primarily aims to provide a degree of symptomatic pain relief by exciting sensory nerves. Recently, Kant et al. (2013) have shown that daily topic application of SP on cutaneous wound of healthy rats increases wound closure and induces faster reepithelialisation (Table 7).

Animal experiments highlight the crucial role of sensory nerve in wound healing and the ability of sensory peptides, SP and CGRP, in modulating wound

Table 6. Animal models of impaired wound healing associated with denervation.

Species	Disease	Wound type	Results	Reference
Male mice, 8 weeks old	Knockout for CGRP Capsaicin-mediated local skin denervation	Full-thickness skin wounds of 8 mm diameter (dorsal midline)	Reduced and delayed wound healing, reduction of VEGF expression in granulation tissue, suppressed neovascularization and angiogenesis Delayed wound healing	Toda et al., 2008
Sprague Dawley rat, 12 days old (developing rats)	Capsaicin mediated systemic denervation (50 mg/kg, s.c. injection at postnatal day 2 and 9)	Full-thickness circular skin wound (below the scapulae)	Capsaicin treatment led to reduced CGRP-immunoreactive sensory innervation which was associated with impaired healing; increased wound area and volume, prolonged scab retention and delayed re-epithelialisation. Denervated tissue exhibited increase of granulation cell proliferation without proportionate increase in apoptosis.	Smith and Liu, 2002
Wistar rat, 8-10 weeks old	Chemical sympathetic denervation with 6-OHDA (hydrobromide salt)	Skin linear incision or full-thickness excisional wound (4 cm^2)	6-OHDA lesion delayed cutaneous wound healing; increased wound contraction, reduced mast cell migration, delayed re-epithelialisation. Authors hypothesize that sympathectomy induced a decrease of neurogenic inflammation.	Kim et al., 1998 ; Souza et al., 2005
CD-1 mice, 3 month old	Streptozotocine-induced diabetic neuropathy	Dorsal skin wound of 3 mm diameter with punch biopsy	Mild decline of DIENF in hairy dorsal skin. Diabetes was associated with slower decline of wound size, failure of plasticity to send axons into newly reconstituted tissue.	Cheng et al., 2013
Male CD57L mice, 8 to 12 week old	Genetically-induced diabetes, db/db	Dorsal full-thickness wound of 1.5*1.5 cm.	Db/db mice had reduced number of epidermal nerve fibre and healed more slowly than non-diabetic mice.	Gibran et al., 2002
Wistar male rat, 8 week old	Neonatal capsaicin treatment-induced sensory denervation	Full thickness wound of 6 mm diameter (dermal punch biopsy)	Sensory denervation: reduced activation of keratinocyte proliferation, impaired follicle cell migration, impaired bulge stem cell progeny migration to the epidermis, slowed down reepithelialisation.	Martínez-Martínez et al., 2012
C57BL/6 female mice, 7 to 12 week old	Surgical denervation of the ear	2 mm punch biopsy wound at the centre of the ear	Surgical denervation affected wound healing and was associated with increased wound area, extensive necrosis and inability to reepithelialise the distal wound margin of the ear hole.	Buckley et al., 2012
Adult female Sprague-Dawley rat	Surgical denervation by section of the inferior epigastric nerve	Full thickness wound of 1 cm diameter on the groin	Healing of denervated wound was delayed with decrease in wound contraction, and reduced count of macrophages and T-lymphocytes in the granulation tissue.	Richards et al., 1999

Table 7. Animal models of improved wound healing associated with nerve stimulation.

Species	Wound type	Treatment	Results	Reference
Adult male Wistar rats (140-160 g)	Full-thickness excisional wound (4 cm^2) on the back	Daily topic application of SP (10^{-7} M, 400 μL) on wound during 14 days	SP stimulated inflammatory cell recruitment and angiogenesis by increased TGF-β1 and VEGF expression. Il-10 was increased in SP treated wound, which led to stop inflammatory phase. SP increased wound closure and induced faster reepithelialisation phase.	Kant et al., 2013
Albinos male and female rats	Dorsal full-thickness incision wound of 1 cm	Daily TENS (transcutaneous nerve stimulation) for 5 days after wound	TENS improved wound healing by accelerating closure of wound edges and reepithelialisation, and by decreasing pro-inflammatory cytokines levels (IL-1β, IL-6 and TNF-α)	Gürgen et al., 2014
Genetically diabetic male mice, 8 to 12 week old	Dorsal full-thickness wound of 1.5*1.5 cm	Topical application of SP dissolved in polyethylene glycol (5%)	Diabetic wound treated with SP healed faster than untreated wound	Gibran et al., 2002

healing.

Conclusion

The skin is a major component of the human body and is the first barrier and protection against outside aggressions. Thus, deciphering cellular and molecular mechanisms involved in wound healing is of paramount importance as skin integrity is crucial in the maintenance of general homeostasis. The skin is a complex tissue, which is highly vascularised and very richly innervated as highlighted in this review. Although skin/nerve interactions are far from being completely understood, much evidence suggests that a proper cutaneous innervation is essential in the process of skin maintenance, protection and healing. Clinical observations in patients with peripheral and central nervous system disorders have greatly helped to understand the roles of the nervous system in skin protection. Moreover, animal models allow dissecting molecular and cellular mechanisms involved in skin protection and repair that are highly regulated and are partly dependent upon the nervous system to be properly orchestrated. A better understanding of these mechanisms is crucial to develop new therapeutic tools and protective strategies that will be useful in the improvement of cutaneous wound healing.

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