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Restoration of the microvasculature and hemodynamics in the oral mucosa wound defects area with and without a piezoelectric polymer membrane

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Abstract. *Introduction.* Restoration of blood supply to tissues is crucial for the regeneration of wound defects. The purpose of this research was to study the effect of piezoelectric polymers on the restoration of microcirculation and hemodynamics in the area of oral mucosa wound defects.
Materials and methods. The study was carried out on 60 Wistar rats divided into four groups (15 rats each): control (intact) and 3 experimental groups. In group 1, the animals had an open wound defect. In groups 3 and 2, the rats had a wound defect covered with a membrane with and without copper modification, respectively. The specimens for subsequent light and electron microscopy were collected on days 3, 7, and 12. We studied qualitative and quantitative indicators of microcirculation and hemodynamics restoration.
Results. Day 3: in all experimental groups, the arteriolo-venular relationship and the pericapillary diffusion index significantly decreased, while the Kernogan index increased. We also observed a significant increase in VEGF expression. Day 7: in experimental group 3, the values of the Kernogan index and the arteriolo-venular relationship were restored, and the pericapillary diffusion index remained significantly higher than in the control group. In the groups 1 and 2, none of the indicators reached the control values. VEGF expression decreased in all groups. On day 12 in group 1, the arteriolo-venular relationship, the Kernogan index and the index of pericapillary diffusion differed from the control group, in contrast to groups 2 and 3, where all the studied parameters were restored. Expression of VEGF in group 1 was significantly less than the control values, and in groups 2 and 3 it was significantly higher.
Conclusion. We revealed that closing of oral mucosa wound defects by a piezoelectric polymer membrane led to the restoration of hemodynamic parameters and promoted active vascular formation.

Keywords: wound defect, oral cavity, inflammation, regeneration, microcirculation, neoangiogenesis

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Восстановление микроциркуляторного русла и гемодинамики в области раневых дефектов слизистой оболочки полости рта при использовании полимерной пьезоэлектрической мембраны и без нее

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Резюме. *Введение.* Восстановление кровоснабжения тканей – неотъемлемая часть регенерации раневых дефектов. Целью исследования являлось изучение влияния использования полимерной пьезоэлектрической мембраны на восстановление микроциркуляции и гемодинамики в области раневых дефектов слизистой оболочки полости рта.

Материалы и методы. Исследование проведено на 60 крысах линии Вистар, разделенных на четыре группы: контрольная (n=15) – интактные животные, 1-я экспериментальная (n=15) – животные с открытым раневым дефектом, 2-я (n=15) и 3-я (n=15) экспериментальные – животные с раневым дефектом, перекрытым полимерной мембраной без модификации и с медным напылением. Забор материала для световой и электронной микроскопии проводили на 3-и, 7-е и 12-е сутки. Изучали качественные и количественные показатели восстановления микроциркуляции и гемодинамики на месте дефекта.
Результаты. На 3-и сутки исследования во всех экспериментальных группах показатели гемодинамики достоверно отличались от контрольных значений – артериоло-венулярное взаимоотношение и индекс перикапиллярной диффузии достоверно уменьшались, а индекс Керногана увеличивался. Значимо увеличивалась экспрессия. На 7-е сутки в 3-й экспериментальной группе восстанавливались значения индекса Керногана и артериоло-венулярного взаимоотношения, а индекс перикапиллярной диффузии оставался значимо больше, чем в группе контроля. В 1-й и во 2-й группах ни один из показателей не достигал контрольных значений. Экспрессия VEGF снижалась во всех группах. На 12-е сутки в 1-й группе артериоло-венулярное взаимоотношение, индекс Керногана и индекс перикапиллярной диффузии в группе контроля отличались показателей от 2-й и 3-й групп, где все исследуемые показатели восстанавливались. Экспрессия VEGF в 1-й группе была значимо меньше, чем контрольные значения, а во 2-й и в 3-й значимо больше.
Заключение. Закрытие ран слизистой оболочки полости рта полимерной пьезоэлектрической мембраной приводило к восстановлению показателей гемодинамики и способствовало активному сосудообразованию.

Ключевые слова: раневой дефект, полость рта, воспаление, регенерация, микроциркуляция, неоангиогенез

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Introduction

Wound healing is a complex dynamic process that is based on the coordinated interaction of connective tissue cells and microvasculature, due to which damaged tissue is effectively restored [1]. Wound regeneration is accompanied by formation of blood vessels in the granulation tissue, which provides nutrition to the wound bed and contributes to the organization of the extracellular matrix [2]. Impaired neoangiogenesis leads to delayed regeneration and negative consequences, i.e., scar tissue deformation at the site of injury [3, 4, 5]. To protect a wound defect from external traumas and thus create favorable conditions for angiogenesis, a number of wound dressings, e.g., hydrogels, films, plates, nanofibers, foams, plasters, sponges, and dressings, can be applied [6]. To close wound defects of the oral mucosa, biosynthetic substitutes for mucosal flaps and skin are used, such as epidermal (for example, Apligraf®), dermal (e.g., Alloderm®, Matriderm®), and composite membranes (e.g., Integra®, Biobrane®, Dermagraft®). However, none of them has all properties of an ideal material for closing wound defects of the oral mucosa [7]. Piezoelectric polymer membranes based on vinylidene fluoride with tetrafluoroethylene have been used to close wound defects of the oral mucosa. It contributes to the restoration of the fibrous component and reduced inflammatory response if wound damage to the oral mucosa has been proven [8].

Thus, the purpose of this research was to study the effect of a piezoelectric polymer membrane based on vinylidene fluoride with tetrafluoroethylene on the restoration of microcirculation and hemodynamics in the area of oral mucosa wound defects.

Materials and methods

The study was carried out on 60 Wistar rats divided into four groups (the control group of intact rats and three experimental groups), each one including 15 rats. The study was approved by the local ethics committee of the Siberian State Medical University (No. 7693/1 from 26.8.2019). The study was performed in compliance with the principles of humanity set out in the Council Directive (86/609/EEC) and the Declaration of Helsinki.

In animals of the experimental groups, we made an excision of a lip mucous membrane flap of 7 × 4 mm. In the animals of experimental group 1, the defect was not covered, while in those of experimental groups 3 and 2, the wound defect was coated with a polymer membrane with and without copper coating, respectively. The surgical intervention technique as well as specimen processing were described in our previous study [8].

The animals were sacrificed on days 3, 7, and 12 of the study by introducing them into hypoxia in a CO_2 chamber. Then, we prepared histological slides and semi- and ultra-thin sections for light and electron microscopy as described in the earlier article [9]. Afterward, we examined the samples with Karl Zeiss Observer D1 light microscope (Karl Zeiss, Germany) and a Zeiss AxioCam ICc5 light microscopy camera (Karl Zeiss, Germany).

To carry out an immunohistochemical study, we prepared serial paraffin sections 4–6-μm thick, then deparaffinized them, and stained with VEGF rabbit recombinant polyclonal antibodies and isotype IgG (Abcam, US). We scored the staining intensity of three cohorts of 100 cells in different fields of view (×400) using the following formula:

Histochemical scores = $\sum P(i) \times i$, where P(i) is the percentage of cells stained with different intensity and *i* is the staining intensity assessed on a 5-point scale from 0 to 4, where 0—no staining, 1—weak staining, 2—moderate staining, 3—strong staining, and 4—very strong staining.

We performed morphometric analysis using 3 parameters: the pericapillary diffusion index, the Kernogan index, and the arterio-venous relationship. The pericapillary diffusion index is a tissue area supplied by one capillary, i.e., the ratio of an average diameter of the capillary to a specific tissue area. The Kernogan index is an indicator of the throughput of the microvasculature, i.e., the ratio of vascular wall thickness to the radius of its lumen. Finally, the arterio-venous relationship was measured using image processing programs Axio Vision (Carl Zeiss, Germany) and ImageJ, version 1.52u (National Institute of Health, USA).

We used ultrathin sections to study the ultrastructure of the microvasculature.

We verified the normal distribution hypothesis with the Kolmogorov–Smirnov test. Since the distribution of the values of all quantitative features did not correspond to the normality tests, we used the Kruskal–Wallis one-way analysis of variance with the median test to compare independent samples and the Wilcoxon signed-rank test for paired comparisons. The results were considered statistically significant at $p<0.05$.

Results

On day 3, in all experimental groups, we visualized arterioles, capillaries, and venules with perivascular edema and sludge of formed elements on the wound defect periphery. The vessels were surrounded by separate disorganized bundles of collagen fibers and cells of the inflammatory infiltrate (Fig. 1A, 3A).

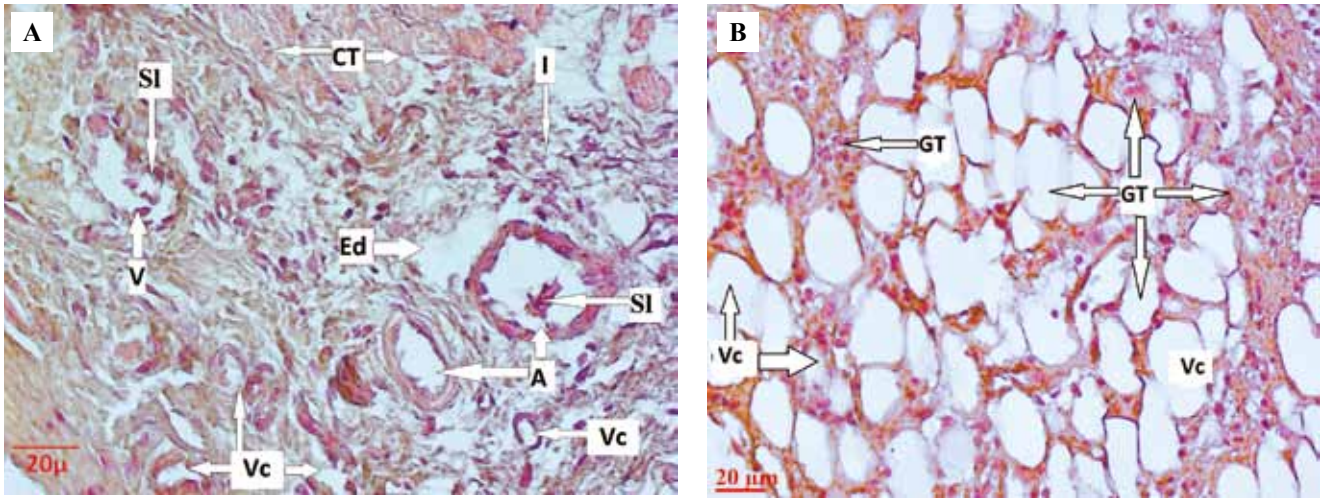

Fig. 1. The mucous membrane of the oral cavity.
A – vessels of the microvasculature with signs of sludge surrounded by lymphohistiocytic infiltration on the wound defect periphery. Histological picture characteristic of all experimental groups on day 3. Experimental group 1. B – newly formed vessels of granulation tissue. Histological picture characteristic of all experimental groups on day 3. Experimental group 3. H&E stain, ×400
Vc – capillary, V – venule, A – arteriole, I – lymphohistiocytic infiltration, GT – granulation tissue, CT – connective tissue, Sl – sludge, T – thrombosis, Ed – edema, Ep – epithelium, BC – cells of the epithelium basal layer, SC – cells of the epithelium spiny layer, GT – layer of superficial epithelial cells, NK – hyperkeratosis, P – papillae

Рис. 1. Слизистая оболочка полости рта крысы.
А – сосуды микроциркуляторного русла с явлениями сладжа, окруженные лимфогистиоцитарной инфильтрацией на периферии раневого дефекта. Гистологическая картина, характерная для всех экспериментальных групп. 1-я экспериментальная группа. 3-и сутки исследования. В – новообразованные сосуды грануляционной ткани. Гистологическая картина, характерная для всех экспериментальных групп. 3-я экспериментальная группа. 3-и сутки исследования. Окраска гематоксилином и эозином, ×400
Vc – капилляр, V– венула, A – артериола, I – лимфогистиоцитарная инфильтрация, GT – грануляционная ткань, CT – соединительная ткань, Sl – сладж, T – тромбоз, Ed – отек, Ep – эпителий, BC – клетки базального слоя эпителия, SC – клетки шиповатого слоя эпителия, GT – слой поверхностных клеток эпителия, НК – гиперкератоз, P – сосочки

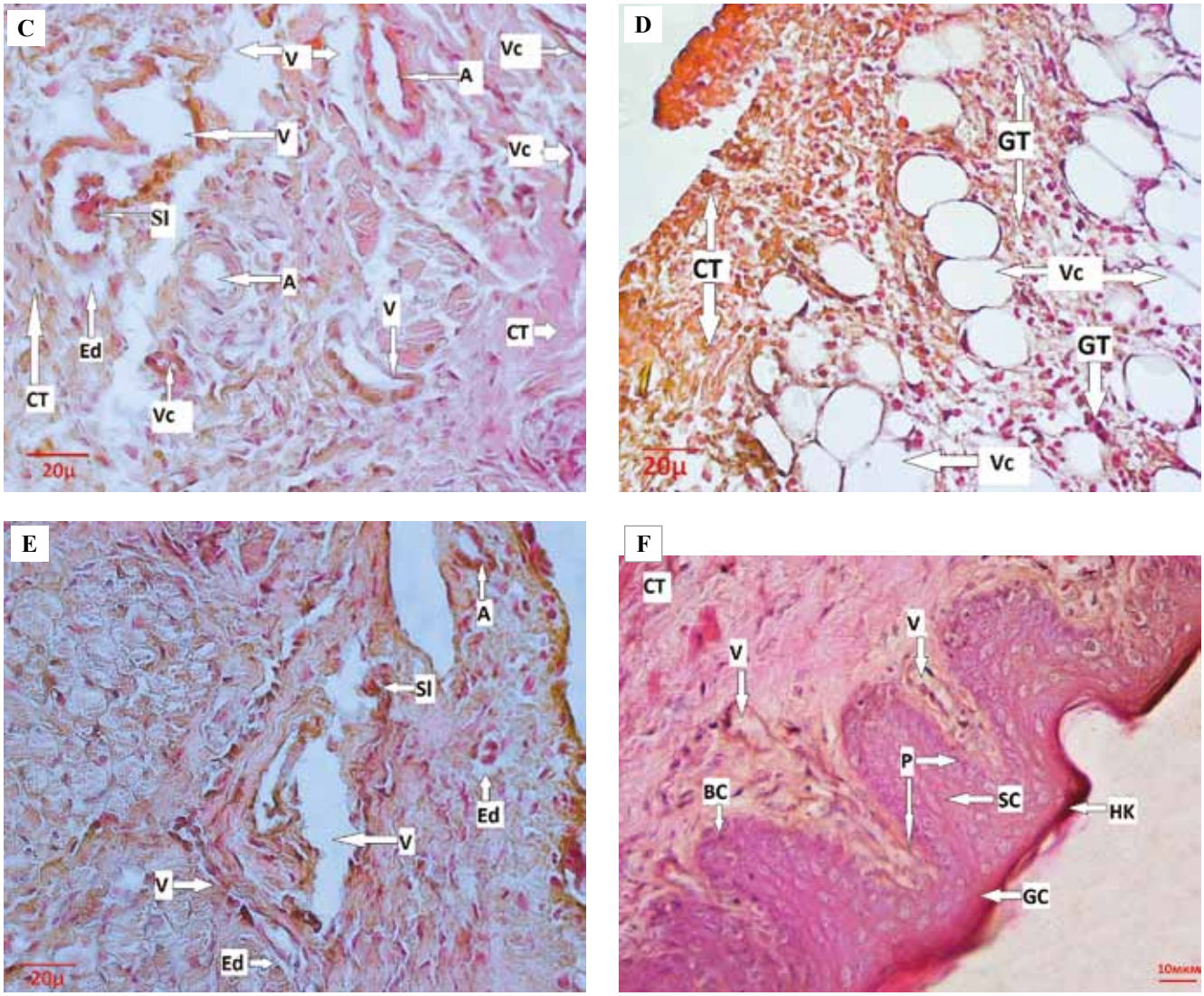

Fig. 1 (end). C – vessels of the microvasculature with sludge and perivascular edema on day 7. Experimental group 1. D – newly formed vessels of granulation tissue on day 7. Experimental group 2. E – vessels with sludge and perivascular edema at the site of the wound defect on day 12. Experimental group 1. F – newly formed vessels at the site of a regenerated wound defect without pathological changes. Histological picture characteristic of experimental groups 2 and 3 on day 12. Experimental group 2. H&E stain, ×400
Vc – capillary, V – venule, A – arteriole, I – lymphohistiocytic infiltration, GT – granulation tissue, CT – connective tissue, Sl – sludge, T – thrombosis, Ed – edema, Ep – epithelium, BC – cells of the epithelium basal layer, SC – cells of the epithelium spiny layer, GT – layer of superficial epithelial cells, NK – hyperkeratosis, P – papillae

Рис. 1 (окончание). C – сосуды микроциркуляторного русла с явлениями сладжа и периваскулярного отека. 1-я экспериментальная группа. 7-и сутки исследования. D – новообразованные сосуды грануляционной ткани. 2-я экспериментальная группа. 7-е сутки исследования. E – сосуды с периваскулярным отеком и сладжем на месте раневого дефекта. 1-я экспериментальная группа. 12-е сутки исследования. F – новообразованные сосуды на месте регенерировавшего раневого дефекта без патологических изменений. Гистологическая картина, характерная для 2-й и 3-й экспериментальных групп. 2-я экспериментальная группа. 12-е сутки исследования. Окраска гематоксилином и эозином, ×400
Vc – капилляр, V– венула, A – артериола, I – лимфогистиоцитарная инфильтрация, GT – грануляционная ткань. CT – соединительная ткань, Sl – сладж, T – тромбоз, Ed – отек, Ep – эпителий, BC – клетки базального слоя эпителия, SC – клетки шиповатого слоя эпителия, GT – слой поверхностных клеток эпителия, НК – гиперкератоз, P – сосочки

Qualitative changes in the vessels were accompanied by quantitative hemodynamic disorders and impaired microcirculation. We observed luminal narrowing of the arterioles and lumen expansion of the venules and capillaries. In this regard, in all experimental groups, the arterio-venous relationship decreased 1.9 times compared to that in the control group [p=0.035] (Table).

Pericapillary diffusion showed a 1.7-fold reduction compared to the intact tissue in all experimental groups (p=0.032), which indicated tissue ischemia (Table). The Kernogan's index increased twice in group 1, 1.8 times in group 2, and 1.6 times in group 3 [p=0.038, p=0.032, p=0.047, respectively] (Table). This indicates an impaired blood flow capacity in the arterioles due to luminal narrowing and wall thickening of the vessel.

We observed new thin-walled vessels in the newly formed granulation tissue (Fig. 1B), their endothelial cells actively expressing VEGF (Fig. 2A, Fig. 2B). At the same time, H-score did not significantly differ between groups 2 and 3 (p=0.077), but it was 1.4 and 4.7 times higher than in group 1 and the control group[p=0.022] (Table).

In the group without wound coating, the endothelial cells of the newly formed vessels did not form microvilli on the luminal side, which indicates impaired transcapillary metabolism. The endothelium and basement membrane of the granulation tissue capillaries were thin; the interendothelial space was enlarged. Endothelial cells were poor in organelles: the endoplasmic reticulum and the Golgi complex were expressed weakly, polysomes were rare, and mitochondria were edematous and increased in size (Fig. 3B).

In experimental groups 2 and 3, there was a large number of micropinocytic vesicles and microvilli in the luminal edge of endothelial cells, which indicated active transcapillary metabolism. Mitochondria, granular endoplasmic reticulum, and Golgi complex were well visualized in the cytoplasm (Fig. 3C).

On day 7 in experimental group 1, we still observed perivascular edema, as well as thrombosis, stasis, and sludge of formed elements (Fig. 1C, Fig. 3D). In groups 2 and 3, we almost did not detect these pathological changes (Fig. 1D).

In experimental group 1, on the ultrastructural level, endothelial cells in the newly formed vessels showed signs of functional immaturity. The granular endoplasmic reticulum and the Golgi complex were practically not visualized; polysomes were rare; in separate clusters, mitochondrial cristae were destroyed (Fig. 3E).

In the groups with wound coating, well-pronounced synthesis organelles and a large number of mitochondria with structured cristae were observed in endothelial cells. Signs of a high transcapillary exchange were preserved. There were multiple microvilli on the luminal surface of endothelial cells, many micropinocytic vesicles; intercellular contacts were formed between pericytes and endothelial

Table | Таблица

Indicators of hemodynamics of the oral mucosa vessels depending on treatment, conventional units, M (Q1:Q3) | Показатели гемодинамики сосудов слизистой оболочки полости рта в зависимости от методики лечения, усл. ед., M (Q1 : Q3)

	Arteriolo-venular relationship | Артериоло-венулярное взаимоотношение	Pericapillary diffusion index | Индекс перикапилярной диффузии	Kernogan index | Индекс Керногана	VEGF
Control | Контроль	0.72 (0.67;0.76)	6.04 (4.65;8.29)	0.61 (0.56;0.65)	80.0 (75.0;85.0)
Day 3 | 3-и сутки				
Group 1 | 1-я группа	0.38 (0.37;0.39)*	3.6 (3.4;3.8)*	1.22 (1.18;1.28)*	275.0 (265.0;290)*
Group 2 | 2-я группа	0.4 (0.39;0.41)*	3.8 (3.6;4.01)*	1.12 (1.1;1.14)*	365.0 (360.0;372.5)*#
Group 3 | 3-я группа	0.44 (0.44;0.45)*	3.6 (3.4;3.75)*	1.01 (1.0;1.04)*	375.0 (370.0;380.0)*#
Day 7 | 7-е сутки				
Group 1 | 1-я группа	0.45 (0.44;0.46)*	3.0 (2.8;3.1)*	1.16 (1.12;1.21)*	165.0 (155.0;175.0)*
Group 2 | 2-я группа	0.52 (0.51;0.53)*	4.3 (4.1;4.4)*#	0.83 (0.81;0.84)*#	255.0 (250.0;265.0)*#
Group 3 | 3-я группа	0.76 (0.74;0.77)#	3.6 (3.5;3.9)*#	0.56 (0.55;0.57)#	275.0 (362.5;282.5)*#
Day 12 | 12-е сутки				
Group 1 | 1-я группа	0.57 (0.56;0.59)*	3.8 (3.1;4.5)*	0.83 (0.82;0.85)*	55.0 (55.0;60.0)*
Group 2 | 2-я группа	0.7 (0.67;0.72)#	4.6 (4.2;4.9)#	0.59 (0.55;0.61)#	130.0 (125.0;140.0)*#
Group 3 | 3-я группа	0.77 (0.75;0.79)#	4.85 (4.59;4.15)#	0.53 (0.52;0.55)#	120.0 (125.0;135.0)*#

* – significant differences compared to the control group (p<0.05)
# – significant differences compared to group 1 (p<0.05)
* – достоверные различия по сравнению с контрольной группой (p<0,05)
# – достоверные различия по сравнению с 1-й группой (p<0,05)

cells. The basement membrane of the vessels was continuous and of uniform thickness (Fig. 3F).

On day 7, in the area of the wound defect, the figures of quantitative indicators of microcirculation gradually turned back to normal .

In experimental group 3, the arterio-venous relationship and the Kernogan's index returned to control values, the latter being twice lower than in group 1 [p=0.045] (Table).

In experimental group 2, the arterio-venous relationship did not change significantly compared to that on day 3 (p=0.065) and was 1.4 times lower than the same indicator in the control group and group 3 (p=0.035). In group 2, the Kernogan's index was 1.4 times lower than in group 1 (p=0.035) and 1.4 times higher than in the control group [p=0.041] (Table).

The index of pericapillary diffusion in experimental groups 2 and 3 was 1.4 and 1.2 times higher than in group 1 (p=0.033), but still differed significantly from the control values [p=0.039] (Table).

In the uncoated group, none of these indicators reached the control values. The arterio-venous relationship did not change significantly compared to the figures on day 3 (p=0.088) and was 1.6 times significantly lower than the same indicator in the control group [p=0.032] (Table).

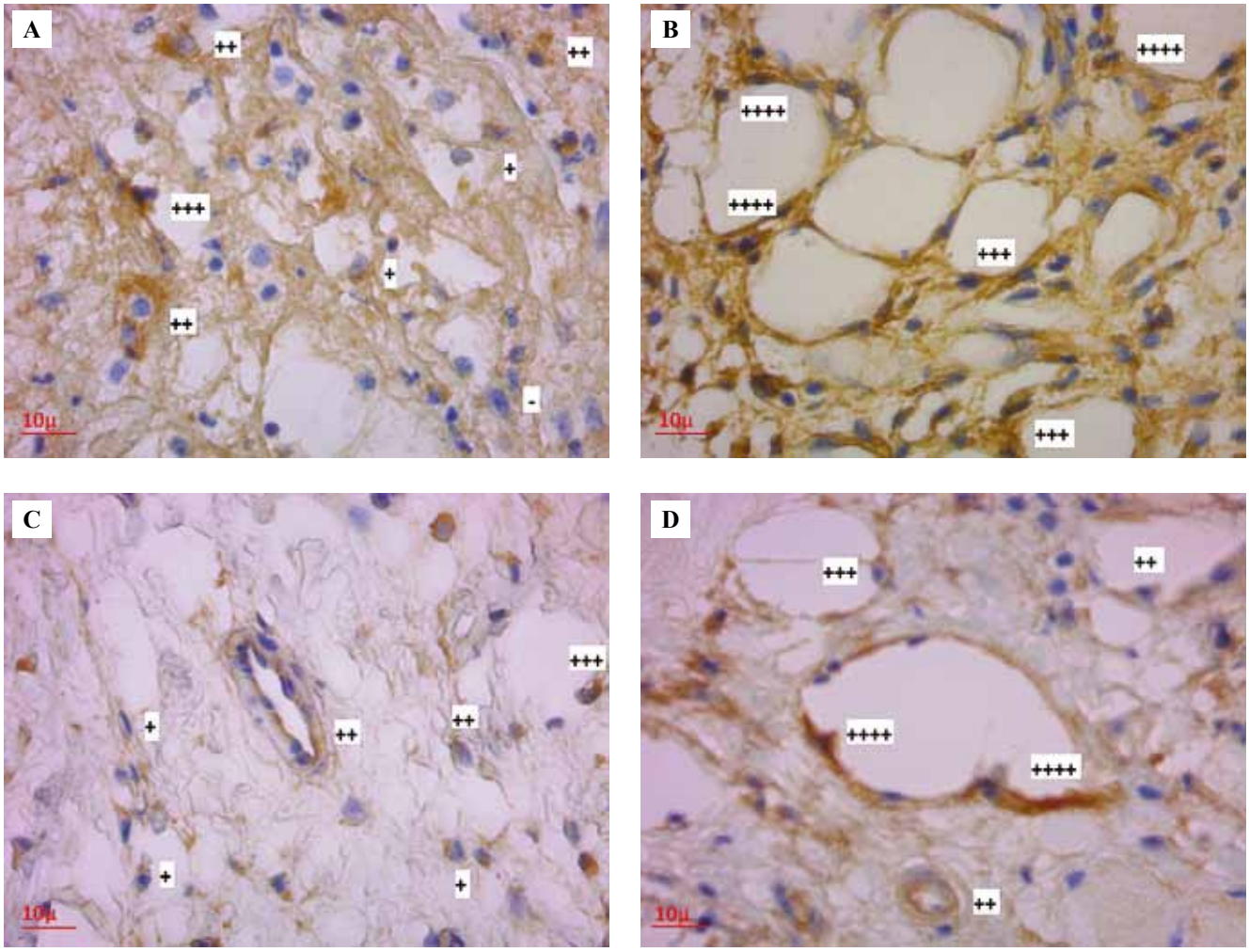

Fig. 2. VEGF expression in endothelial cells of granulation tissue, staining of nuclei with hematoxylin.
A – experimental group 1 on day 3. B – histological picture characteristic of experimental groups 2 and 3 on day 3. Experimental group 3. C – histological picture characteristic of experimental groups 2 and 3 on day 7. Experimental group 2. D – experimental group 1 on day 7. ×900
++++ – very strong staining, +++ – strong staining, ++ – moderate staining, + – weak staining, – – no staining

Рис. 2. Экспрессия VEGF в эндотелиоцитах грануляционной ткани, докрашивание ядер гематоксилином.
A – 1-я экспериментальная группа. 3-и сутки исследования. B – гистологическая картина, характерная для 2-й и 3-й экспериментальных групп. 3-я экспериментальная группа. 3-и сутки исследования. C – гистологическая картина, характерная для 2-й и 3-й экспериментальных групп. 2-я экспериментальная группа. 7-е сутки исследования. D – 1-я экспериментальная группа. 7-е сутки исследования. ×900
++++ – очень сильное окрашивание, +++ – сильное окрашивание, ++ – умеренное окрашивание, + – слабое окрашивание, – – нет окрашивания

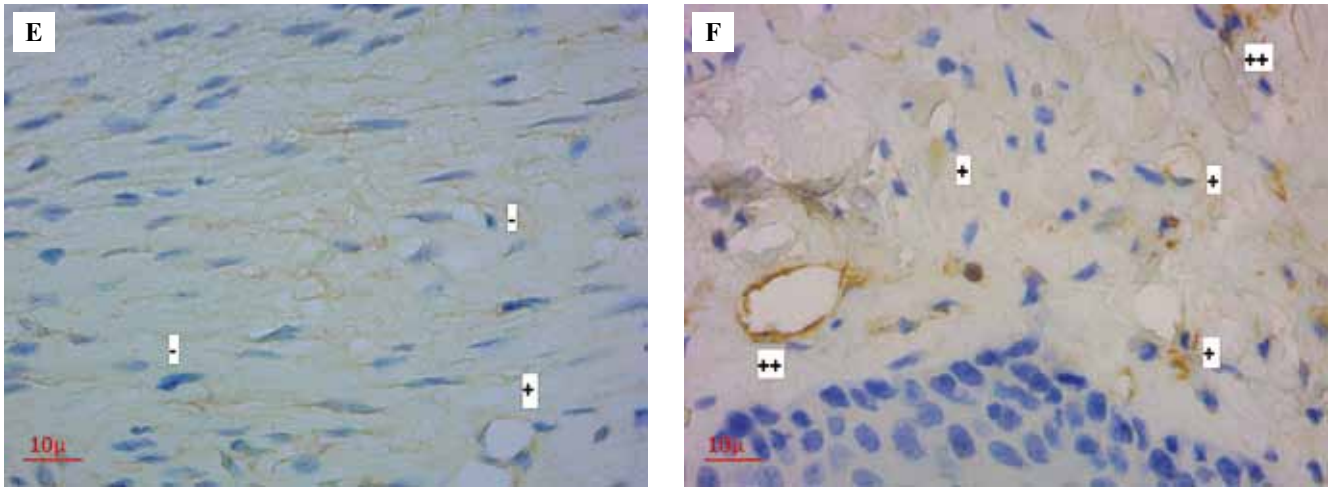

Fig. 2 (end). E – experimental group 1 on day 12. F – histological picture characteristic of experimental groups 2 and 3 on day 12. Experimental group 3. ×900
++++ – very strong staining, +++ – strong staining, ++ – moderate staining, + – weak staining, – – no staining

Рис. 2 (окончание). E – 1-я экспериментальная группа. 12-е сутки исследования. F – гистологическая картина, характерная для 2-й и 3-й экспериментальных групп. 3-я экспериментальная группа. 12-е сутки исследования. ×900
++++ – очень сильное окрашивание, +++ – сильное окрашивание, ++ – умеренное окрашивание, + – слабое окрашивание, – – нет окрашивания

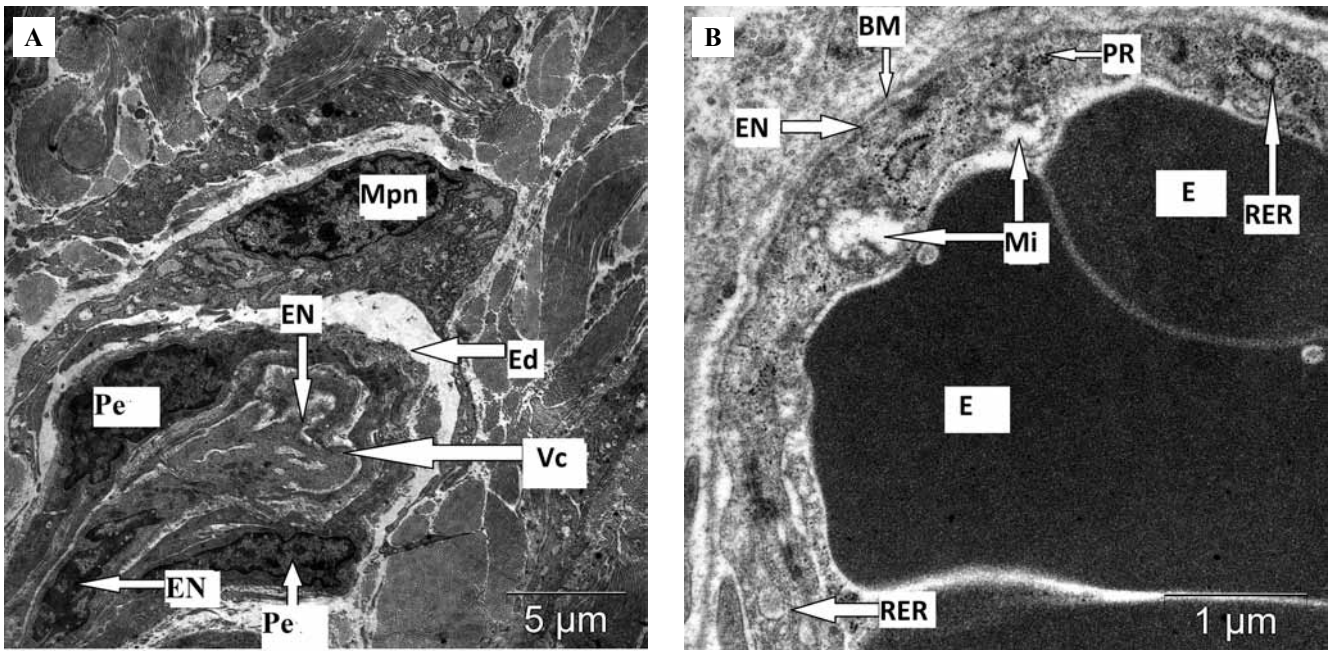

Fig. 3. Electron micrographs of vessels in the area of the oral mucosa wound defect.
A – narrowed postcapillary on the periphery of the wound defect with signs of perivascular edema on day 3. Experimental group 3, ×5000. B – capillary with sludge in the emerging granulation tissue with thinned endothelial lining and signs of transcapillary metabolism disorders on day 3. Experimental group 1, ×20 000
Vc – capillary, Mpn – macrophage, Pe – pericyte, EN – endothelial cells, Ed –perivascular edema, E – erythrocyte, BM – basement membrane, RER – granular endoplasmic reticulum, Mi – mitochondria, PR – polysomes, C – intercellular contact, MV – microvilli, Vs – micropinocytic vesicles, G – Golgi complex, CF – collagen fibers

Рис. 3. Электронные микрофотографии сосудов микроциркуляторного русла в области раневого дефекта слизистой оболочки полости рта крысы.
A – суженный посткапилляр на периферии раневого дефекта с признаками периваскулярного отека. 3-я экспериментальная группа. 3-и сутки исследования, ×5000. B – капилляр со сладжем в формирующейся грануляционной ткани с истонченной эндотелиальной выстилкой и признаками нарушения транскапиллярного обмена. 1-я экспериментальная группа. 3-и сутки исследования, ×20 000
Vc – капилляр, Mpn – макрофаг, Pe – перицит, EN – эндотелиоцит, Ed – периваскулярный отек, E – эритроцит, BM – базальная мембрана, RER – гранулярный эндоплазматический ретикулум, Mi – митохондрия, PR – полисомы, C – межклеточный контакт, MV – микроворсинки, Vs – микропиноцитозные пузырьки, G – комплекс Гольджи, CF – коллагеновые волокна

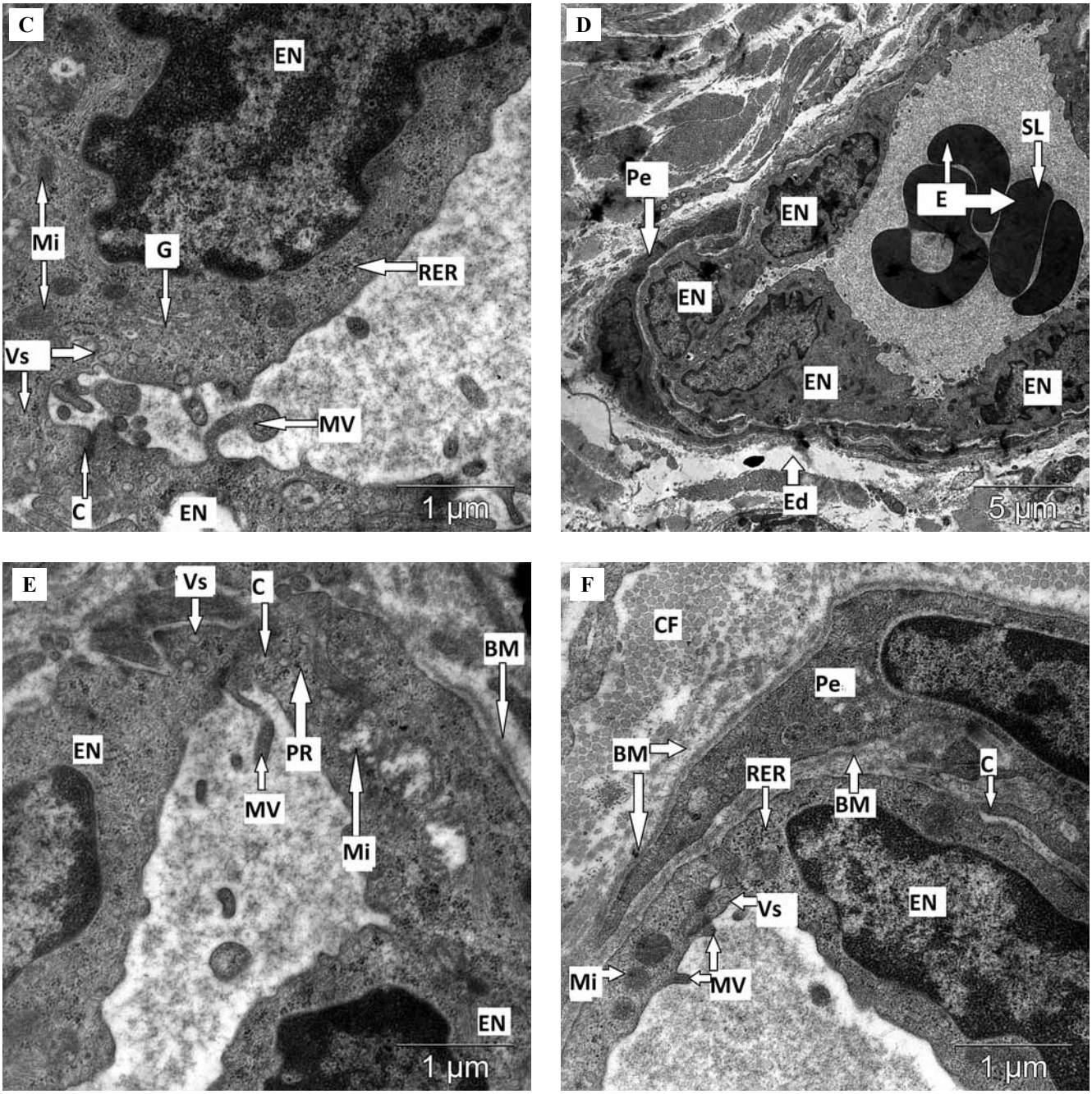

Fig. 3 (continuation). C – capillaries of granulation tissue with signs of activation of transcapillary exchange and synthetic processes. Histological picture characteristic of groups 2 and 3 on day 3. Experimental group 3, ×20 000. D – venule in the area of the regenerating wound defect with the sludge of formed elements and perivascular edema on day 7. Experimental group 1, ×5000. E – narrowed capillary with mitochondrial destruction and thinned basement membrane on day 7. Experimental group 1, ×5000. F – interdigitation between the capillary endothelial celle and pericyte at the site of the wound defect. Experimental group 2, ×20000

Рис. 3 (продолжение). С – капилляры грануляционной ткани с признаками активации транскапиллярного обмена и синтетических процессов. Микроскопическая картина, характерная для 2-й и 3-й экспериментальных групп. 3-я экспериментальная группа. 3-и сутки исследования, ×20 000. D – венула в области регенерирующего раневого дефекта со сладжем форменных элементов и периваскулярным отеком. 1-я экспериментальная группа. 7-е сутки исследования, ×5000. E – суженный капилляр с деструкцией митохондрий и истонченной базальной мембраной. 1-я экспериментальная группа. 7-е сутки исследования, ×5000. F – интердигитация между эндотелиоцитом и перицитом капилляра на месте раневого дефекта. 2-я экспериментальная группа, ×20 000

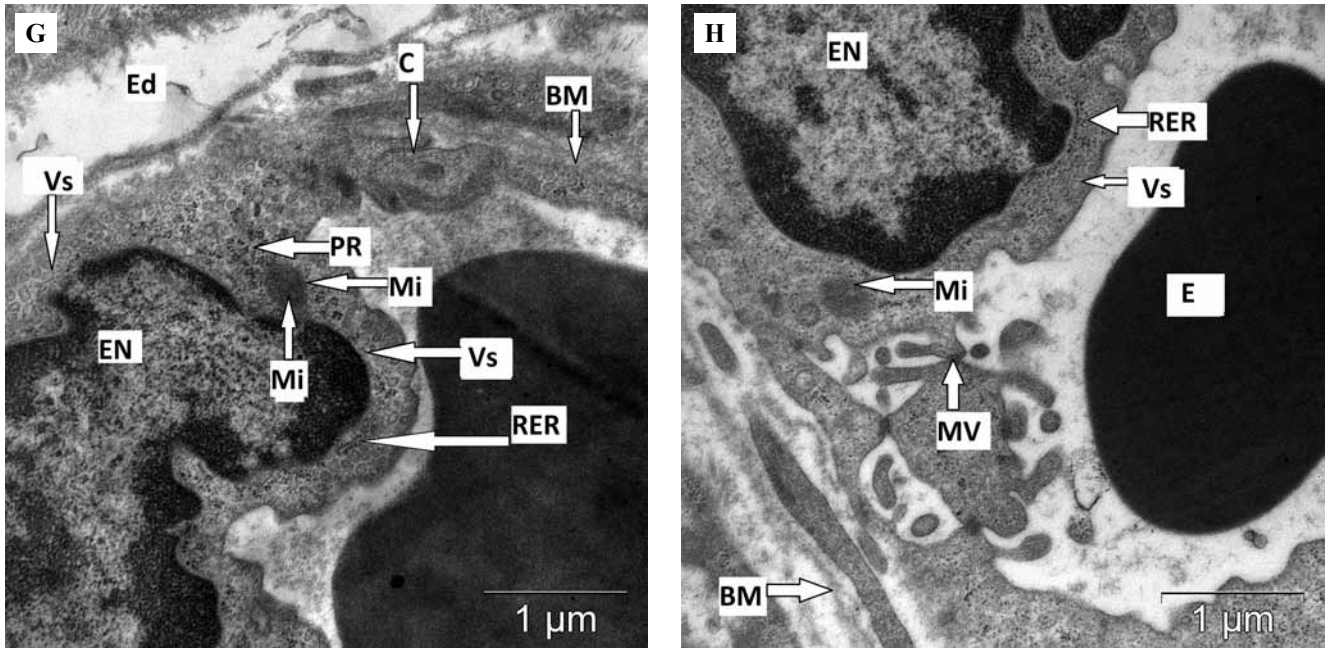

Fig. 3 (end). G – capillary in the area of the wound defect with signs of perivascular edema on day 12. Experimental group 1, ×20 000. H – capillary with signs of high transcapillary exchange at the site of the wound defect on day 12. Histological picture characteristic of groups 2 and 3. Experimental group 3, ×20 000

Рис. 3 (окончание). G – капилляр в области раневого дефекта с признаками периваскулярного отека. 1-я экспериментальная группа. 12-е сутки исследования. ×20 000. H – капилляр с признаками высокого транскапиллярного обмена на месте раневого дефекта. Гистологическая картина, характерная для 2-й и 3-й экспериментальных групп. 3-я экспериментальная группа. 12-е сутки исследования, ×20 000

VEGF expression decreased in comparison with that on day 3, being insignificantly different between experimental groups 2 and 3 [Fig. 2C] (p=0.079). However, it was 1.7 and 3.6 times significantly higher than in group 1 (Fig. 2D) and control group [p=0.038, p=0.035] (Table).

On day 12, in the uncoated group, we observed the narrowing and plethora of the vessels, as well as perivascular edema (Fig. 1E). The basement membrane of the capillaries was thin. In endothelial cells, the number of microvilli and micropinocytic vesicles was small (Fig. 3G).

In the coated groups, the newly formed vessels were detected in the connective tissue at the site of the wound defect, being especially developed in the area of the lamina propria papillary layer (Fig. 1F). In mature vessels, there were no morphological signs of hemodynamic disorders (i.e., thrombosis, sluggishness, and stasis). The mature vessels were surrounded by structured connective tissue fibers; there was no perivascular edema. We visualized a continuous basement membrane of uniform thickness. In endothelial cells, the quantity of organelles was sufficient, and they were of a typical structure. There was an active transcapillary exchange, as evidenced by a large number of micropinocytic vesicles and microvilli on the luminal surface of endothelial cells (Fig. 3H).

In groups 2 and 3, all the discussed quantitative hemodynamic parameters reached control values (Table).

In group 1, the arteri-venous relationship and the pericapillary diffusion index were 1.3 and 1.6 times lower than the control values, respectively (p=0.034, p=0.042), and the pericapillary diffusion index was 1.6 times higher (p=0.045) than in the control group (Table).

On day 12, in all experimental groups, VEGF expression decreased compared to that on day 7. In experimental group 1, The H-score VEGF index became 1.45 times lower than the control values [p=0.035] (Fig. 2E). Between groups 2 and 3 this indicator did not differ significantly [p=0.077] (Fig. 2F), but was 1.6 and 2.4 times significantly higher than in group 1 and control group [p=0.035, p=0.042] (Table).

Discussion

In this study, we identified the main mechanisms of microvasculature and the restoration of hemodynamics in the area of the oral mucosa wound defect with and without the use of a piezoelectric polymer membrane.

On day 3, the vascular response indicated the first phase of wound defect regeneration, i.e., inflammation [9]. So, in all experimental groups, on day 3, we observed expanded venules and capillaries, as well as luminal narrowing of the arterioles on the periphery of the wound defect. It resulted in a changed arterio-venous relationship and contributed to the progression of congestion and tissue ischemia in the wound area. A decrease in the index of pericapillary diffusion due to luminal narrowing of the capillary and an increase in the Kernogan index due to wall thickening and luminal narrowing of the arterioles, which occurred due

to edema of their intima, also led to an increase in edema and tissue ischemia. As a result, acidosis developed and free radicals accumulated in the wound defect area, which was a signal for the cells of the lymphohistiocytic series migration to the inflammation focus. The cells exit from the bloodstream into the surrounding tissues was facilitated by the expansion of postcapillary venules and, as a result, the thinning of their walls [10].

The VEGF signaling pathway was induced under hypoxia and acidosis, which was indirectly evidenced by the studied hemodynamic parameters, i.e.,- a reduced index of pericapillary diffusion, reflecting a decrease in the area of tissue supplied by one capillary; an increased index of pericapillary diffusion, indicating a deterioration in the throughput of arterioles; and an altered arteriolovenular relationship indicating venous congestion [11]. In turn, an increased concentration of VEGF also contributed to an increment in vascular permeability and an accretion in edema [12].

At the same time, granulation tissue formed in the area of the wound defect, where we visualized a large number of newly formed vessels. An increase in VEGF expression indicated neoangiogenesis [13]. Simultaneously, in the coated groups, VEGF expression was more pronounced, indicating more active vascular formation, which was due to the protection of the wound defect from the effects of traumatic factors through a polymer membrane. Angiogenesis depended on the inflammatory response, since VEGF was synthesized not only by endothelial cells, but also by the cells of lymphohistiocytic infiltration, therefore, VEGF expression was maximum in all groups on day 3, which corresponded to the 1st phase of wound regeneration, i.e., the inflammation [14].

On day 7, in groups treated with a piezoelectric polymer membrane, the values of the Kernogan index, pericapillary diffusion index, and arteriolovenular relationship gradually returned back to normal. In this regard, the severity of edema and congestion decreased, which led to the normalization of acid-base balance. In the uncoated group, these indicators differed significantly from the control values, there was perivascular edema and altered vessels with thrombosis and sludge of formed elements in many fields of vision. On the ultrastructural level, we observed signs of impaired capillary exchange, which were not detected in the coated groups. VEGF expression decreased in comparison with that on day 3, confirming that the phase of active angiogenesis in the area of the wound defect turned to the stage of maturation. A decrease in VEGF synthesis was associated with the restoration of hemodynamics, especially in the coated groups, since an increase in its concentration was due to tissue hypoxia during its prolonged ischemia [15].

On day 12, hemodynamic parameters turned back to normal values in the coated groups. We visualized altered vessels of typical ultrastructure with sludge and thrombosis. Regression of granulation tissue capillaries was a logical step in wound regeneration. It included selective apoptosis of cells of the newly formed vessels [16]. Since neoangiogenesis slowed down at the final stage of wound regeneration, VEGF expression was still higher than the control values.

In the uncoated group, there were signs of impaired microcirculation in the wound defect area: none of the morphological parameters of hemodynamics reached the control values, which indicated impaired blood supply to the tissues. VEGF expression was lower than in the control group, where it was secreted in small amounts by cells in the intact mucosa, such as macrophages and fibroblasts [17]. A decrease in VEGF activity probably led to increased apoptosis of pericytes and endothelial cells, which resulted in the involution of vessels in the area of the wound defect and the formation of poorly vascularized scar tissue [18].

Conclusion

We revealed that closing oral mucosa wounds with a piezoelectric polymer membrane led to the restoration of hemodynamic parameters and promoted active vascular formation, in contrast to the situation when the defect remained open and continued to be subjected to traumatic effects from aggressive environmental factors.

Compliance with ethical principles

The study was approved by the local ethics committee of the Siberian State Medical University (No. 7693/1 from 26.8.2019). The study was carried out in compliance with the principles of humanity set out in the Council Directive (86/609/EEC) and the Declaration of Helsinki.

Author contributions

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