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MORPHOLOGICAL CHANGES IN THE WOUND PROCESS AFTER IMPLANTATION OF SILVER ALLOY PIERCINGS IN LABORATORY ANIMALS

Purpose of the study. *To prove the effect of selenium alloy piercing on the course of the wound process in the soft tissues of the oral cavity through morphological studies of microdissections in experimental animals.* ***Materials and methods***. *Experimental studies were conducted on mature rabbits of the Chinchilla breed weighing from 2.7 to 3.2 kg. The procedures were in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, March 18, 1986), EU Council Directives (November 24, 1986) and Protocol No. 6 of the KhNMU Ethics Committee of June 5, 2013. Implantation of a silver alloy piercing into the soft tissues of the tongue and cheek. Histologic analysis of changes in the wound channel was performed on days 7, 14, 28, and 60. Morphological studies were performed at the Central Research Laboratory and at the Department of Pathological Anatomy of KhNMU. The material was fixed in 10% formalin, treated with alcohol, and embedded in paraffin, sections 4-5 μm. Analysis and photographs were taken with an Olympus BX-41 microscope (Japan).* ***Scientific novelty.*** *On the 7th day, purulent-necrotic detritus, initial epithelialization at the edges, granulation tissue with capillaries, fibroblasts and an infiltrate of lymphocytes and neutrophils were observed in the wounds of the tongue and cheek. On the 14th day, the epithelium is thickened with hyper- and parakeratosis, acanthosis, and microabscesses; granulation tissue matures, collagen fibers are bundled, and cellular elements are represented by fibroblasts, macrophages, and neutrophils. On day 28, epithelialization is uneven, with microabscesses and inflammation; granulation tissue matures, with young connective tissue in the deeper parts. On day 60, the epithelium is uneven, sometimes thickened or thinned; granulation tissue is voluminous, with bundled collagen fibers and secondary granulations inside and on the surface of the epithelial layer.* ***Conclusions.*** *The healing of the wound channels of the tongue and cheek after implantation of silver piercing clips is accompanied by inflammation and repair. On days 7-14, purulent-necrotic detritus, neutrophil infiltration, granulation tissue formation, and the onset of epithelialization dominate. On day 28-60, epithelialization is uneven with microabscesses and necrosis, indicating chronicity of the process. The granulation tissue matures, but secondary granulations make recovery difficult. This indicates long-term irritation from silver clips and requires caution when choosing piercing materials.*

Key words: *piercing, silver alloy, experiment, granulation tissue, inflammation*

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МОРФОЛОГІЧНІ ЗМІНИ РАНОВОГО ПРОЦЕСУ ПІСЛЯ ІМПЛАНТАЦІЇ ПІРСИНГУ ЗІ СРІБНОГО СПЛАВУ У ЛАБОРАТОРНИХ ТВАРИН

Мета дослідження. *Довести вплив пірсингу зі срібного сплаву на перебіг ранового процесу в м'яких тканинах порожнини рота за рахунок морфологічних досліджень мікропрепаратів у експериментальних тварин.* ***Матеріали та методи***. *Експериментальні дослідження проводили на статевозрілих кролях породи " Chinchilla " масою від 2,7 до 3,2 кг. Процедури відповідали Європейській конвенції щодо захисту хребетних тварин (Страсбург, 18 березня 1986 року), директивам Ради ЄС (24 листопада 1986 року) та протоколу № 6 комісії з етики ХНМУ від 5 червня 2013 року. Вживляння пірсингу зі срібного сплаву в м'які тканини язика та щоки. Гістологічний аналіз змін у рановому каналі проведено на 7-й, 14-й, 28-й та 60-й дні. Морфологічні дослідження здійснювали в Центральній НДЛ та на кафедрі патологічної анатомії ХНМУ. Матеріал фіксовано в 10% формаліні, оброблено спиртом, імпрегновано парафіном, зрізи 4–5 мкм. Аналіз та фотографування виконали мікроскопом Olympus BX-41 (Японія).* ***Наукова новизна.*** *На 7-й день у ранах язика та щоки спостерігається гнійно-некротичний детрит, початкова епітелізація по краях, грануляційна тканина з капілярами, фібробластами та інфільтратом з лімфоцитів і нейтрофілів. На 14-й день епітелій потовщений із гіпер- і паракератозом, акантозом та мікроабсцесами; грануляційна тканина дозріває, колагенові волокна пучкові, клітинні елементи представлені фібробластами, макрофагами та нейтрофілами. На 28-й день епітелізація нерівномірна, з мікроабсцесами та запаленням; грануляційна тканина дозріває, у глибоких відділах – молода сполучна тканина. На 60-й день епітелій нерівномірний, місцями потовщений або стоншений; грануляційна тканина об'ємна, з пучковими колагеновими волокнами та вторинними грануляціями всередині і на поверхні епітеліального шару.* ***Висновки.*** *Загоєння ранових каналів язика та щоки після імплантації срібних пірсингових кліпс проходить із запаленням і репарацією. На 7–14 день домінує гнійно-некротичний детрит, інфільтрація нейтрофілами, формування грануляційної тканини та початок епітелізації. На 28–60 день епітелізація нерівномірна з мікроабсцесами і некрозом, що свідчить про хронізацію процесу. Грануляційна тканина дозріває, але вторинні грануляції ускладнюють відновлення. Це вказує на тривале подразнення від срібних кліпс і потребує обережності при виборі матеріалів для пірсингу.*

Ключові слова: *пірсинг, срібний сплав, експеримент, грануляційна тканина, запалення.*

Problem statement. Piercing, especially in the oral cavity (tongue, cheeks, lips), has gained wide popularity in recent decades among young people and people of different age groups [1, p.99]. At the same time, this phenomenon is accompanied by an increase in the number of complications associated with the insertion and prolonged wearing of foreign bodies in soft tissues [2, p.679]. The implantation of piercings into the oral mucosa creates a constant mechanical and chemical irritant that triggers inflammatory and destructive processes, contributes to the disruption of normal tissue regeneration, the development of secondary infection, and the formation of chronic pathological changes [3, p.128]. Particular attention should be paid to the choice of piercing material, as it largely determines the course of reparative processes [4, p.329]. The use of silver clips has a dual aspect: on the one hand, silver is known for its antiseptic properties, on the other hand, its prolonged presence in the tissues of the oral cavity can cause irritation, imbalance of cellular reactions and chronicity of the inflammatory process [5, p.75]. At the same time, the mucous membrane of the tongue and cheek is highly vascularized and innervated, which increases the risk of developing edema, pain, local complications, and even systemic reactions [6, p.2; 9, p. 327]. The lack of morphological studies that would comprehensively analyze tissue changes under the influence of silver piercing elements makes this problem particularly relevant to modern medicine [7, p.6097, 8, p.200]. Thus, the study of morphological changes in the tissues of the tongue and cheek during implantation of silver clips is extremely important from the point of view of clinical practice, prevention of complications and optimization of the choice of materials for piercing [10, p.167].

Objective of the study. To find out the effect of silver alloy piercing on the dynamics of the wound process in the soft tissues of the oral cavity through morphological studies of microdissections in experimental animals.

Materials and methods of the study. Experimental studies were conducted on sexually mature rabbits of the Chinchilla breed weighing from 2.7 to 3.2 kg. All experimental procedures met the requirements of the European Convention for the Protection of Vertebrate Animals (Strasbourg, March 18, 1986), the directives of the Council of the European Economic Community (Strasbourg, November 24, 1986) and were approved by Protocol No. 6 of the Ethics and Bioethics Committee of Kharkiv National Medical University on June 5, 2013.

The silver alloy piercing was implanted into the soft tissues of the tongue and cheek of the experimental animals. To study changes in the wound channel, histological analysis was performed on days 7, 14, 28, and 60 after implantation. Morphological studies of the soft tissues of the tongue and cheek were performed at the Central Research Laboratory and the Department of Pathological Anatomy of Kharkiv National Medical University. The research was aimed at studying the morphological features of the inflammatory process caused by silver alloy piercing clips. The material was fixed in a 10% solution of neutral formalin, treated with alcohol, impregnated with paraffin, and sections of 4-5 μm thickness were prepared. The analysis, description of microsections, and photographs were performed using an Olympus BX-41 microscope (Japan).

Research results and discussion. On day 7, the longitudinal section of the tongue wound canal shows purulent-necrotic detritus in its lumen. At the edges of the wound canal, a layer of epithelium is determined, which begins to regenerate. In the epithelialized areas of the wound canal, the multilayered squamous keratinized epithelium shows acanthotic growths and signs of hyperkeratosis and parakeratosis. In the deeper parts of the wound canal, the process of epithelialization has not yet begun. The wound walls are diffusely infiltrated with neutrophilic granulocytes. The tissues surrounding the wound demonstrate circulatory disorders, which is manifested by vascular hemorrhage and stasis (Fig. 1)

Under the zone of necrosis in the corresponding areas of the wound canal, granulation tissue is detected, characterized by edema, a large number of thin-walled capillaries, a small number of fibroblasts, and the presence of thin, scattered, delicately fuchsinophilic fibers. There are more fibroblasts in the marginal parts of the wound than in the central parts. The granulation tissue vessels are moderately dilated, with thin basement membranes. The granulation tissue cells include lymphocytes, macrophages, and numerous neutrophilic granulocytes.

On the 7th day, in most cases, purulent-necrotic detritus is observed in the cheek wound channel. The epithelial regenerate is thin, intermittent, and localized focally. The subepithelial granulation tissue is not formed along the entire length of the wound, contains numerous capillary vessels and cells of the lymphoplasmacytic, macrophage and fibroblastic series, as well as a significant number of neutrophilic granulocytes. Fibrous structures are weakly stained with fuchsin, located chaotically, mainly around the vessels (Fig. 2)

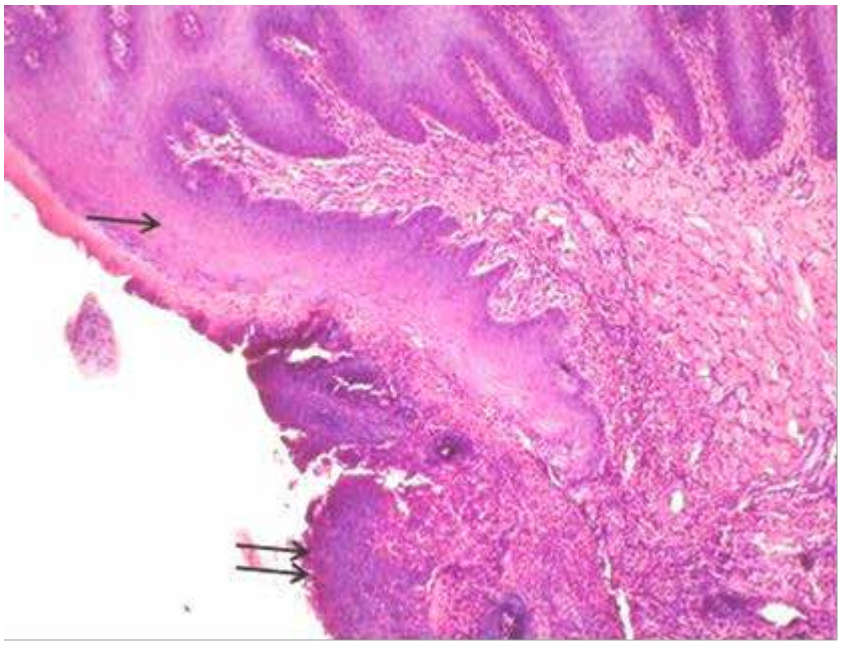

Fig. 1. Regenerating epithelium at the edges of the wound canal (arrow). The canal wall is infiltrated by neutrophils (two arrows). Day 7, hematoxylin and eosin staining, ×100

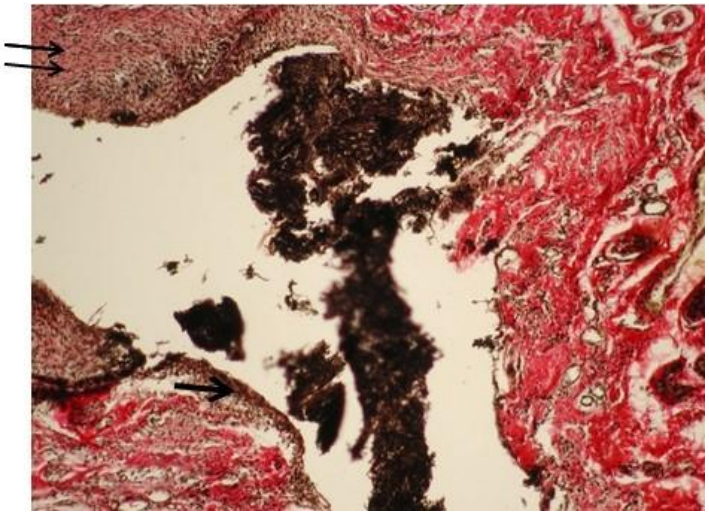

Fig. 2. Thin, discontinuous epithelial regenerate, poorly connected to the underlying tissue (arrow). Granulation tissue is not along the entire length of the wound canal (two arrows). Fibrous structures are slightly fuchsinophilic. Day 7, Van Gieson stain, ×100

On the 14th day, purulent-necrotic detritus is detected in the dilated lumen of the tongue wound canal. Regenerating epithelium covers the wound only at its edges. Multilayered flat keratinized epithelium demonstrates acanthotic overgrowth and signs of hyper- and parakeratosis. In deep areas of the wound canal, the process of epithelialization has not yet begun. The wound walls are diffusely infiltrated with neutrophilic granulocytes, and the surrounding tissues show circulatory disorders, in particular, vascular hemorrhage and stasis (Fig. 3).

Under the necrotic zone in the wall of the wound canal, granulation tissue is formed, characterized by edema, a large number of thin-walled capillaries with full blood flow, a small number of fibroblasts, and the presence of thin, scattered fuchsinophilic fibers. There are more fibroblasts in the marginal parts of the wound than in the central parts. The vessels of granulation tissue are moderately dilated, their basement membranes are thin. Among the granulation tissue cells there are lymphocytes, macrophages and numerous neutrophilic granulocytes (Fig. 4).

On the 14th day, in most cases, purulent exudate is observed in the lumen of the wound canal. The epithelial lining of the cheek wound is intermittent. The regenerating epithelium is thickened, with mani-

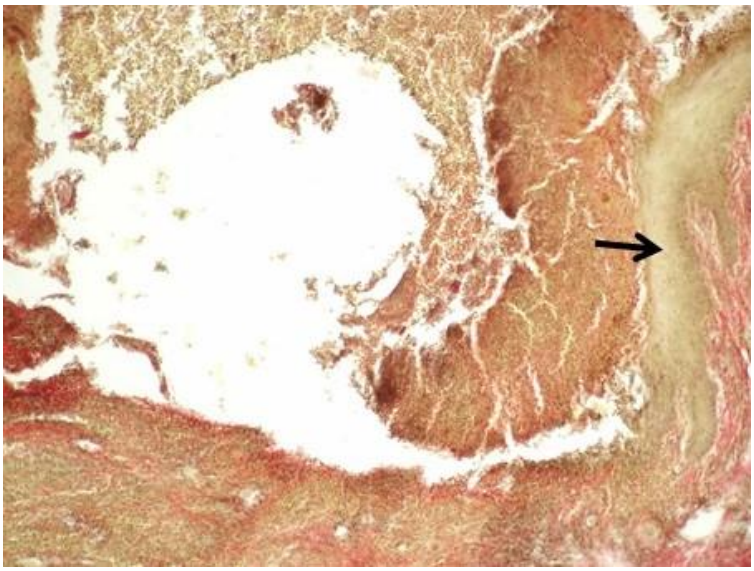

Fig. 3. Transverse section of the tongue wound canal is partially epithelialized. The multilayered squamous keratinized epithelium is characterized by the formation of acanthotic growths, as well as signs of hyper- and parakeratosis (arrow). Silver alloy piercing. 14 days. Van Gieson's stain. ×100

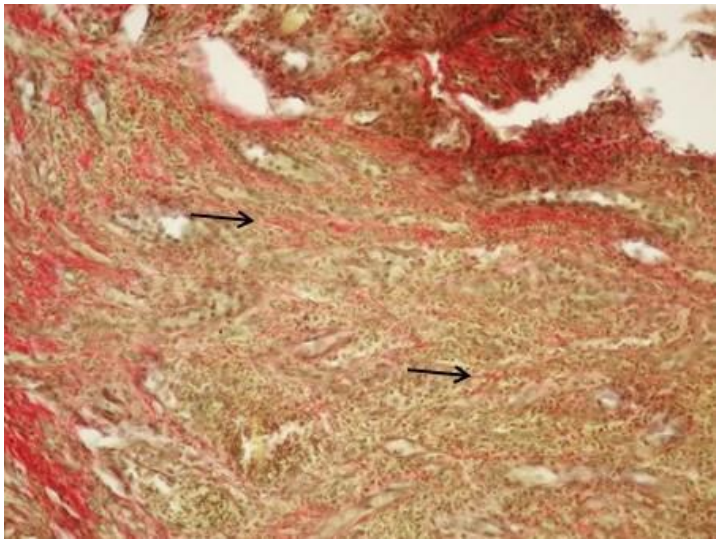

Fig. 4. Granulation tissue with numerous thin-walled capillaries, fibroblasts, and thin, delicate fuchsinophilic fibers (two arrows). Silver alloy piercing, 14 days old, Van Gieson's stain, ×200

festations of hyper- and parakeratosis and acanthosis. Under the epithelium in the wound wall, granulation tissue with full-blooded capillaries and cells of the lymphoplasmacytic, macrophage, and fibroblastic series, as well as numerous neutrophilic granulocytes, is detected. The fiber structures are thin and randomly arranged (Fig. 5).

On day 28, purulent exudate is observed in the lumen of the tongue wound canal. The walls of the canal are epithelialized; the multilayered squamous epithelium shows acanthotic growths and signs of hyper- and parakeratosis. There are microabscesses in the epithelium. The wound walls and surrounding tissues show signs of inflammation and local circulatory disorders. In the marginal areas of the canal, under the epithelium, there is maturing granulation tissue: collagen fibers are fuchsinophilic and have a bundle organization. Fibroblasts, single macrophages, and neutrophilic granulocytes predominate among the granulation tissue cells. Immature granulation tissue is observed in the areas of the wound canal passage through the transverse striated muscles of the tongue (Fig. 6).

On day 28, epithelialization was observed in the transverse section of the cheek wound canal, but the multilayered squamous keratinized epithelium of the regenerate was absent in some places and had uneven thickness. In some areas, the epithelium is thin, with

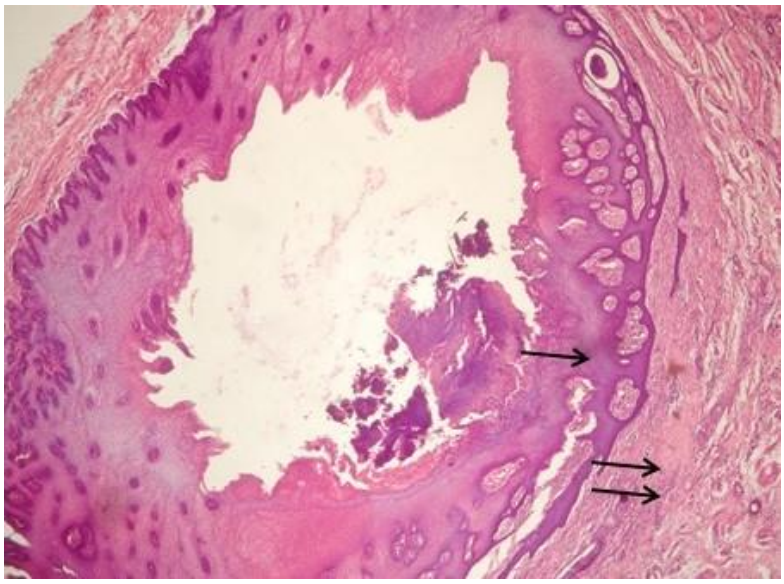

Fig. 5. Epithelial regenerate of cheek wound with hyper-, parakeratosis and acanthosis (arrow). There are numerous neutrophils in the granulation tissue (arrows). Silver alloy piercing, day 14, hematoxylin and eosin staining, ×100

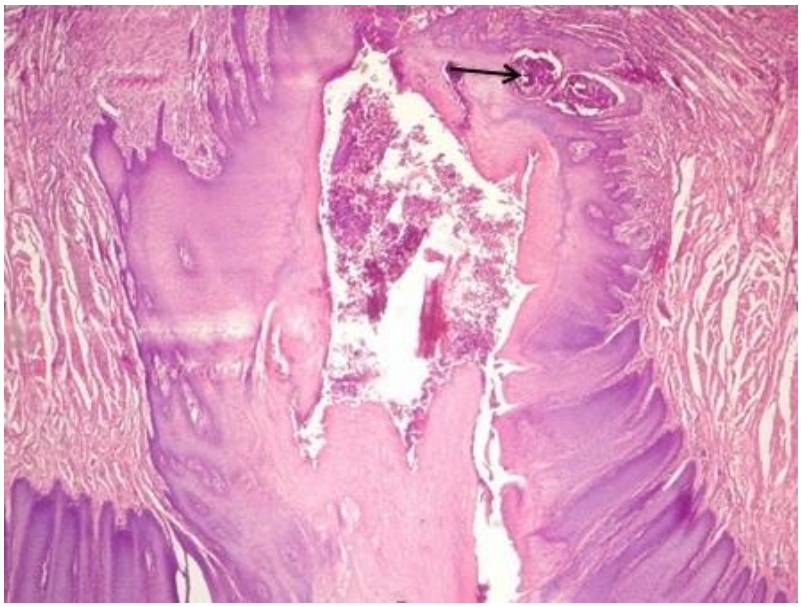

Fig. 6. The epithelial regenerate shows signs of hyperkeratosis and acanthosis. Intraepithelial microabscesses are detected (arrow). Silver alloy piercing. Day 28. Hematoxylin and eosin staining. ×40 cheek

a small number of cell layers, in others it is significantly thickened, with manifestations of hyper- and parakeratosis and acanthotic growths (Fig. 7).

In the wall of the wound canal, there are areas of necrosis, under which there is granulation tissue rich in blood vessels and cellular elements. In areas with well-formed epithelialization, the subepithelial granulation tissue is predominantly represented by uniformly oriented, gently fuchsinophilic fibrous structures that form bundles, with a small number of fibroblasts and a limited number of vessels.

In some cases, young connective tissue is detected in the deep parts of the granulation tissue. On the 60th day, uneven epithelialization is observed on the section of the wound canal of the tongue: in some areas the epithelial layer is significantly thickened, in others it is thinned or absent. The granulation tissue in the canal wall is voluminous, with weakly fuchsinophilic collagen fibers (Fig. 8).

On day 60, a transverse section of the cheek wound canal shows a thickened epithelial regenerate with manifestations of hyperkeratosis and acanthosis. There are foci of inflammation and necrosis in the epithelium, which leads to the formation of secondary volumetric granulations both within the epithelial layer and on its surface (Fig. 9).

During the observation of the healing of the wound channels of the tongue and cheek, consistent morpho-

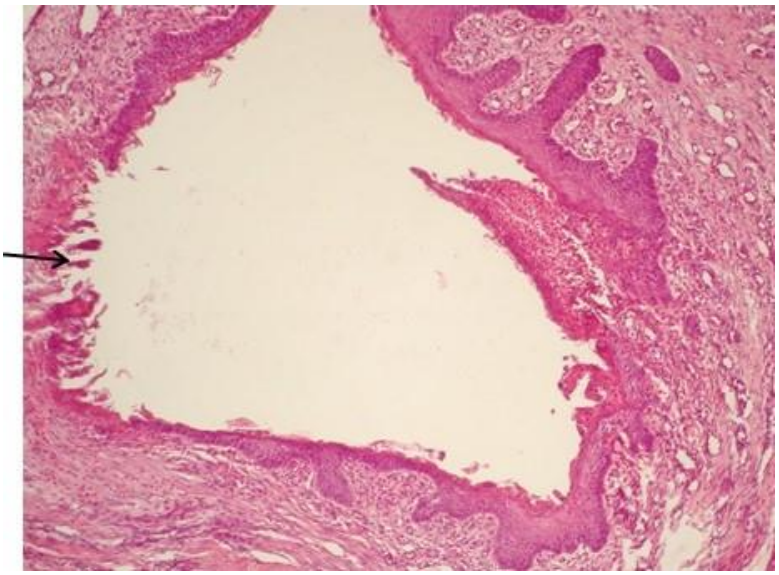

Fig. 7. Transverse section of the cheek wound. The multilayered squamous epithelium of the regenerate is absent in some places and of uneven thickness (arrow). Day 28, hematoxylin and eosin staining, ×100

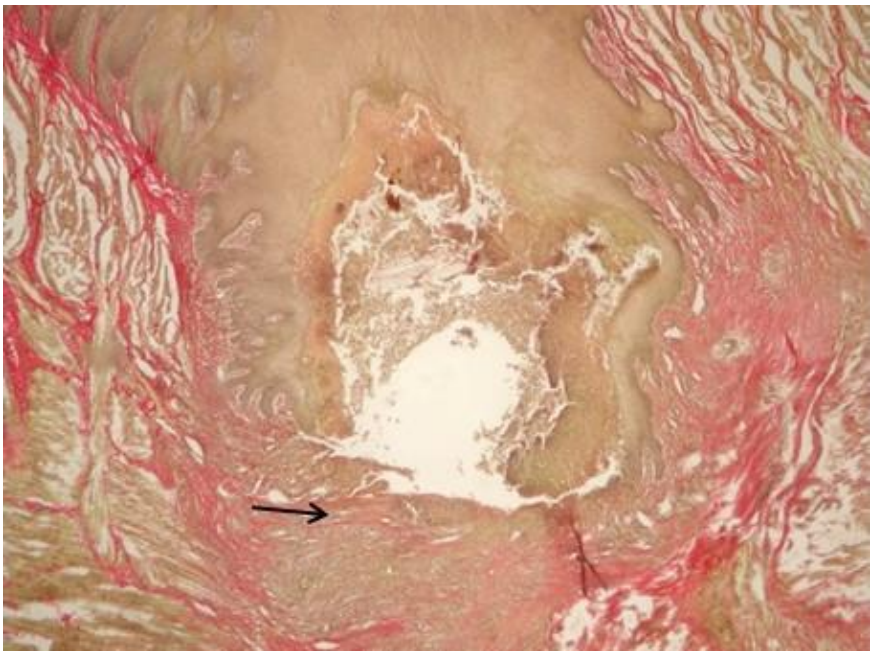

Fig. 8. Transverse section of the tongue wound canal with incomplete epithelialization; voluminous granulation tissue with weakly fuchsinophilic collagen fibers (arrow). Silver alloy piercing, 60 days, Van Gieson's stain, ×40

logical changes were noted. On day 7, purulent-necrotic detritus prevails in the wound lumen, the epithelium is thin, intermittent, the walls are infiltrated by neutrophils, granulation tissue with capillaries, fibroblasts and delicate fuchsinophilic fibers, more fibroblasts in the marginal areas. On day 14, the epithelialization of the wound edges progresses, the epithelium is thickened with signs of hyper- and parakeratosis and acanthosis, and microabscesses are formed.

The granulation tissue matures, collagen fibers are bundled, and cellular elements are represented by fibroblasts, macrophages, and neutrophils. On the 28-60th day, epithelialization is uneven: in some areas, the epithelium is thickened, in others it is thinned or absent, with signs of inflammation and necrosis, and secondary granulations are formed. The granulation tissue is voluminous, with bundled collagen fibers and a predominance of fibroblasts.

Conclusions. The healing of the wound channels of the tongue and cheek after implantation of silver piercing clips is characterized by consistent morphological changes that reflect the stages of inflammatory and reparative processes. In the initial stages (7-14 days), purulent-necrotic detritus, intense infiltration

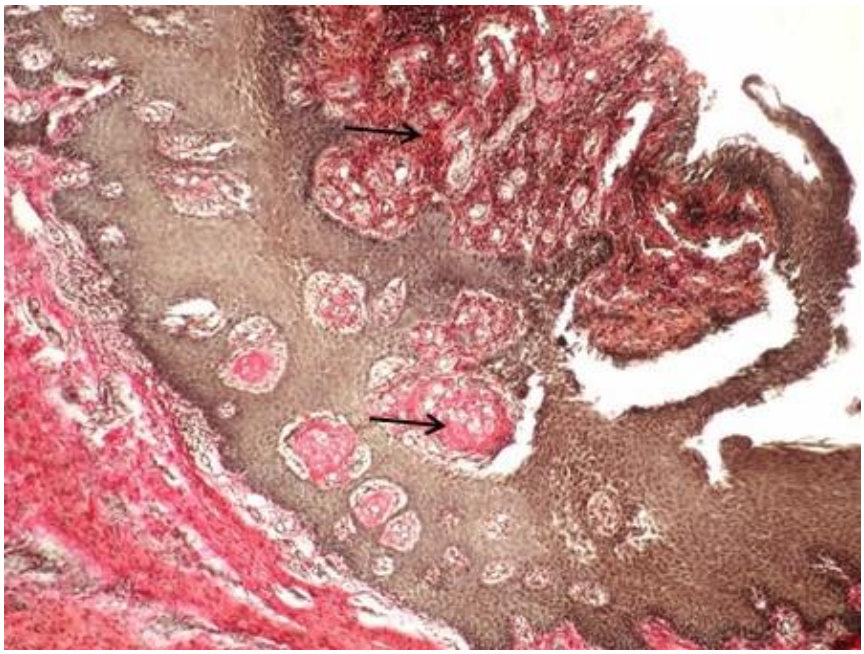

Fig. 9. Fragment of the wall of the tongue wound canal: thickened epithelial regenerate with hyperkeratosis and acanthosis; voluminous granulations intraepithelially and on the surface (arrows). Silver alloy piercing, 60 days, Van Gieson's stain, ×100

with neutrophils, and the formation of granulation tissue with active vascularization and the beginning of epithelialization, accompanied by acanthosis, hyper- and parakeratosis, dominate. Later (28-60 days), the epithelialization becomes uneven, with areas of thickened, thinned or absent epithelium, the presence of microabscesses, inflammation and necrosis, which indicates chronicity of the inflammatory process. The granulation tissue matures, forming bundled collagen fibers and predominantly fibroblasts, but secondary granulations are observed, which can complicate complete tissue repair. The data obtained indicate prolonged irritation and impaired normal tissue healing when using silver piercing clips, which requires attention when choosing materials for implantation in the oral cavity.

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