# MINI-REVIEW

# Ticks and the effects of their saliva on growth factors involved in skin wound healing

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**ABSTRACT**

Ticks are unique hematophagous arthropods and possess an astounding array of salivary molecules that ensure their unnoticed and prolonged attachment to the host skin. Furthermore, ticks are very effective vectors of a diverse spectrum of pathogens. In order to feed, tick chelicerae cut the host epidermis and their hypostome penetrates through the layers of the skin. As a result of laceration of the skin and rupturing blood vessels, a pool of blood is formed in the dermis, serving for intermittent blood sucking and secretion of saliva. Cutaneous injury caused by tick mouthparts should normally elicit wound healing, a complex biological process coordinated by interaction among different host cells, numerous signalling pathways and by a variety of soluble factors including growth factors. Growth factors, endogenous signalling proteins involved in various biological events, are key players in all phases of the skin repair process. Maintaining feeding site integrity by overcoming sequential phases of wound healing is particularly important for ixodid ticks and is governed by bioactive molecules in their saliva. Tick saliva is a complex mixture of proteins, peptides, and non-peptide molecules and its composition depends on the feeding phase, tick developmental stage, gender and/or the presence/absence of microbial agents. In addition to already demonstrated anti-haemostatic, anti-cytokine and anti-chemokine activities, anti-growth factors activities were also detected in saliva of some tick species. In consequence of counteracting host defences by ticks, tick-borne pathogens can be transmitted to and disseminated in the host. Elucidation of the complex interplay between ticks – pathogens – host cutaneous immunity could lead to improved vector and pathogens control strategies. Additionally, tick saliva bioactive molecules have a promising therapeutic perspective to cure some human diseases associated with dysregulation of specific cytokines/growth factors and alterations in their signalling pathways.

**KEYWORDS:** Ticks, saliva, growth factors, wound healing, host immunity

## INTRODUCTION

Ticks are hematophagous ectoparasites with immense medical and veterinary importance due to transmission of a wide spectrum of pathogens and direct damage of host skin. However, based on discovering toxins in tick saliva that are similar to those found in venomous animals, such as spiders or snakes, ticks should also be referred to as venomous blood-feeding arthropods (Cabezas-Cruz and Valdés, 2014). To successfully obtain a blood meal, ticks as telmophages lacerate all epidermis layers by penetration of chelicerae and hypostome and, by rupturing blood vessels, they create a blood pool in the dermis. A continuous feeding process till repletion, lasting for several hours to days, is boosted and

ensured by tick salivary bioactive molecules. Tick saliva represents a very complex fluid secreted by salivary glands into the bite site and contains an incredible number and variety of proteins, peptides and non-peptide molecules that: i) facilitate the feeding process by overcoming haemostasis and wound healing processes; ii) interfere with host innate and adaptive immune defences; ii) support transmission and local establishment of pathogens in the immunomodulated feeding site (reviewed in Kotál et al, 2015; Kazimírová et al; 2017; Mans et al, 2017; Šimo et al, 2017; Nuttall, 2018; Wikel, 2018); iii) can induce paralysis or other toxicoses in vertebrate hosts (Cabezas-Cruz and Valdés, 2014; Pienaar et al, 2018).

Skin injury elicited by tick feeding initiates immediately a repair process which is composed of several stages generally grouped into sequential overlapping phases: inflammation, proliferation and tissue remodelling (Canedo-Dorantes and Canedo-Ayla, 2019). Each phase is conducted by a complex interplay among several resident skin cells including keratinocytes, fibroblasts, endothelial cells, macrophages, platelets and infiltrating immune cells and is performed and controlled by an equally intricate signalling network of numerous cytokines, chemokines and growth factors (Sorg et al, 2017; Larouche et al, 2018).

Growth factors (GFs), naturally occurring signalling molecules, are involved in infinite biological events including cell growth, proliferation, migration and differentiation, and are irreplaceable in the wound healing process. GFs play critical roles in modulating inflammatory responses, enhancing granulation tissue formation, and in inducing angiogenesis. They are essential for successful extracellular matrix (ECM) formation and tissue remodelling processes (Park et al, 2017).

Despite the undoubted key role of GFs in wound healing, data about interactions between ticks and GFs are very scarce. This mini-review contains information about effects of tick salivary molecules on vertebrate GFs, key players of wound healing, and discusses their possible therapeutic potential.

**SKIN**

The interaction between ticks, tick-borne pathogens and the host immune response is a complex and multifactorial event taking place in the vertebrate host skin. The skin is no longer considered just as a mechanical barrier, but represents a sophisticated intricate communicative line between inner and external environment of individuals. The discoveries of the ‘skin immune system’, ‘keratinocytes as potent sensors of infectious intruders and danger signals’ and more recently of ‘the skin microbiome’ highlighted the skin as complex multitasking organ with unique immunologic properties (DiMeglio et al, 2011). Under constant exposure to environmental stimuli, the skin performs numerous tasks to preserve

homeostasis and integrity of the organism. This ability of multifunctionality is closely linked to the skin anatomical structure. Skin consists of three major layers – epidermis, dermis and hypodermis. The epidermis, a four layered stratified epithelium, houses mostly keratinocytes (90%), melanocytes and immune cells, such as Langerhans cells and dendritic epidermal T-lymphocytes. The composition of dermis is more varied with greater cell diversity (fibroblasts, dendritic cells, macrophages, natural killer cells, mast cells, T-lymphocytes) and thin and thick collagen fibres, respectively. Close to a basement membrane, there is a papillary layer of dermis with high fibroblast density and thin collagen fibres followed by reticular dermis consisting of thick collagen fibres and low cell density. Hypodermis, the white dermal adipose tissue, consists of pre- and mature adipocytes surrounded by fibroblasts, nerves and blood vessels (Pasparakis et al, 2014; Rognoni and Watt, 2018).

Despite serving as a significant and effective barrier, the skin is still the major entry portal for most pathogens transmitted by arthropod vectors such as ticks, mosquitoes, sand flies, etc. during their feeding, taking advantage of their ability to penetrate skin and bypass the host defence responses in wounds evoked by mechanical injury (Boulanger, 2018).

## WOUND HEALING

As a response to damage or disturbance of the skin's barrier function, wound healing is initiated. Wound healing is an evolutionarily conserved, complex, multicellular process that, in skin, aims to protect and restore tissue integrity. Formation of new tissue and wound closure involves the coordinated efforts of myriad cell types including resident skin cells as well as infiltrated immunocompetent cells and is executed and regulated by a complex signalling network involving numerous GFs, cytokines and chemokines (Behm et al, 2012; Rodrigues et al, 2019). Sequential overlapping, and functionally coordinated phases of acute cutaneous wound healing are activated within seconds of injury and evolve over days, weeks, and months until skin integrity is restored (Greaves et al, 2013; Eming et al, 2014).

## HAEMOSTASIS/INFLAMMATION PHASE

Upon the tick hypostome penetration into the skin, a clotting cascade is initiated with the aid of activated platelets, resulting in the formation of a coagulation fibrin clot and ensures haemostasis. The fibrin clot also provides a basic matrix to support inflammatory (neutrophils and monocytes) or other resident skin cells (fibroblasts, endothelial cells) influx. Activated platelets, trapped in the clot, express adhesion receptors for interaction with fibroblasts and endothelial cells and for recruitment of circulatory cells and simultaneously release various GFs (Figure 1) and chemokines to regulate all

downstream phase events of wound healing (Eming et al, 2014; Golebiewska and Poole, 2015). Of GFs, platelets secrete platelet-derived growth factor (PDGF), transforming growth factor-α (TGF-α), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), heparin-binding EGF (HP-EGF), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) that interact, promote and chemotactically attract cells into the wound. PDGF attracts neutrophils to the wound; VEGF and FGF endothelial cells and initiate angiogenesis.

Along with haemostasis, early inflammatory reactions trigger innate defensive reactions at the wound site, specifically neutrophils, the first immune cells entering the wound followed by monocytes, which convert into macrophages by TGF-β. Both neutrophils and macrophages control and kill invading pathogens and amplify inflammatory response by secretion of antimicrobial molecules, cytokines and GFs. Production of FGF, EGF, TGF-β, VEGF and PDGF leads to tissue granulation and transition to the proliferative phase (Greaves et al, 2013).

**PROLIFERATIVE PHASE AND REMODELLING PHASE (MATURATION)**

The proliferative phase involves numerous important cellular and molecular components that contribute to granulation tissue formation and ECM and initiation of angiogenesis. The proliferative phase is characterized by: i) fibroplasia (proliferation of fibroblasts and their differentiation to myofibroblasts, deposition of EMC) driven by TGF-β1, EGF, FGF-2, PDGF and cytokines; ii) re-epithelization (differentiation, replication and migration of keratinocytes) stimulated by EGF, hepatocyte growth factor (HGF), FGFs, IGF-1, TGF-β1; and iii) angiogenesis coordinated by VEGF, VEGF-A, FGF-2, PDGF, TGF-β1 (Greaves et al, 2013; Canedo-Dorantes and Canedo-Ayla, 2019; Rousselle et al, 2019). Restoration of damaged blood vessels and growth of new ones is an essential part of healing, as it provides nutrition and oxygen to the cells in the wound. Finally, a transition from granulation tissue to mature scar occurs, characterized by continued collagen synthesis and collagen catabolism. The remodelling phase is characterized by the active reorganization of the ECM, a reduction in the total number of capillaries and replacement of type III collagen by type I collagen.

According to above mentioned studies on the wound healing process it is evident that besides the importance of cell-cell and cell-matrix interactions, different GFs are involved and play critical roles in all stages of the repair process.

**GROWTH FACTORS INVOLVED IN WOUND HEALING**

GFs comprise a large group of regulatory proteins affecting cellular growth, proliferation, differentiation, and/or migration. They can be secreted as fully functional molecules or as molecules that require further posttranslational processing in order to be activated. These chemical messengers attach to specific cell surface receptors and via this interaction mediate inter and intracellular signalling pathways by paracrine, autocrine, juxtacrine, or endocrine mechanisms (Barrientos et al, 2008; Fabi and Sundaram, 2014). GFs are important in both normal physiological processes such as wound healing and abnormal processes such as cancer. In the skin wound healing, GFs synthesized by fibroblasts, keratinocytes, platelets, lymphocytes, and mast cells play critical roles in modulating inflammatory responses, enhancing granulation tissue and ECM formation, inducing angiogenesis and tissue remodelling (Barrientos et al, 2008; Behm et al, 2012). Supplementary Table S1 shows cell sources, cell targets and some mechanisms of action that have been identified for the key GFs involved in wound healing. The expression and function of GFs in normal skin wound healing correlate spatially and temporally with the phase-specific recruitment of different cells.

However, GFs deficiencies, including reduced levels, are responsible for chronic non-healing wounds. On the other hand, abnormal GFs expression is associated with impaired wound healing or excessive scarring (fibrosis) (Lichtman et al, 2016; Park et al, 2017).

**TICK COUNTERMEASURES AGAINST GROWTH FACTORS**

All blood-feeding arthropods, especially ticks, face challenges from host haemostasis, inflammation, and initiation of immune responses that could impair subsequent blood feeding. The feeding process of hard ticks is a sequence of behavioural changes from questing and engaging the host, finding a suitable feeding site, penetration of the skin, blood pool creation and feeding to repletion. Thus, host resident skin cells are exposed to both, mechanical injury from insertion of mouthparts and biological actions of saliva molecules facilitating blood meal acquisition. To secure uninterrupted blood uptake, ticks suppress and evade the complex physiological host immune and homeostatic responses that are raised against them. Cutaneous injury and ruptured blood vessels should normally elicit a wound healing. Repairing progression would cause tick rejection and/or disrupt tick feeding, however, ticks evolved the ability to overcome all host defence responses, including wound healing so as to maintain the feeding lesion in dermis and complete the feeding process. Tick salivary glands produce and secrete a cocktail of different molecules (Figure 1) that interfere with various components of haemostasis, wound healing, and both arms of the immune system of the vertebrate hosts, including enzymes, cytokines, complement and even GFs (Kazimírová and Štibrániová, 2013; Wikel, 2018; Štibrániová et al, 2019). Studies of Hajnicka et al (2011) and Slovák et al (2014) determined a direct impact of salivary gland

extracts (SGE) derived from different hard tick species on several GFs involved in wound healing. They discovered various anti-TGF-β1, -PDGF, -HGF and -FGF-2 activities depending on the tick species (see Table 1). Specific binding activity was confirmed just for TGF-β1. No anti-EGF, -granulocyte macrophage colony stimulating factor (GM-CSF), -keratinocyte growth factor (KGF) and -VEGF activities have been demonstrated in any of the tick SGE. Of all tested ticks, SGE of *Amblyomma variegatum* and *Hyalomma excavatum* showed a richer repertoire of GF-binding molecules than the other tested species. In addition to anti-GF effects, SGE derived from ticks with long mouthparts displayed *in vitro* anti-proliferative and cytotoxic activities associated with actin filaments disruption in different cell lines. The anti-GFs activities identified by Hajnická et al (2011) could be one of the explanations of suppressive effects of *Dermacentor variabilis* SGE and saliva on fibroblast migration and ability to repair an injury that were previously observed by Kramer et al (2008). Tick SGE/saliva also reduced pro-migration signalling triggered with extracellular signal-regulated kinase (ERK) in PDGF-stimulated fibroblasts. On the other side, *D. variabilis* saliva enhanced basal- and PDGF-stimulated migration of macrophage-derived IC-21 cell line without affecting ERK signalling and suppressed macrophage ability to phagocytose zymosan particles (Kramer et al, 2011).

The ERK signalling pathway, like other MAPK serine/ threonine kinases pathways (JNK, p38MAPK), regulates cell migration induced by GFs, including PDGF, VEGF, EGF, FGF and insulin. Thus, ticks could conquer wound healing by inhibiting the signalling activity as shown earlier. In addition, SGE of *D. variabilis* was found to display the same effects on renal epithelial OK cells (not concerned in wound healing) suggesting that tick saliva might be valuable in controlling the migration of many cell types, including endothelial cells during angiogenesis and invasiveness of cancer cells (Kramer et al, 2008). Studies that focused on beneficial effects of tick saliva and their therapeutic potential are mentioned below.

In *Amblyomma americanum* saliva three insulin-like GFs binding-related proteins (IGFBP-rPs) were identified and termed as AamIGFBP-rP1 and AamIGFBP-rP6S (short) and AamIGFBP-rP6L (long), respectively, according to comparison with human IGFBP-rPs. IGFBP-rPs, belonging to the IGF system, are responsible for cell differentiation and growth in vertebrates. The RNAi-mediated silencing of AamIGFBP-rPs suggested that these three proteins may be involved in physiological functions linked to the tick feeding process, perhaps trough possible AamIGFBP-rP1 stimulation of prostacyclin secretion from salivary glands into the tick saliva (Mulenga and Khumthong, 2010). Both AamIGFBP-rP6 are highly expressed during the first 24 h of feeding and are probably associated with formation of the feeding site, but are downregulated as ticks continue to feed. AamIGFBP-rP1, which is upregulated after feeding commences, may be associated with regulating continuous events such as blood ingestion and/or

maintenance of the tick-feeding lesion. A study with recombinant AamIGFBP-rP1 revealed its ability to bind insulin, but no IGFs, and showed no antihaemostatic or antimicrobial functions (Radulovič et al, 2015). Native AamIGFBP-rP1 protein is an immunogen and, as previously observed, conservation of AamIGFBP-rPs amino acid sequences amongst other hard tick species makes them potential targets for universal anti-tick vaccine development (Mulenga and Khumthong, 2010: Radulovič et al, 2015). Bakshi et al (2019) showed that rAamIGFBP-rPs belong to the pro-inflammatory *A. americanum* saliva proteins or, in other words, pro-host defence proteins, which stimulate both PBMC-derived and mouse RAW 267.4 macrophages to *in vitro* express pro-inflammatory markers such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α. On the contrary, anti-inflammatory or anti-host defence tick saliva proteins, such as the two serpins derived from *A. americanum*, were found to enhance expression of anti-inflammatory cytokines (IL-10 and TGF-β) in the macrophages pre-activated by LPS or rAamIGFBP-rPs. Mice paw oedema test confirmed *in vivo* functional countervailing of tick saliva pro- and anti-inflammatory proteins (Bakshi et al, 2019).

Despite having much shorter feeding times, argasid (soft) ticks may manipulate host wound healing responses as well. *Ornithodoros brasiliensis*, a nidicolous soft tick, is frequently allied with human and canine toxicosis syndrome up to severe reactions and with notably sluggish healing lesion at the bite site, which can take several weeks to heal. Saliva and/or salivary gland homogenates from *O. brasiliensis* ticks significantly delayed *in vivo* wound healing of rat skin in the classical model of excisional induced skin lesions and showed a strong cytotoxic and antiproliferative activity on cultured endothelial cells *in vitro*. However, the mechanisms of the described effects are unknown (Reck et al, 2013).

## THERAPEUTIC POTENTIAL OF TICK SALIVA

Disregarding the parasitic life style and transmission of pathogens, the ability of ticks to defeat every stage of wound healing and host defences by a wide array of saliva constituents shift them to a spotlight as promising sources of new drugs (Sousa et al, 2015; Chmelar et al, 2019; Štibraniova et al, 2019). Among the various tick saliva activities, anti-angiogenic properties and also cytotoxic effects on various cell types were observed. Angiogenesis is a crucial event involved in many physiological processes from early embryonic to adult stages including the recovery of haemostasis during wound repair (Wietecha and Dipietro, 2013). Yet, in some severe pathological processes including cancer (Welti et al, 2013), angiogenesis has become a target for treatment. Of GFs, VEGF-A is the most important mediator of angiogenesis. Amblyomin-X, a 15 kDa recombinant Kunitz-type serine protease inhibitor of coagulation factor Xa (FXa) derived from *Amblyomma cajennense* ticks (Batista et al, 2010) was found to possess anti-tumour and anti-metastatic activities (Chudzinski-Tavassi et al, 2010; Ventura et al, 2013) and also

anti-VEGF-A activity. Amblyomin-X treatment did not affect VEGF-A induced cell migration, but inhibited VEGF-A induced angiogenesis in the mouse dorsal subcutaneous tissue as well as in the chicken chorioallantoic membrane. Amblyomin-X also reduced cell adhesion and tube formation and inhibited VEGF-A induced endothelial PECAM-1 expression (Drewes et al, 2012).

Another potent anti-angiogenesis factor with a potential to control cancer is the Troponin I-like molecule (HLTnI) identified in saliva of *Haemaphysalis longicornis* by You et al (2001). Recombinant HLTnI significantly suppressed the capillary development in human vascular endothelial cells (HUVEC) in a dose-dependent manner in VEGF competitive angiogenesis assay *in vitro* (Fukumuto et al, 2006). The mechanism of this effect is unclear, however, the structure similarity with human troponin I suggests an interaction of HLTnI with receptors for VEGF and FGF-2 on endothelial cells. On the other hand, HLTnI can facilitate blood feeding of ticks by inhibition of angiogenesis and moreover, it can be a potent target molecule to control ticks due to its strong immunogenicity.

Ixolaris, an effective Kunitz domain anticoagulant derived from salivary glands of *Ixodes scapularis* targeting the FVIIa/TF complex (Francischetti et al, 2002), displays promising anti-tumour therapeutic usage. Ixolaris was found to decrease U87-MG tumour growth *in vivo* in a xenograft model in nude mice and also reduce tumour vascularization and expression of VEGF (Carneiro-Lobo et al, 2009, 2012).

**SIGNALLING PATHWAYS**

Protein Kinase B (PKB)/Akt and extracellular signal-regulated kinase (ERK) signalling pathways can both mediate GF-induced cellular migration, and aberrant regulation of Akt and ERK pathways can contribute to cancer phenotypes (Gan et al, 2010; Liu et al, 2012). The MEK/ERK pathway, for example, is associated with more than 30% of human cancers (Bonjardim, 2017).

Saliva from *D. variabilis* was observed to inhibit basal and agonist-induced Saos-2 and MB-231 migration and invasion. Saliva of this tick species had no effect on EGF-stimulated Akt and ERK activity in MB-231 cells. In the Saos-2 cells, saliva suppressed EGF-activation of Akt, however, only basal ERK activity was affected in MB-231 cells (Poole et al, 2013).

TGF-β1, via specific serine/threonine kinase receptors, activates the common SMAD- dependent signalling pathway (Massagué, 2012; Fabregat et al, 2014) and/or non-SMAD pathways regulated by ERK, p38 MAPK, JNK, and PKB/AKT (Walton et al, 2012). The dysregulation of the TGF-β1 pathways leads to numerous human diseases and disorders, including cancer, fibrosis and inflammation (Pickup et al, 2013). SGE derived from

*Dermacentor reticulatus* and *Hyalomma anatolicum excavatum* ticks was observed to effectively decrease human (hu)TGF-β1-induced signalling pathways, canonical as well non-canonical in SiHa and HeLa cervical cancer cell lines, varying in their response to huTGF-β1. Furthermore, SGE of *D. reticulatus* significantly inhibited huTGF-β1-induced ERK1/2 activation, but not Akt activation. On the other hand, SGE of *H. anatolicum excavatum* ticks did not display any evident effect on ERK1/2 or AKT activation induced by huTGF-β1 (Holikova et al, 2018). The results suggest that the anti-GF activities of SGE depend not only on the origin of the treated cells but also on the tick species from which the SGEs originate. Additionally, crosstalk between signalling pathways of different GFs has been described and isolation and identification of specific tick molecules responsible for anti-GFs activities is highly desirable.

## CONCLUSIONS

GFs and their signalling pathways are considered as crucial players in diverse physiological processes, including wound healing. However, any aberration in these pathways is associated with many immunological disorders and diseases, even cancer. Tick saliva comprises a complex cocktail of bioactive compounds that facilitate their feeding process and transmission of pathogens. Saliva of some tick species showed anti-GFs activities, but the knowledge on the mechanisms of action of tick salivary molecules on GFs involved in wound healing in vertebrate skin is still limited. However, tick saliva molecules with immunomodulatory, anti-inflammatory, anti-clotting, anti-platelet, anti-cytokine, anti-GFs as well as anti-tumour and anti-angiogenic properties represent important sources for discovering and designing new therapeutics for various pathways of the mammalian physiology.

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## COMPETING INTERESTS

None declared.

## ABBREVIATIONS

GFs: Growth factors

ECM: Extracellular matrix

EGF: Epidermal growth factor

FGF: Fibroblast growth factor

HP-EGF: Heparin-binding EGF

HGF: Hepatocyte growth factor

IGF-1: Insulin-like growth factor-1

PDGF: Platelet-derived growth factor

TGF-α Transforming growth factor-alpha

TGF-β: Transforming growth factor-beta

VEGF: Vascular endothelial growth factor

GM-CSF: Granulocyte macrophage colony stimulating factor

KGF: Keratinocyte growth factor

SGE: Salivary gland extract

ERK: Extracellular signal-regulated kinase

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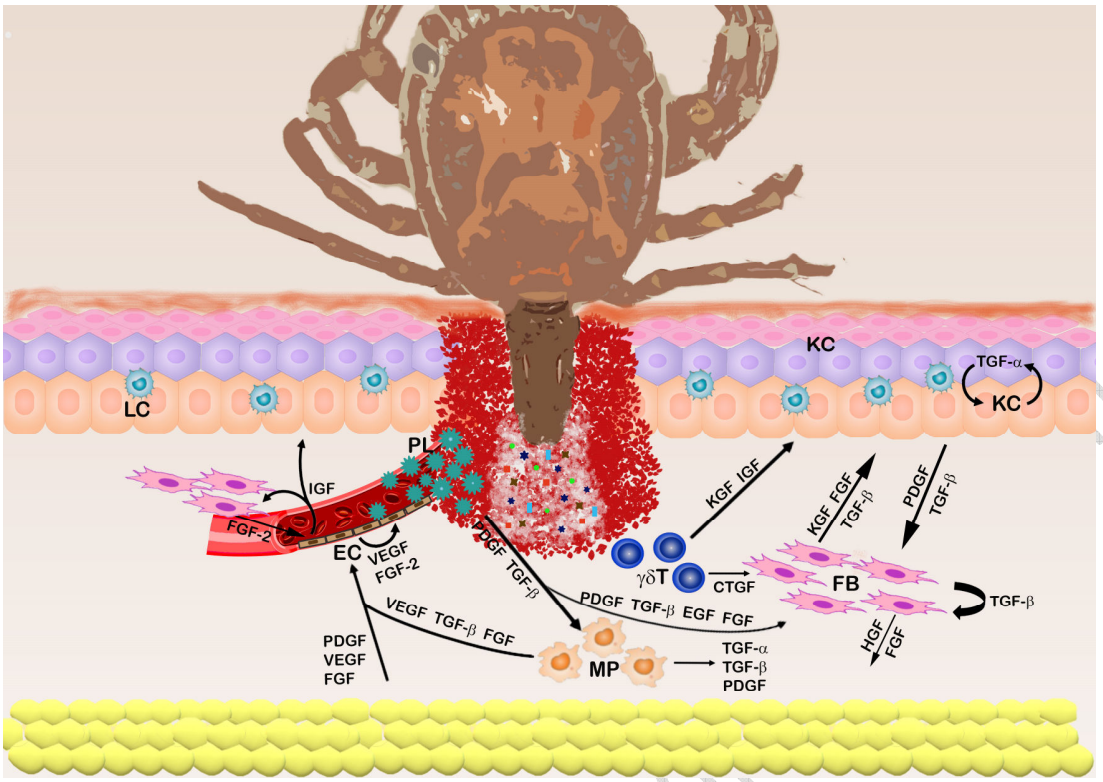


**Figure 1.** Upon the tick hypostome penetration into the host skin, activated platelets, resident skin cells and infiltrated immunocompetent cells secret numerous GFs into the wound edge. Platelets (PL) within the plug release PDGF, TGF-β, EGF, IGF, which are chemotactic for neutrophils, monocytes-macrophages and promote the proliferation of fibroblasts (FB), keratinocytes (KC) and endothelial cells (EC) in the wound. Due to a sophisticated network of sensors, KC recognize foreign or microbial agents and tissue damage and transmit warning by producing mediators (cytokines, chemokines, growth factors) and alert skin-resident immune cells, for instance, Langerhans cells (LC), type of dendritic cells resident in the epidermis representing the main antigen presenting cells. The γδ T cells in dermis act as immune sentinels and moreover produce GFs important in wound healing, such as connective tissue growth factor (CTGF), FGF, KGF and IGF1. After injury, tissue-resident macrophages (MP) activate migration of circulating monocytes into the wound, which convert into MP by TGF-β. MP undergo phenotypic changes throughout the healing process, in the early stages they differentiate into the pro-inflammatory M1 subset of MP associated with phagocytic activity, scavenging as well as secrete pro-inflammatory cytokines and growth factors (FGF-2, PDGF, VEGF) to mobilize more immune cells and stimulate the proliferation of KC, FB and EC. During the proliferation stage of wound healing, M1 cells transform into a functionally and phenotypically anti-inflammatory M2 cells orchestrating the interaction with EC, FB and KC and GFs govern proliferation and differentiation of FB, KC and EC leading to tissue and vessels formation. Maintaining feeding site integrity by overcoming wound healing processes is particularly important for ixodid ticks and is governed by bioactive molecules in their saliva. Saliva of some tick species displayed anti-TGF-β, -PDGF, -FGF-2, -HGF, -VEGF activities.

**Table 1.** Anti-growth factor activities in salivary gland extracts of different tick species (modified according to Hajnická et al, 2011; Slovák et al, 2014).

| Growth factors | *Amblyomma variegatum* | | *Dermacentor reticulatus* | | *Rhipicephalus appendiculatus* | | *Hyalomma excavatum* | | | *Ixodes ricinus* *Ixodes scapularis* |
|---|---|---|---|---|---|---|---|---|---|---|
| | F | M | F | M | F | M | F | M | N | F |
| **TGF-β1** | ++++ | ++++ | ++++ | ++++ | ++++ | ++ | ++++ | ++ | ++++ | - |
| **FGF-2** | ++++ | ++++ | ++ | ++ | + | + | ++++ | ++++ | + | - |
| **PDGF** | ++++ | ++++ | - | - | - | - | ++++ | + | +++ | ++++ |
| **HGF** | +++ | ++ | +++ | + | +++ | ++ | ++ | ++ | ++ | - |
| **EGF** | - | - | - | - | - | - | nd | nd | nd | - |
| **VEGF** | - | - | - | - | - | - | nd | nd | nd | - |
| **GM-CSF** | - | - | - | - | - | - | nd | nd | nd | - |
| **KGF** | nd | nd | nd | nd | nd | nd | - | - | - | nd |

Activities are recorded as: nd (not done); **-** (undetectable); + (1–25%); ++ (26–50%); +++ (51–75%); ++++ (76–100%). Growth factor effects are shown as % reduction in OD reading compared with the control in specific ELISAs. F – adult females; M – adult males, N – nymphs