The role of myofibroblasts in the healing of chronic wounds

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Introduction
In recent years, there has been an increase in the number of patients with chronic purulent-necrotic wounds, due to the formation of antibiotic-resistant strains of microorganisms and the preservation of a significant number of wound complications [7, 15]. The treatment of chronic wounds is a significant burden on the health care system - both in the intensity of treatment and in its cost [6]. A significant amount of work has been devoted to the study of the mechanisms of regenerative processes in chronic wounds [1, 2, 3]. However, the role of cellular regulation in the pathogenesis of the restoration of the morphofunctional state of a chronic wound in terms of its damage remains undisclosed [2, 3]. Today, the therapeutic potential of different types of stem cells is being discussed, but in terms of therapy, the most effective cell population has not yet been determined. In recent years, experimental material has been accumulating that shows the ability of mesenchymal stem cells (MSC) to produce anti-inflammatory factors, growth factors that increase the proliferative activity of epithelioctyes and fibroblasts [7]. The role of myofibroblasts in the healing of chronic purulent-necrotic wounds has not been fully elucidated.
The aim of the study was to evaluate the role of myofibroblasts in the healing of chronic purulent-necrotic wounds in the treatment of mesenchymal stem cells using the immunohistochemical method.

Materials and methods
On the basis of the veterinary clinic (vivarium) National Pirogov Memorial Medical University, Vinnytsya performed an experimental study on 120 rats. All animals were divided into the following groups: I - control (without treatment); II - the use of classical wound healing (decasan); III - the use of clones of mesenchymal stem cells (from the umbilical cord); IV - the use of mesenchymal stem cell clones (cloned in inert gases). The study was performed in accordance with international conventions on the protection of animals used for experimental and other scientific purposes (Strasbourg, 1985), as well as in accordance with the provisions of the Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsya (Minutes № 6 of 20.06.2019).

To assess morphological changes in chronic wounds, fragments measuring 0.5 cm²1.0 cm²1.0 cm were excised from the wound edges, followed by fixation in 10 % neutral formalin solution. The samples were prepared according to standard methods. Histological sections 5-7 μm thick were stained with hematoxylin and eosin, Mason's trichrome [5, 16]. Microscopy and photographing of histological specimens were performed using a light microscope OLIMPUS BX 41 at magnifications of 40, 100, 200 and 400 times. Microscopy assessed the condition and cell composition of chronic wounds, the presence and nature of reparative changes. Images were obtained and processed, morphometry and statistical processing were performed using the program “Quick PHOTO MICRO 2.3”.

Immunohistochemical study was performed using paraffin blocks and DAKO reagents with monoclonal antibodies markers of intermediate filaments, mesenchymal cells and myofibroblasts - vimentin (Clone V9) and smooth muscle actin (αSMA, Clone 1A4), transmembrane protein of endothelial cells, stem and embryonic fibroblasts - CD34 (Clone QBEnd 10) with visualization system En VisionTMFLEX. The results of the study were evaluated taking into account the distribution of expression of vimentin, αSMA and CD34 in cells, the intensity of the reaction and the nature of the interaction with other structural elements. Evaluation of the immunohistochemical response was performed in 10 fields of view at 200 and 400-fold magnification. The intensity of expression was assessed by a semi-quantitative method based on the severity and integrity of the color of the cytoplasm according to the following scheme: low, moderate and strong, given the localization of pathological changes. Pieces of wounds obtained from experimental animals without corrective therapy were used as controls. To quantify vimentin, smooth muscle actin and endothelial cell marker CD34, a semi-quantitative method was used, according to which 4 categories were distinguished: 0 (-) - negative reaction (staining <5 % of cells), 1 (+) - weak staining (positively stained 10-30 % of cells), 2 (+++) - moderate reaction (most positively stained cells - 31-60 %) and 3 (++++) - intense staining (>60 % of cells or almost all cells are positively stained). The expression coefficient (EX) was calculated for each observation according to the formula: KE=Σ(i x v)/100, where i is the intensity of staining in points (0 to 3), v is the percentage of stained cells (0 to 100 % of the most expressed by the reaction in 10 fields of view at ?400) for each value [9].

Statistical processing of morphometric parameters was performed using the standard software package “Statistica 6.1” from StatSoft (SRC National Pirogov Memorial Medical University, Vinnytsya, licensed № BXXR901E246022FA) and Excel. Differences between samples were determined using Student's t-test, and the mean values for each trait and their standard deviations were determined. The level of p<0.05 was determined as probable in all tests.

Results
On the 28th day of the experiment we received chronic purulent-necrotic wounds in 97.0 % of experimental animals, which were characterized by classic signs of chronic progressive inflammation: the presence of a large area of fibrinoid necrosis with admixtures of polymorphonuclear leukocytes, macrophages, lymphocytes, lymphocytes and lymphocytes. At the same time both in the central departments (bottom of a wound), and in edges the multilayered flat keratinized epithelium was practically not defined due to considerable necrotic changes of epithelocytes.

In the groups using decasan, MSC and MSC-IG on the third day of treatment (31 day of observation) around the wound edges and in the bottom recorded the appearance of a narrow layer of poorly differentiated cells (Fig. 1), the number of which differed significantly from the control group.

Fig. 1. Layers of vimentin-positive poorly differentiated fibroblasts at the bottom of a chronic wound. MSC-IG, 3 day of treatment (31 day of observation). Immunohistochemical (IHC) reaction with vimentin, x200.
confirmed by immunohistochemical research (Table 1). As can be seen from Table 1, in decasan, MSC, and MSC-IG groups significantly increased the number of αSMA-positive myofibroblasts, CD34-expressing endothelial cells, and mesenchymal cells (vimentin-positive fibroblasts), especially in the MSC-IG group (p<0.001).

The bottom of the wound at this observation period consisted in all experimental groups of three distinct layers: leukocyte-necrotic layer, a narrow surface layer containing vascular loops, and a deeper layer containing vertical vessels with surrounding cell clusters in the form of horizontally located and an amorphous intermediate. In longitudinal sections, the layer of horizontal fibroblasts was manifested by the concentration of cellular elements of the fibroblastic series: immature fibroblasts, myofibroblasts, fibroblasts that had an approximate course perpendicular to the wound edge. Cross sections of cell nuclei were oval and rod-shaped. Among the latter, slit-like lumens were identified, so they can be defined as endothelial cells that formed the walls of newly formed vessels in the immediate vicinity of the wound and gave a positive reaction on immunohistochemical labeling on CD34 (Fig. 2).

On day 7 of the experiment, morphological changes in chronic purulent-necrotic wounds of the control group corresponded to previously established, with a slight decrease in the thickness of the purulent-necrotic layer and a large number of randomly arranged fibroblasts, mast cells, macrophages, lymphocytes and plasma cells. A feature of this observation period in the groups with corrective treatment of MSC and MSC-IG was a significant decrease in mononuclear cells despite an increase in the number of cells of mesenchymal origin with active vasculogenesis, which was confirmed by immunohistochemical analysis (Fig. 3, Table 2), in which probable values were obtained (p<0.001) compared with the control and other groups, but the expression of αSMA and CD34 in animals treated with decasan and MSC was not significant (p>0.05) compared with the untreated group.

In the control group at this time of the experiment in micropreparations revealed severe edema, accumulation of lymphocytes, leukocytes, macrophages in the superficial departments and in immature granulation tissue. Histological examination revealed cellular detritus surrounded by a well-defined pyogenic membrane, abundantly infiltrated with leukocytes. At coloring on collagen fibers loosening of fibrous structures with their infiltration by neutrophilic leukocytes, a large number of lymphocytes, macrophages were defined. The fullness of the vessels was preserved with the expansion and thickening of their walls due to edema.

In the groups of decasan, MSC and MSC-IG on the 7th day of the experiment (35 day of observation) edema and vascular hyperemia, compared with the 3rd day, were significantly reduced. Coagulation necrosis in the center of the wound becomes homogeneous, eosinophilic, homogeneous, as a result of fluid loss and drying, cracks

**Fig. 2.** Endothelial cells that form the walls of newly formed blood vessels. MSC-IG, 3 days after treatment (31 day of observation). IHC reaction with CD34, x200.

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**Fig. 3.** Proliferation of myofibroblasts. MSC-IG, 7 days after treatment (35 day of observation). IHC reaction with smooth muscle actin (αSMA), x200.
appear in it, sometimes covered with endothelium. In a zone of a necrosis vessels with segmentally destroyed walls and agglutinin erythrocytes and thrombi with signs of the initial organization are found. On the periphery of the necrotized areas there are a large number of macrophages in the form of “granular balls”, in the area of resorption of foci of hemorrhage - many siderophages and newly formed vessels of the microcirculatory tract. On the seventh day of the experiment (35 day of observation) according to our data in the control group with chronic progressive purulent-necrotic wounds in comparison with animals receiving decasan, MSC and MSC-IG there was no replacement of granulation tissue (vascular loops) with fibrous (horizontal fibrosis), in addition, we recorded a large number of plasma cells, which together, in our opinion, supports the chronicity of the inflammatory process.

On the 14th day of the experiment, histological analysis of the skin in the wound area showed that the first experimental group retained the same signs of exacerbation of chronic inflammation, such as infiltration of neutrophils and necrosis. The cell composition has undergone significant changes. In the groups MSC-IG and MSC on day 14 in 80 % and 70 % of experimental animals was almost complete cleansing of wounds from purulent-necrotic detritus and the formation of connective tissue scar with epithelialization, with similar changes in these terms were found in the control group only 10 % of rats and 60 % with decasan.

After treatment with decasan, MSC and MSC-IG observed attenuation of dystrophic changes in collagen fibers. However, in the MSC-IG group, a significant decrease in both dystrophic changes in smooth myocytes and an increase in the number of smooth muscle elements was observed at this observation period, which was confirmed by immunohistochemical data using SMA (Fig. 4). In addition, at this time, the expression markers of myofibroblasts, endothelial and mesenchymal cells were significantly higher in the MSC and MSC-IG groups compared to the control (p<0.001), except for CD34 in the decasan group, whose enhancement of labeling was insignificant. The most effective immunohistochemical parameters were found in experimental animals treated with MSC-IG (Table 3).

Thus, for 2 weeks in the chronic wounds studied by us, further replacement of granulations with fibrous tissue continues, which is as follows: 1) by gradually replacing from the depth to the surface of the deep layers of granulations a strong layer of horizontal fibroblasts with the simultaneous development of collagen fibers, 2) by the development in the granulation tissue of thin vertical collagen strands (in the layer of vertical vessels), 3) by forming a superficial, weakly expressed horizontal layer of collagen fibers on the border with the leukocyte-necrotic layer. These processes are simultaneous, but the main role in the replacement of granulations and wound healing belongs to the first of them.

Thus, on the 14th day of the experiment in the group of MSC and MSC-IG animals, the process of vascular formation actively took place in the immediate vicinity of the

<table>
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<th>Table 2. Intensity of αSMA, CD34 and vimentin expression in the treatment of chronic purulent-necrotic wounds (at the rate of 0.1 mm²) on the 7 day of the experiment.</th>
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<td>CD34</td>
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<td>Vimentin</td>
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**Fig. 4.** Increased number of myofibroblasts around blood vessels, 14 days after treatment (MSC-IG, 42 day of observation). IHC reaction with smooth muscle actin (αSMA), x200.

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wound bottom by forming microvessels with narrow lumen, maturation and differentiation of endothelial cells and fibroblastic cells.

On the 30 day of the experiment (58 day of observation) in the control group in 70 % of rats, the epithelium that grew on the edge of the chronic wound often necrotized and was permeated with leukocytes. However, epithelial growths in the granulation tissue, sometimes quite deep, persisted and, apparently, could be a source of new, re-growth of epithelium on the wound surface. In some wounds, the epithelium covers the granulation tissue and at the same time grows into it in the form of "tongues" in the shape of a wedge. Usually such pictures were observed at sharply expressed inflammatory process - in superficial layers of granulation fabric. Dystrophic changes and formation of an insignificant polymorphic capillary network were determined in the surrounding tissue.

Thus, on the 30th day of the experiment in 70 % of experimental animals in the control group there was no epithelialization of the wound, replacement of granulation tissue with fibrous, which indicated the progression of purulent inflammation and prolongation of the first phase of wound healing. In the days and edges of the chronic wound of rats of the control group was determined young connective tissue, which contained numerous cellular elements: macrophages, fibroblasts, lympho- and plasma cells, a small number of neutrophilic granulocytes. Some of the blood vessels found here were dilated. Stasis was often observed in the venules.

In contrast to the first group in experimental animals with the use of decasan, MSC and MSC-IG, the inflammatory cellular response was virtually absent, which was confirmed by quantitative analysis of cellular elements in the area of chronic wound. When comparing the activity of fibrillogenesis, it should be noted that in the group with MSC-IG fibroblastic reaction was stabilized for 30 days compared to previous dates. In the control group, delayed maturation of connective tissue was noted, as evidenced by the remnants of granulation tissue in the wound area. Compared with the control group with the use of decasan, MSC and MSC-IG granulation tissue at this time of observation was almost non-existent, which indicates an earlier period of recovery and stabilization of the wound process.

The results of immunohistochemical analysis (Table 4) confirmed the morphological data and showed a significant predominance of αSMA- and CD34-positive cells in the groups of decasan, MSC and MSC-IG (p<0.05 and p<0.001, respectively), despite insignificant increase in vimentin-positive cells in the decasan group (p<0.05), it should be noted that the animals injected with MSC-IG obtained the best results.

Therefore, in the dynamics in the groups with corrective treatment with decasan, MSC and MSC-IG with the progressive thickening of the deep layer of horizontal fibroblasts and the fibrous layer, the relationship between the layers of granulation tissue gradually changes. At the end of the 2nd month, in some wounds, the layer of horizontal fibroblasts and the actual fibrous layer is several times thicker than the layer of vertical vessels, which gradually thinned. These changes in the thickness of individual layers of granulation are more pronounced at the edges of the wound, where the layer of horizontal fibroblasts, gradually increasing, reaches in places the surface of the wound; here the growing epithelium lies not on the layer of loose granulation tissue, as observed in earlier terms, but on the layer of fibroblasts horizontally located to the epithelium, where the formation of the papillary layer of the skin is already visible.

Thus, at a later term of wound healing (from the 1st month and later) in the granulation tissue the following changes occur: the layer of vertical vessels gradually thins; the amount of amorphous intermediate in it decreases; at the same time the number of fibroblasts increases not only near the vessels, but also in between. Accelerating the development of the fibroblastic stage with the introduction of MSC and MSC+IG into the wound is important both for earlier restoration of the structure and function of damaged tissues and to prevent late wound complications, as failure to regenerate or fibrosis prolongs inflammation or causes its chronic course. In addition, the use of MSC and MSC+IG in the treatment of chronic wounds promotes accelerated epithelialization, according to our data, it has already begun on day 3 and was quite intense on day 7 (35 day of observation).

Discussion
Our research results are comparable with the literature on the stages of changes in the cells involved in the inflammatory process. According to modern ideas, the wound process is divided into 3 phases: inflammation, regeneration and reorganization of the scar with epithelialization [8, 12]. In the inflammatory phase, vascular reactions that characterize the mechanism of inflammation.

### Table 4. Intensity of αSMA, CD34 and vimentin expression in the treatment of chronic purulent-necrotic wounds (at the rate of 0.1 mm²) on the 30 day of the experiment.

<table>
<thead>
<tr>
<th>Cellular composition</th>
<th>Control</th>
<th>Decasan</th>
<th>MSC</th>
<th>MSC-IG</th>
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<tbody>
<tr>
<td>αSMA</td>
<td>0.614±0.050</td>
<td>0.768±0.023†</td>
<td>0.782±0.064†</td>
<td>0.910±0.031*</td>
</tr>
<tr>
<td>CD34</td>
<td>0.231±0.012</td>
<td>0.412±0.060†</td>
<td>0.370±0.050†</td>
<td>0.408±0.056†</td>
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<tr>
<td>Vimentin</td>
<td>1.364±0.105</td>
<td>1.515±0.110^</td>
<td>1.790±0.071†</td>
<td>1.850±0.092†</td>
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predominate, and then the wound is cleaned of dead tissue. The second phase involves the formation of granulation tissue. Scar reorganization and epithelialization are the main components of the final stage of the wound process [13]. Regarding the formation of connective tissue in wound healing, we observe certain general patterns of its development, so first in response to damage there is an inflammatory reaction with cytokine synthesis and stimulation of fibroblasts to proliferate with secretion of collagen, glycosaminoglycans and extracellular fibrillogenesis. Then two important processes occur simultaneously: wound contraction and connective tissue remodeling with resorption of excess fibrils. The final stage can be characterized by three main processes: 1) complete involvment of connective tissue; 2) stabilization of the scar; 3) chronicity of the process and progression of fibrosis. Undoubtedly, the leading role in all the above processes belongs to the fibroblast, as the main cellular element of connective tissue. Fibroblast produces proteoglycans, fibronectin, participates in the metabolism and structural stability of collagen, reticular and elastic fibers, their interaction with epithelial differon. Immature (stem cells, poorly differentiated and young) fibroblasts and mature forms (myofibroblast, actively synthesizing fibroblast, fibroblast and fibrocyte) are usually distinguished. Mature fibroblasts maintain homeostasis between the extracellular matrix and cells, performing synthetic, resorbing and regulatory functions in connective tissue remodeling. Myofibroblasts are stellate cells with an active nucleus. The granular endoplasmic reticulum and the Golgi complex are developed in the cytoplasm. A unique feature is the degree of development and organization of the cytoskeleton, represented by bundles of parallel microfilaments. In their composition, along with cytoplasmic β-actin, there is also α-SMA, the amount of which is directly proportional to the local level of transforming growth factor beta (TGFβ) [4, 13]. In response to transforming growth factor β (TGFβ), Wnt ligands, biomechanical and profibrotic signals, fibroblasts can acquire a myofibroblast phenotype with marked expression of smooth muscle α-actin fibers (α-SMA). Myofibroblasts can also arise from bone marrow progenitor cells or through epithelial-mesenchymal junction (EMT) and endothelial-mesenchymal junction (endoMT) [13].

Prolonged inflammatory process, which we observed in the first group of observations, led to changes in the maturation of granulation tissue with inhibition of wound epithelialization, indicating a violation of epithelial-mesenchymal interactions. Also in our study, myofibroblasts were less common in the control group at a later date, which slowed wound contraction. However, in the decasan, MSC, and MSC-IG groups, there was a significant increase in αSMA-positive myofibroblasts, CD34-expressing endothelial cells, and mesenchymal cells (vimentin-positive fibroblasts), especially in the MSC-1 group (p<0.001). In the control group, the fibroblastic response was inhibited by prolongation and exacerbation of chronic inflammation with a significant decrease in the number of myofibroblasts in contrast to the groups using decasan, MSC and MSC-IG where the development of fibroblastic stage of the wound process was observed in most animals. The reduction of the purulent component established by us on the 3rd day of observation was more pronounced with the use of decasan. We found on the 7th day a significant increase in the number of fibroblasts after treatment of MSC-IG in the chronic wound indicates that the introduction of MSC-IG, provides a faster transition to the fibroblastic stage of wound inflammation. In the control group it was found that on the seventh day of the experiment the number of neutrophils was 2 times (p<0.05) higher than with decasan, MSC and MSC-IG, while the number of fibroblasts was significantly higher when using MSC-IG (208±3.9). At the same time, in the early observation period (3-7 days) the fibroblastic response was significantly (p<0.05) more active in the group with MSC and MSC-IG, which is consistent with the data of other authors [10, 11].

One of the essential signs of restoration of organ function is the formation of a vascular bed in the damaged area, so the rate of vascular formation in granulation tissue is one of the criteria for assessing the positive properties of the drug [17]. As is known [17, 18], vasculogenesis is associated with progenitor endothelial cells, which are precursors of endothelial cells, and which are well identified by the CD34 marker in the form of single cells, their small clusters and chains. In the groups with corrective therapy with decasan, MSC and MSC-IG, we were able to see the positive dynamics of vascular formation from progenitor endotheliocytes (vasculogenesis) from the proliferation of these cells to the formation of small spaces among them, which lengthen, forming a network of future vessels. Endothelial cells with membrane-cytoplasmic staining CD34 with the formation of capillary-type vessels were well visualized in the stromal component of the wound bottom, where they found chaotically located vessels of small caliber with a significant predominance of capillaries. We obtained reliable data on the predominance of CD34-positive cells in the group with MSC-IG (0.730±0.074, p<0.001) compared with the control group, which is consistent with the results of other researchers [4, 6, 18]. According to the literature [12], the growth of epithelium on the wound surface begins in the first stage of healing, but in our study it should be noted that it occurred very slowly in the control group. New collagen fibers appear in chronic wounds on day 7, their number increases rapidly, reaching a maximum of 7-14 days, and then the intensity of synthesis decreases to 30 days. Collagen, after its synthesis by fibroblasts, binds in the extracellular substance into bundles of fibrils and bundles of fibers, gradually filling the entire space between cells. The involution of the connective tissue begins and the wound process passes into the third phase - the phase of scarring and reorganization of the scar, which in the uncomplicated
course of the wound process usually takes 10-12 to 20 days. Fibroblasts in the conditions of physiological and pathological involution of connective tissue can function as fibroblasts, participating in the resorption of collagen fibers [8, 12]. The changing balance between collagen synthesis and its destruction underlies the mechanism of wound healing [12]. In the remodeling phase, collagen degradation increases and synthesis decreases, which was confirmed by our data with the use of MSC-IG and is consistent with other authors [7, 8, 12]. By synthesizing collagen, elastin, glycoproteins and proteoglycans, fibroblasts provide the support-mechanical function of the skin, producing signaling molecules that affect vascular permeability and metabolism, perform trophic function [4, 12, 14]. According to the results of our study on the 14th day of the experiment in the granulation tissue with the use of MSC and MSC-IG was less coarse connective tissue fibers, stained according to Mason in blue than in the group using only decasan. Microscopic examination of the wound canal of the dermis with the use of MSC showed faster dynamics of purification from the purulent process. The fibroblastic reaction with the use of decasan and MSC in the process of wound healing is quite pronounced, but with the use of decasan there was a tendency to form a coarser connective tissue scar. The tissue sections obtained for the study were usually well-developed granulation tissue with an increased number of microvessels compared to the first group and a decrease in the number of neutrophils, macrophages, lymphocytes, plasma cells and mast cells. The ability of MSC to stimulate fibroblast proliferation has been repeatedly confirmed experimentally [10, 12, 14]. Macrophage-fibroblastic interaction leads to migration and accelerated proliferation of fibroblasts, their differentiation, synthesis and secretion of collagen and other components of the matrix, active fibrillogenesis. In our study, we also observed stimulation of decasan, MSC and MSC-IG fibroblast activity and vascular formation in young connective tissue, which improved wound oxygenation and accelerated connective tissue maturation followed by remodeling and epithelialization of chronic purulent necrotic necrosis. In the control group there was a delay in the inflammatory response in the monocyte-macrophage stage, which not only increases the risk of purulent complications, but also prevents the completion of fibrosis and, consequently, the restoration of wound scar strength.

Morphometric studies showed that on the 30th day of the experiment (58 days of observation) the number of neutrophils, plasma cells, lymphocytes and macrophages in the control group numerically exceeds similar rates of inflammatory response in the groups of decasan, MSC and MSC-IG. A similar tissue reaction was also characteristic of degranulating mast cells involved in the release of inflammatory mediators, which indicated the progression of the chronic process in the wound. In the control group, the young connective tissue between the stratified fragments of capillaries contained mainly amorphous substance and cellular elements. The number of fibers was small, they had a different direction. While in the groups with corrective treatment, they were already oriented parallel to the wound surface. From the second month of the experiment, the number of collagen fibers around the fibroblasts forming vertical compact bundles in the intermediate substance increased. Thus, in the late stages of wound healing in the granulation tissue creates a fairly correct arrangement of fibrous structures, vertical and horizontal, intersecting in mutually perpendicular directions. However, special importance in wound healing (contraction), of course, belongs to the more developed deep layers of horizontal fibroblasts and fibrous fibers. It is these elements that replace the disappearing granulation tissue and in the late stages of healing perform and tighten to a greater or lesser extent the wound defect. The importance of horizontal structures and their role in wound healing is also recognized by other researchers [10, 12]. In the chronic wounds of the control group studied by us, despite the long time, after simulation of chronic purulent-necrotic wound (up to 2 months or more), complete closure and epithelialization was not observed, despite the growth of fibrous fibers.

Thus, pathomorphological studies indicate that the use of decasan, MSC and MSC-IG in the treatment of wounds leads to rapid suppression of purulent inflammation, edema and accelerates the processes of reparative regeneration. Granular tissue remodeling is faster with MSC and MSC-IG, and myofibroblasts, which in our study were positively labeled with αSMA, are a key link in wound healing, they not only maintain the homeostasis of the intercellular matrix of the dermis, providing its remodeling and renewal, but also play a significant role in maintaining the physiological state of other layers of the skin. Accelerating the development of fibroblastic stage with the introduction of MSC and MSC-IG into the wound is important for earlier restoration of the structure and function of damaged tissues and to prevent late wound complications, as failure to regenerate or fibrosis prolongs inflammation or causes its chronic course. In addition, the use of MSC and MSC-IG in the treatment of chronic wounds promotes accelerated epithelialization, according to our data it has already begun on day 3 and was quite intense on day 7 (35 days of observation), due to the synthesis of MSC of various growth factors that stimulate proliferation mesenchymal and epithelial differon of skin.

Further studies of the involvement of myofibroblasts in the restructuring of chronic wounds using the latest molecular biological markers in the use of MSC and MSC-IG will allow differentiated pharmacocorrection and increase the effectiveness of treatment of chronic wounds.

**Conclusion**

1. According to our data, in 97.0 % of experimental animals on the 28th day of the experiment morphological changes corresponded to chronic purulent-necrotic
inflammation with the presence of three main phases of the wound process: alteration, exudation and proliferation.
2. The results of the study proved the positive effect of 0.025 % decasan solution in the treatment of experimental wounds and the high efficiency of MSC and MSC-IG, which was confirmed by the dynamics of morphological changes in chronic wounds. The use of decasan showed positive dynamics of morphological parameters in the early stages (3-7 days), while MSC and MSC with IG were effective at all stages of the study.
3. Mesenchymal stem cells and MSC-IG accelerated the healing process of experimental chronic purulent-necrotic wounds after 3 days of treatment (31 day of observation). At this time there was a faster cleaning of the wound surface from purulent-necrotic tissues, accelerating the formation of granulation tissue. On the seventh day of treatment under the influence of MSC and MSC-IG revealed faster formation and maturation of granulation tissue, reducing wound area and accelerating epithelialization processes.
4. On the 14th day of treatment and the 30th day of treatment, complete epithelialization of wounds was detected in 60 and 70 % of experimental animals using decasan, 75 % and 80 % with MSC and 90 % and 95 % with MSC-IG, compared with the control group, where epithelization occurred in only 10 and 30 % of experimental animals, respectively (p<0.001).
5. The results of the study proved the positive effect of MSC in the treatment of experimental chronic purulent-necrotic wounds, their high efficiency in combination with inert gases was established, which was confirmed by a significant increase and stabilization of vimentin expression in fibroblasts, CD34 in endothelial cells and ?SMA in myofibroblasts (0.910±0.031, 0.408±0.056, 1.850±0.092, p<0.001).

References
подальшого пошуку нових методів лікування, що стимулюють репаративні процеси в хронічних ранах, в тому числі із застосуванням морфологічних методів дослідження. Роль клітинної регуляції в патогенезі відновлення морфофункционального стану хронічної рані в умовах її пошкодження залишається не розкритою. Тому, метою нашого дослідження стала оцінка ролі міофібробластів у засвоєні хронічних гнійно-некротичних ран при лікуванні мезенхімальними стовбуровими клітинами за допомогою імуногістохімічного методу. В експерименті нами отримана модель хронічної гнійно-некротичної рані, яка відповідає усім вимогам щодо якісних показників при вивченні морфологічних змін в хронічних ранах та в подальшому може використовуватися як базова на доклінічному етапі досліджень. За допомогою гістологічного та імуногістохімічного методів вивчили стан хронічних гнійно-некротичних ран у 120 щурів. Хронічну рану моделювали за оригінальною методикою автора: під час формування стандартного дефекту шкіри в міжлопатковій ділянці щура діаметром 1 см, на оточуючі тканини накладалася ішемізуюча металоконструкція з ціллю зменшення кровотоку в ділянці рану, що призводило до значного уповільнення термінів загоєння. Лікування починали від 28 доби з початку накладання ран, що клінічно та гістологічно відповідало хронізації ранового процесу. Статистична обробка морфометричних параметрів здійснювалася за допомогою стандартного програмного пакета "Statistica 6.1". Встановлено, що позитивна динаміка загоєння хронічних ран, при застосуванні 0,025 % розчину декасану, спостерігалась переважно на ранніх термінах (3-7 доби), в той час як мезенхімальні стовбурові клітини (МСК) та МСК клоновані в інертних газах (МСК-ІГ) були ефективними на всіх етапах дослідження. Використання МСК та МСК-ІГ створюють сприятливі умови для нормального перебігу регенераторних процесів і епітелізації ран, забезпечуючи протинабряковий та протизапальний ефекти з активацією міофібробластів, що підвищує ефективність загоєння хронічних гнійно-некротичних ран. Показано перспективи використання МСК при лікуванні хронічних ран.

Ключові слова: хронічні рані, лікування, мезенхімальні стовбурові клітини, мофібробласти, морфологічні зміни, імуногістохімія.