MOLECULAR COMPONENTS, IMMUNE AND STEM CELLS IN SOFT TISSUE REGENERATION

Plekhova N.G.,
Korolev D.V.,
Shumatov V.B.

Pacific State Medical University
(Ostryakova Ave. 2, Vladivostok 690002,
Russian Federation)

Corresponding author:
Natalia G. Plekhova,
e-mail: pl_nat@hotmail.com

ABSTRACT

Wound healing is a spatiotemporal and highly regulated process that is divided into four continuous and overlapping stages: hemostasis, inflammation, repair (proliferation) and remodeling. All stages are controlled by various body systems and depend on the regulatory role of immune and stem cells. Despite significant progress in understanding the cellular and molecular mechanisms of inflammation, the role of the immune microenvironment in the regeneration process remains unclear. On the one hand, the critical importance of the cellular and molecular components of the immune system in the reparative response of tissues, including the degree of scarring, restoration of structure and function of organs, has been proven, and on the other hand, little data is presented on the loss of tissue regeneration ability associated with the immune competence evolution. The review presents the key cellular and molecular mechanisms of the immune response and of the stem cells participation soft tissue repair process during their interaction with the extracellular matrix. An analysis of the latest scientific data on the participation of components of the immune microenvironment and of stem cells in soft tissue repair process was carried out based on the publications presented in Google Scholar, Medline, PubMed, Scopus and Web of Science. It has been shown that the nature of this response and its duration have a significant impact on the outcome of repair – from incomplete recovery (scarring or fibrosis) to full regeneration. It is indicated that various types of immune and stem cells take part in the soft tissue repair and remodeling processes, and their interaction must be precisely controlled. The review data may provide the basis for the development of new therapeutic approaches for soft tissue repair through immune regulation or the use of stem cells and extracellular vesicles.

Key words: *immune microenvironment, stem cells, soft tissues, regeneration, intercellular interaction*

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МОЛЕКУЛЯРНЫЕ КОМПОНЕНТЫ, ИММУННЫЕ И СТВОЛОВЫЕ КЛЕТКИ В РЕГЕНЕРАЦИИ МЯГКИХ ТКАНЕЙ

Плехова Н.Г.,
Королев Д.В.,
Шуматов В.Б.

ФГБОУ ВО «Тихоокеанский государственный медицинский университет» Минздрава России (690002, г. Владивосток, просп. Острякова, 2, Россия)

Автор, ответственный за переписку:
Плехова Наталья Геннадьевна,
e-mail: pl_nat@hotmail.com

РЕЗЮМЕ

Статья посвящена 500-летию со дня рождения величайшего врача и учёного XVI века Габриэле Фаллопио (Фаллопия), революционера-морфолога, внёсшего неоценимый вклад в развитие науки, одного из основателей фундаментальной анатомии. И хотя прежде всего Фаллопий известен как анатом, описавший маточные («фаллопиевы») трубы, круг интересов учёного был гораздо шире, а вклад в анатомию – несоизмеримо более значительным. Фаллопий сделал множество важных открытий в анатомии, ряд анатомических структур носят его имя. Кроме того, Габриэле Фаллопио был талантливым педагогом и известным практикующим врачом, хирургом и фармацевтом. Особо следует отметить, что Фаллопий считал себя учеником Андреаса Везалия. Данных, подтверждающих факт личного знакомства Фаллопия и Везалия, не имеется, но есть документальное подтверждение кратковременной переписки упомянутых учёных. В своём знаменитом труде «Анатомические наблюдения» («Observationes anatomicae», 1561) Фаллопий указал на ошибки Везалия и его неточности в анатомических описаниях, подвергнув корректной критике везалиевскую «De humani corporis fabrica». Сохранился ответ Везалия с комплиментами в адрес Фаллопия как учёного. В любом случае, несомненным фактом является то, что Фаллопий был приверженцем методов Везалия в прикладной науке и преподавании анатомии и последовательно внедрял их в практику на протяжении всей своей жизни.

Ключевые слова: *история анатомии, история медицины, Габриэле Фаллопио, фаллопиевы трубы, медицинская терминология*

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The process of soft tissue regeneration after injury is accompanied by activation of various cells: platelets, neutrophils, macrophages, endothelial cells, keratinocytes and fibroblasts, as well as secretion of biologically active substances (growth factors, cytokines, chemokines and others) necessary for coordination of intercellular interactions. This process sequentially involves four coordinated steps: haemostasis, inflammation, repair (proliferation) and remodelling, which are controlled by different body systems (fig. 1) [1]. The regulatory role of immune cells in the development of the initial stages of tissue healing determines the efficiency of subsequent repair and remodelling [1, 2]. Despite significant progress in understanding the cellular and molecular mechanisms of repair, the question remains: «Why is there a tendency for incomplete healing (substitution) and scarring of damaged tissue and not complete regeneration?». The important role of the immune system in the reparative response, including the development and severity of scarring, has been proven on the one hand, and on the other hand, data on the loss of tissue regenerative capacity associated with the evolution of immune competence have been obtained. In clinical practice, the disruption and chronisation of regeneration in traumatic soft tissue defects (trauma, postoperative wounds, infected wounds) represent a serious problem. Understanding the mechanisms of regeneration, in particular the regulatory role of the extracellular matrix over tissue homeostasis, is necessary to develop ways to treat such defects. The relationship between tissue healing and immune response depends on the organ localisation of the process, the period of life of the organism (embryonic, neonatal, postnatal) and can have both negative and positive effects [3]. This review presents the major cellular and molecular mechanisms of the immune response involved in the soft tissue healing process. The nature of the immune response and its duration have a significant influence over the outcome of repair and determine the completeness of the regenerative process – incomplete (scarring or fibrosis) or complete (restitution) recovery.

The action of exogenous etiological factors of mechanical (trauma, wounding), thermal or chemical nature in soft tissues initiates primary alteration and leads to a prolonged period of increasing vascular permeability with necrosis of endothelial cells at the level of arterioles. Haemostasis with clot formation and exudative reaction with release of blood plasma and inflammatory infiltrate cells into the paravasal space are activated in the area of injury. The early transient response of increased vascular permeability is caused by the action of histamine, progesterone, leukotriene E4, serotonin, and bradykinin (fig. 1). One of the leading mediators of delayed and persistent reaction is the slow-reacting substance of anaphylaxis, which includes various leukotrienes, is secreted by mast cells and causes proteolysis of basal microvascular membranes [4]. Vascular dilation with increased endothelial permeability facilitates the migration of monocytes and neutrophils attracted by chemokines, growth factors and cytokines secreted by platelets aggregated in the lesion during haemostatic clot formation [4].

Neutrophils

When tissues are damaged, the cells of innate immunity provide immediate defence against potential pathogens (fig. 1), and even in the absence of pathogens, the immune response, initially triggered by molecular signals from damaged cells, can cause aseptic (sterile) inflammation [5]. Recruitment of neutrophils and the homing of these cells to the focus of injury are provided by pro-inflammatory cytokines, in particular tumour necrosis factor α (TNF-α), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), arachidonic acid derivatives – leukotrienes and prostaglandins, as well as complement components C3a and C5a [2, 6-8]. The process of effective wound healing requires the active participation of neutrophils expressing pattern-recognising receptors for microbe- and pathogen-associated molecules (MAMP/PAMP microbe-/pathogen-associated molecular pattern), as well as damage-associated molecular pattern (DAMP) [2, 6]. Such cells are phagocytised by macrophages via β2 integrins, which induces in them the release of TGF-β_1, which stimulates myofibroblast differentiation, promoting collagen synthesis and reducing the area of damage [9]. The presence of neutrophils at the injured area is generally limited to the phase of active inflammation; their longer presence in physical trauma and/or ongoing infection has a detrimental effect and prevents effective wound healing [2, 10]. Expression of the DAMP and MAMP pattern-recognising receptors by neutrophils in combination with cytokine release further enhances the inflammatory response at the injured area. In this case, the universal nuclear transcription factor «kappa-bi» (NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells), which controls the expression of immune response, apoptosis and cell cycle genes, is activated in neutrophils [11, 12]. The toxic arsenal of neutrophils directed primarily against pathogens, when released as a result of necrosis rather than apoptosis, leads to damage to the extracellular matrix, which affects blood coagulation and other mechanisms involved in wound healing [2, 8, 10, 13]. The negative influence of neutrophils can be manifested in the initiation of secondary soft tissue damage, including reperfusion, which increases the influx of these cells and the formation of persistent inflammation [14]. Another example of undesirable effects of neutrophils is the excessive formation of neutrophil extracellular traps (NET), which is considered as an inhibitor of wound healing in diabetic patients [15]. NET is an effector function of neutrophils with release of bactericidal granule components into the cytoplasm, histone modification, resulting in chromatin decondensation, nuclear envelope and cytoplasmic membrane disruption with the participation of gazdermin D protein. Subsequently, chromatin is ejected outside the cell and a structure is formed of modified nucleus chromatin surrounded by bactericidal granule proteins and cytoplasm. Uncontrolled NET formation is a provocative factor in the development of many inflammatory and autoimmune diseases [15].

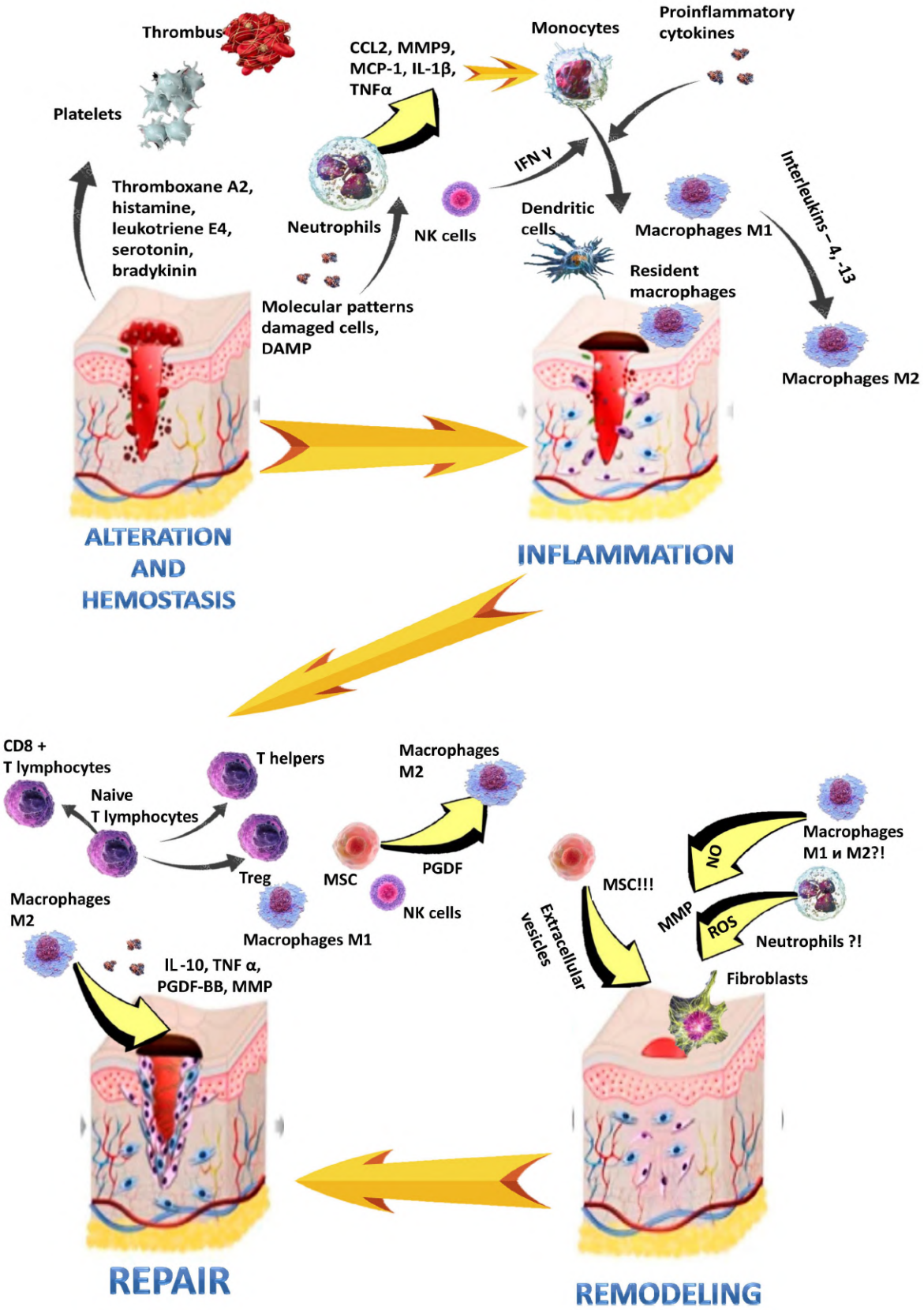

FIG. 1.

Immune microenvironment and intercellular signaling during soft tissue regeneration. Stages of healing including hemostasis, inflammation, repair and remodeling. CCL2 – C-C motif ligand 2; MMP – matrix metalloproteinase; MCP-1 – monocyte chemoattractant protein-1; IL - interleukin; TNF-α - tumour necrosis factor α; IFN-γ - interferon gamma; NK – natural killer; DAMP – damage-associated molecular pattern; Treg – regulatory T-cells; MSC – mesenchymal stem cells; PDGF – platelet-derived growth factor; NO – nitrogen oxide; ROI – reactive oxygen intermediates

The physiological role of neutrophils in wound healing is not only in the clearance of pathogens, but also in the removal of remnants of damaged cells, including red blood cells. In soft tissue repair, neutrophils have no direct effect on collagen synthesis or granulation tissue formation, but their production of cytokines, including TNF-α, can promote re-epithelialisation and wound closure [16]. Generally, the claim that neutrophils have no effect on wound healing through their regulatory effect on the synthesis of connective tissue components is rather controversial. The experimental model of aseptic wounds, for example, revealed that neutrophils take part in inflammation that ends in scarless regeneration [17]. Furthermore, a decrease in the number of neutrophils in this type of soft tissue injury without bacterial involvement correlates with high levels of anti-inflammatory cytokine interleukin (IL) 10, vascular endothelial growth factor (VEGF) and accelerated wound epithelialisation [18, 19]. VEGF secreted by neutrophils stimulates angiogenesis and promotes tissue repair [20]. There are several mechanisms to control the effect of neutrophils on the induction of repair, particularly through the scavenging of free radicals generated by hyperactivated neutrophils by superoxide dismutase-3 (SOD-3) mesenchymal stem cells (MSC) [21]. Additionally, MSCs themselves can slow neutrophil migration through TNF-6 protein gene expression and IL-10 production. Epidermal growth factor in saliva has been known to reduce neutrophil recruitment and activity, which explains the positive effect of licking wounds in animals [22]. The positive effect of neutrophils on wound healing is also their effect on cell hyperproliferation and prevention of malignisation [23]. A pronounced soft tissue inflammatory response with the presence of neutrophils that neutralise bacteria may be crucial for the control of the commensal microbiota and subsequent epitheliocyte proliferation [17]. The involvement of the powerful oxidative potential of neutrophils (production of reactive oxygen intermediates (ROIs)) is also important: in addition to their bactericidal effect, they additionally supply oxygen to proliferating cells [24]. Neutrophils support monocyte chemoattractant protein-1 (MCP-1) and chemokine ligand 3 (CCL3, C-C motif ligand 3) synthesis-mediated additional recruitment of macrophages and T lymphocytes to the injury focus [25]. During wound infection, neutrophils through the release of carboanhydrase, elimination of DAMP and MAMP alter the microenvironment, which also promotes healing processes [6]. After bacteria and necrotic tissue are removed, neutrophils undergo apoptosis or necrosis and are engulfed by macrophages via efferocytosis [26, 27]. Some neutrophils leave the injured area and return to the circulatory system by reverse migration. If neutrophils are not eliminated from the injured area, secondary necrosis develops with the release of pro-inflammatory and cytotoxic molecules. Therefore, the number and activity of these cells requires strict regulation, which is a challenge, especially in the chronic course of severe trauma.

Recently, another mechanism of neutrophil involvement in the resolution of inflammation during tissue injury has been actively discussed [2, 28]. The response in the form of migration to the inflammation location in these cells is often switched from exploratory patrolling to coordinated formation of dense clusters, so-called «swarming», which further disrupts the architecture of the surrounding tissue [28]. The response in the form of cell aggregation (their self-organisation) occurs as a result of signal transduction by paracrine chemoattractants of neutrophils themselves and primarily the inflammatory mediator leukotriene B4 [LTB4]. The mechanism of neutrophil swarm coordination is triggered in part by sustained calcium flux from necrotic tissue, which requires perception of a damage signal involving adenosine triphosphate (ATP). This «calcium alarm» signal propagates rapidly in the nascent neutrophil cluster in a contact-dependent manner via connexin-43 (Cx43, connexin 43) half-channels, which mediate the active release of ATP molecules. As a consequence, the biosynthesis of chemoattractants in the growing cluster is enhanced, which promotes coordinated cell movement and swarming. The regulatory mechanisms of swarm growth limitation are implemented with the participation of ROIs and possibly through modulation of ion channel activity [29]. There may also be similarities with neutrophil extracellular traps, the formation of which is enhanced by ATP involvement and varies with cell population density [30]. Similarly, with respect to the resolution phase, it is not clear whether monocytes or macrophages can resolve the recruitment cascade into neutrophil swarms.

Macrophages

Infiltration of the injury focus by neutrophils and monocytes occurs in response to the appearance of chemoattractants, namely protein fragments of extracellular matrix (MCP-1) and inflammatory chemokines (CXCL8, CCL2, CCL3, CCL4, CCL5, CCL11, CXCL10). Monocytes differentiate into active macrophages, which in turn synthesise cytokines and chemokines, causing the subsequent mobilisation and recruitment of lymphocytes into the wound bed with the development of further intercellular communication (fig. 1) [1]. In the meantime, resident macrophages near capillaries in the focus of injury recognize signals of extracellular matrix and start to express purine-producing receptors on their surface, which promote the attraction of other cells [31]. Based on their different roles in the healing process, macrophages can be roughly divided into two types: inflammatory (type M1), tissue utilising and remodelling (type M2) [32, 33]. M1-type cells produce pro-inflammatory cytokines, have high phagocytic activity, can engulf apoptotically altered neutrophils and remove pathogens and dead cells, whereas M2-type cells have anti-inflammatory effects

and regulate angiogenesis, fibroblast regeneration, myofibroblast differentiation and collagen production [33]. The polarisation of macrophages into the M1 phenotype is influenced by interferon γ (IFN-γ), interleukins (IL-2, IL-3, IL-12), TNF-α, as well as by lipopolysaccharides and agonists of Toll-like receptors (TLR) [33, 34]. These cells secrete pro-inflammatory interleukins (IL-1β, IL-6, IL-12, IL-23), TNF-α, chemokines (C-X-C motif), their ligands (CXCL-9 and CXCL10) and participate in inflammatory responses. Cytokines such as IL-4, IL-10, IL-13, TGF-β and granulocyte-macrophage colony-stimulating factor (GM-CSF) induce polarisation of M2 macrophages, which secrete anti-inflammatory molecules, promoting tissue regeneration [35].

In the early stages of injury, M1 macrophages expressing high levels of receptors for lymphocyte complex antigen 6 (Ly6c) and CC-chemokine 2 (CCR2) are the main activated phenotype in the wound microenvironment. They act predominantly as phagocytes as they remove cell fragments and pathogens and cleanse wounds of detritus. When large numbers of apoptotic neutrophils are phagocytosed, these cells can induce the transition of microenvironment components from inflammatory to proliferative [36-38]. A prospective randomised study concerning the treatment of second-degree deep burns with recombinant GM-CSF revealed that an increase in the number of tissue macrophages is associated with an enhanced local immune response and accelerated healing [39]. Although M1 macrophages have strong antibacterial activity, their persistence at the wound area may result in the secretion of matrix metalloproteinase 9 (MMP9) and tissue damage [40]. For this reason, timely polarisation of macrophages of the M1 phenotype into M2 is very important for accelerating the wound healing process; it takes place with the participation of T-helper type 2 (Th2) cytokines, apoptotic cells and with the synergistic effects of nucleotides and extracellular matrix components [41].

New data regarding the mechanism of M1 macrophage community formation in the wound bed are of interest. Swarming of these nitric oxide (NO)-producing cells may help prevent neutrophil accumulation by blocking cellular respiration and reducing the ATP:ADP (adenosine diphosphate) ratio [42]. The coordination of neutrophils resulting in their «swarming» in wounds (mentioned above) is protective, as it allows the formation of «plugs» or physically isolates sterile tissue from potential microbial invasion [43]. A community of tissue macrophages in «swarming» can actively «mask» small tissue lesions and prevent the leakage of danger signals, such as ATP in particular, to deter further tissue damage by clusters of neutrophils [44]. «Community»-type (quorum) reactions of neutrophils and macrophages reflect the group behaviour of cells and the synchronised response of the mammalian immune system to tissue damage.

Conversely, M2 macrophages at the repair stage produce cytokines that promote neutrophil apoptosis and switch from a pro-inflammatory (M1) to anti-inflammatory (M2) phenotype, while phagocytosing the debris of dead cells. The mechanism of switching from one phenotype to another has also been demonstrated to involve the scavenger receptor class B1 and/or under the control of exosomes, with the latter promoting skin wound healing by enhancing angiogenesis, re-epithelialisation and collagen formation [45, 46]. In addition to phagocytic functions, M2 phenotype macrophages participate in the regeneration process by actively synthesising TGF-β and PDGF, which influence the formation of granulation tissue [37]. M2 phenotype macrophages are responsible for the formation of new extracellular matrix with fibroblast activation and blood vessel formation. Activated fibroblasts secrete IL-1 and insulin-like growth factor (IGF-1), which participate in the initiation of the proliferative phase. Thus, macrophages play a crucial role in tissue regeneration through phenotypic polarisation and are involved in almost all its stages.

Mast cells (MS) originate from bone marrow and are located around blood vessels in the dermis, peripheral nerves, sebaceous and sweat glands [47]. They are key effectors of allergic reactions and determine the body's resistance to bacterial invasion. Upon tissue injury, activated MSs release pre- or *de novo* synthesised mediators such as histamine, serotonin, chymotrypsin, elastase and trypsin from secretory granules into the inflammatory microenvironment [47]. Histamine promotes skin wound healing by increasing the expression of basic fibroblast growth factor (bFGF) to attract macrophages and stimulate angiogenesis during inflammation [48]. The combination of trypsin with protease-activated receptors 2 (PAR2) of vascular endothelial cells causes telangiectasia and mediates neutrophil infiltration of the injured area [49]. Inflammatory mediators such as TNF-α, MMP-2 and IL-8 synthesised by MS influence neutrophil recruitment and macrophage activation [50]. The presence of excessive MS, however, can interfere with wound healing. Specifically, in type 2 diabetes mellitus, high expression of the MS receptor to IL-3 causes chronic inflammation of the skin, and prolonged activation of these cells promotes fibrosis [51]. MS also influence the proliferation stage, especially the formation of abundant granulation tissue (e.g., keloids and hypertrophic scars), angiogenesis, stimulate wound re-epithelialisation and the transition from acute to chronic inflammation [47]. It is noteworthy that different microenvironmental stimuli of the injury focus can lead to functional differences in MS, which allows us to distinguish phenotypes of actively producing anti-inflammatory mediators, secreting mediators without degranulation and degranulating mastocytes [52].

In the late stages of healing, growth factors and cytokines synthesised by MS affect the phenotype of fibroblasts, inducing the emergence of myofibroblasts that provide the transition from fibroplasia to contraction and final wound healing [53]. During tissue

remodelling, these cells can activate collagen synthesis by fibroblasts, which may be associated in part with tryptase, which has been demonstrated on human dermal fibroblasts to stimulate type I collagen synthesis [50]. In contrast, MSs produce and release potent proteolytic enzymes such as matrix metalloproteinases, initiating the degradation of the extracellular matrix. It has also been found that inhibition of histamine synthesis by MS reduces the content of hydroxyproline in granulation tissue and delays wound epithelialisation [50, 52].

Dendritic cells (DCs)

When soft tissue, particularly skin, is injured, various DC phenotypes are recruited to the injury focus, including epidermal Langerhans cells (ECs), dermal and plasmacytoid DCs. Dendritic cells attach to neighbouring keratinocytes using adhesion molecules, or E-cadherins; they are capable of self-renewal, and exposure to inflammatory stimuli enhances their migration and proliferation (fig 1) [54]. The formation by DC of long and complex dendritic structures between keratinocytes contributes to their rapid response to tissue damage [55]. Despite the fact that the participation of DCs in the processes of healing and regeneration of soft tissues remains a subject of study, their important role in the process of the foreign substance recognition, modulation of macrophage homeostasis and immunoregulation of regeneration processes has been revealed. In diabetic foot ulcers, healing improves with increasing amounts of DCs, indicating their positive effect over the microenvironment in the inflammatory focus [56]. Significantly accelerated healing of pressure sores in patients with high DC content in the marginal epidermis of the wound when combined with the use of drugs containing zinc [57]. DC subtypes such as $CD141^+$ stimulate CD8+ T-cell immunity by secreting IL-12 and promote differentiation of type 1 T-helper cells [54, 55, 58]. The CD1C-positive DC subtype presents antigens to $CD4^+$ cytotoxic T-cells. Furthermore, resident DCs in the injury focus express Toll-like receptors (TLR-7 and TLR-9) and induce an early inflammatory response [4]; the absence of these cells in the microenvironment negatively affects acute inflammatory responses and delays wound healing [58]. A burn wound model in DC-deficient mice showed a significant delay in the healing process associated with inhibition of early cell proliferation, low levels of TGF-β1 and neoangiogenesis in the wound beds. The essential role of DC in accelerating wound healing has thus been revealed, which is probably related to the secretion of factors that activate cell proliferation. With the development of single cell sequencing, additional opportunities are opening up to study the origin of DCs, their development, and their involvement in reparative regeneration processes in the skin.

NK cells are recruited to the focus of injury and are able to secrete immune response effectors. The key functions of these cells comprise identification of foreign, virus-infected and metabolically altered cells and induction of their apoptosis or lysis [59]. Activated cytotoxic NK cells cause lysis of target cells, including secreting death-inducing cytokines [59]. The interaction between NK cells and MSCs during regeneration has recently become an important area of studies. Undifferentiated MSCs suppress proliferation, cytokine release and cytotoxicity of NK cells, and under appropriate conditions can maintain and enhance their regenerative functions, in particular influencing neoangiogenesis and proliferation [60].

T lymphocytes

A subpopulation of regulatory T-cells (Treg) maintains the body's immune tolerance and inhibits the activity of potentially autoreactive T-cells, modulating the immune response and preventing autoimmune diseases. These cells also have many non-immune functions, including regulation of stem and progenitor cell activity. Tregs appear to be key cells in tissue repair and regeneration (fig. 1). It was revealed that the increased content of IL-33 synthesised by Treg influences the regulation of differentiation of fibroblast and adipocyte progenitor cells, and the decreased content of this cytokine is the main cause of unsuccessful tissue regeneration in ageing mice [61]. The presence of Treg cells in soft tissues is associated with increased regeneration rate, increased immune tolerance and induction of proliferation of resident T-cells in tissues through increased amphiregulin synthesis, resulting in an immunosuppressive microenvironment [62]. Stimulation of regeneration by Tγδ cells also through association with stem cells is not excluded [63]. Their participation in wound healing was demonstrated on the basis of the dynamics of the content of regeneration activators synthesised by them. [64]. Other types of T-cells, such as cytotoxic ($CD8^+$) and T-helper ($CD4^+$) cells, are also important activators of soft tissue regeneration. The peak content of CD4- and CD8-positive T-cells in the wound bed of the skin on days 5–10 and 7–10 after injury has been revealed and it has been demonstrated that these cells may play different regulatory roles [65]. T-helpers accompany the enhancement of regenerative processes in wounds, while cytotoxic T-lymphocytes are associated with impaired healing [66]. T-cells mediate regulation of the regeneration process through the release of a wide range of cytokines affecting both macrophages and fibroblasts.

Stem cells (MSC)

MSCs are also involved in the soft tissue regeneration process, affecting the wound microenvironment and regulating immune-inflammatory responses (fig. 1). At the moment, stem cell therapy is of particular interest in regenerative medicine [67]. MSC are trophoblasts that are found in virtually all tissues of the body to maintain a pool of many cell types including haematopoietic, epithelial, tumour, nerve, hepatocytes and endothelial cells. The unique features of MSC

include the ability to self-renew with asymmetric division and multidirectional differentiation and determine the modulation of tissue metabolism and regeneration, including through interaction with immune cells. While over-reactivity of innate immune cells during inflammation impairs tissue regeneration, MSCs fulfil an immunomodulatory role by producing various regulatory cytokines such as interleukins (IL-4, IL-7, IL-10), IFN-γ and prostaglandin E2 (PGE2) [68].

Signal transduction involving molecular mediators. One of the most important intercellular signals includes the regulation of stem cell activity during soft tissue regeneration using the N-terminal Janus kinase c-Jun (JNK) [69]. JNK mediates intracellular stem cell responses to stimuli from the extracellular microenvironment. JNK function is required to achieve a delicate balance between stem cell death and survival and promotes soft tissue repair and remodelling. Transplantation of preconditioned stem cells enhances soft tissue regeneration by a balanced antioxidant defence mechanism through activation of JNK signalling [70]. Signal transduction via JNK plays a critical role in the regulation of MSC differentiation into keratinocytes and promotes tissue regeneration.

The phosphatidylinositol-3-kinase, α-serine-threonine and serine-threonine protein kinase signalling pathway (PI3K/Akt/mTOR) is the mammalian target of rapamycin (mTOR) [71]. Phosphorylation of the amino acids tryptophan (Tr308) and serine (Ser473) converts Akt to an activated form that controls a multitude of cellular regulatory processes, including cell survival and cellular metabolism. The extracellular matrix provides protective mechanisms for the induction of stem cell differentiation by aberrant mTOR activation. Strategies targeting indirect mTOR activation can be used to enhance epithelial cell migration into damaged areas and accelerate soft tissue regeneration.

In soft tissue homeostasis and regeneration, Wnt/β-catenin, a signalling pathway that is involved in the regulation of tissue homeostasis by controlling cell proliferation, differentiation and apoptosis, has attracted the attention of researchers in recent decades. This extracellular signal transduction pathway has been implicated in the regulation of stem cell function and tissue repair, as well as in the progression of chronic inflammatory diseases [72]. Within the nucleus, β-catenin binds to T-cell transcription factor enhancers, thereby promoting transcription of specific genes and specific transduction of Wnt/β-catenin. Activation of the β-catenin-dependent pathway enhances the proliferation and function of epithelial and mesenchymal stem cells, promoting soft tissue regeneration [73]. Selective enhancement of Wnt/β-catenin signal transduction may be an effective strategy for the induction of soft tissue regeneration. Furthermore, from a therapeutic point of view, the role of nuclear factor erythroid 2-related factor-2 associated protein (Nrf2), which is a major mediator of redox homeostasis, has been discussed in soft tissue regeneration. This factor is expressed in a wide range of cells including stem cells, endothelial cells and fibroblasts. In damaged tissues, excess ROIs suppresses stem cell proliferation, stimulates apoptosis and impairs regeneration [74]. The activation of the antioxidant defence system in keratinocytes shows the important role of the factor Nrf2 in preventing the accumulation of ROIs; in its deficiency, epithelial wound healing is prolonged. While this evidence suggests an important role for Nrf2 signal transduction during soft tissue repair and regeneration, more studies are required to understand the function of Nrf2 in this process.

Extracellular vesicles in the immunomodulation of tissue regeneration

Regeneration after injury requires two main conditions: the formation of a pro-inflammatory microenvironment to neutralise the injury and remove necrotised tissue and an anti-inflammatory microenvironment, with conditions for tissue regeneration through migration, proliferation and differentiation of different cell types, increased vascularisation and nutrient supply. A correlation between the increase in the number of small extracellular vesicles (sEVs), which contain various bioactive molecules, including cytokines, lipids and nucleic acids and exert paracrine effects, and the activity of cell proliferation and migration processes in the damaged tissue has been evidenced [75]. Nanoscale extracellular vesicles are involved in the regulation of intercellular communications during microenvironment formation and have attracted the attention of researchers as a promising cell-free therapeutic strategy. The MSC therapeutic activity is provided by the production of extracellular vesicles, influence on the proliferation and functional activity of cells of the microenvironment, creation of a favourable immune microenvironment in the focus of damage and enhancement of tissue regeneration [75, 76]. A study in a collagen-induced arthritis model revealed that sEVs MSC effectively inhibited IL-17A and stimulated IL-10, reducing the frequency and intensity of bone erosions, and may be a promising new cell-free therapy strategy in the treatment of rheumatoid arthritis. Small extracellular vesicles derived from human bone marrow-derived MSCs significantly reduce the expression of pro-inflammatory genes.

CONCLUSION

Tissue damage and regeneration depend on the severity of the body's defence response involving cells of innate immunity and their active components, which is crucial for successful recovery. Spatial and temporal regulation of the functional state of immune cells involving the extracellular matrix and tissue-specific cells, including stem cells, is essential for the successful outcome of tissue regeneration.

A thorough understanding of immunomodulatory and pro-regenerative activators and their multiple functions is crucial, in particular for their successful application as therapeutic agents in developing strategies to stimulate tissue regeneration. Additional data are required for such developments, for example, if we consider macrophages of the M2 phenotype as a target, excessive activation and infiltration of the lesion focus by these cells do not contribute to tissue resistance to foreign pathogens and may impair the process of tissue healing. The mechanisms underlying this dual function are not well understood. Other immune cells, such as DC subpopulations, which have different effects on tissue regeneration depending on their functional state, or T lymphocyte populations, have similar effects. Cytotoxic $CD8^+$ T-cells have unfavourable effects towards tissue regeneration, whereas T-helpers ($CD4^+$) and Treg, to the contrary, enhance this process. The use of research technologies to study the mechanisms of coordination and functioning of various subpopulations of immune cells, in particular, sequencing of individual cells, will allow to clarify the degree of their participation in the mechanism of regulation of the microenvironment during tissue regeneration. Since the functioning of the immune system gradually decreases with age, there is a need to investigate in the elderly the relationship between the decreased ability of soft tissues to regenerate and the characteristics of their defence response. Such data may have implications for improving tissue repair with the involvement of targeted therapies.

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Conflict of interest

The authors of this article declare no conflicts of interest.

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Information about the authors

Natalia G. Plekhova – Dr. Sc. (Biol.), Head of the Central Research Laboratory, Pacific State Medical University, e-mail: pl_nat@hotmail.com, https://orcid.org/0000-0002-8701-7213

Dmitry V. Korolev – Cand. Sc. (Med.), Research Assistant at the Department of Hybrid Educational Technologies in Surgery, Institute of Simulation Certification, Pacific State Medical University, e-mail: doc.korolevdv@mail.ru, https://orcid.org/0000-0003-3789-6632.

Valentin B. Shumatov – Dr. Sc. (Med.), Professor, Rector, Pacific State Medical University, e-mail: mail@tgmu.ru, https://orcid.org/0000-0003-3836-390X