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The course of the wound process after intraoral piercing with gold alloy clips in the experiment

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Abstract

Background. Oral piercing remains an urgent problem in modern dentistry due to its growing popularity among young people. Studies show that about 5-20% of young people have oral piercings, with the tongue as the most common site, requiring attention to oral health risks. High-risk complications include tooth chipping, gum recession, tissue trauma, systemic infections, and aspiration or impaction of jewelry, necessitating immediate intervention and prevention.

Aim. To determine the effect of gold alloy clips on the wound healing process in cheek and tongue tissues after implantation in experimental animals.

Materials and methods. The study involved 8 chinchilla rabbits, following European standards for animal protection and the Ethics Committee protocol of Kharkiv National Medical University (№6, June 5, 2013). Gold clips were implanted in the soft tissues of the

tongue and cheek. Histologic analysis was performed on days 7, 14, 28, and 60 at the Central Research Laboratory and Department of Pathological Anatomy. Samples were fixed in 10% neutral formalin, dehydrated, embedded in paraffin, sectioned at 4-5 μm, and stained with hematoxylin and eosin for general assessment and Van Gieson for connective tissue. Sections were examined and photographed using an Olympus BX-41 microscope.

Results. On day 7, initial epithelialization of the tongue and cheek wound canals was observed, with epithelium covering less than a third of the canal and granulation tissue containing collagen bundles and single cells. By day 14, epithelialization progressed, covering over half the canal with high regenerative cells and ordered collagen bundles. On days 28 and 60, canals were fully epithelialized, with thickened epithelium showing acanthosis and hyperkeratosis, and subepithelial tissue as mature connective tissue with ordered collagen bundles, single cells, and no inflammation or microcirculatory disorders.

Conclusions. Epithelialization of tongue and cheek wound canals progresses in stages, completing by day 60 with epithelial thickening, acanthosis, and hyperkeratosis. Subepithelial granulation tissue matures into connective tissue with ordered collagen bundles, sparse cells, and reduced vessels. The morphology indicates effective regeneration without inflammation or microcirculatory issues, confirming high recovery potential of oral tissues.

Keywords: intraoral piercing; gold alloy; rabbits; granulation tissue; purulent inflammation

Introduction. Oral piercing remains an urgent problem in modern dentistry due to its growing popularity among young people [1]. According to epidemiological data, this practice is prevalent among adolescents and people aged 18-35 years, often as a form of self-expression or cultural influence, which leads to an increase in the number of complications [2]. Studies show that about 5-20% of young people have oral piercings, with the tongue being the most common location, which calls for attention to the potential oral health risks [3]. However, there is a high risk of complications such as tooth chipping, gum recession, soft tissue injuries, and systemic infections [4]. This is especially important in the context of increasing cases of jewelry aspiration or impaction, which requires immediate intervention and preventive measures [5]. In addition, the problem is gaining social significance, as piercings can affect psychological well-being, social adaptation, and professional opportunities, especially in the medical or educational fields [6]. In general, the relevance of the topic emphasizes the need for an interdisciplinary approach: dentists, infectious disease specialists, and public organizations should develop educational programs, standards for

jewelry materials, and monitoring protocols to minimize risks and improve the quality of life of patients [7].

The aim of the study was to determine the effect of gold alloy piercing clips on the dynamics of the wound process in the cheek and tongue tissues after implantation in experimental animals.

Materials and methods. The study was conducted on chinchilla rabbits (total number - 8 animals). All experimental procedures were in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, March 18, 1986), the Council of the European Economic Community Directive on the Protection of Vertebrate Animals (Strasbourg, November 24, 1986) and were approved by Protocol No. 6 of the meeting of the Ethics and Bioethics Committee of Kharkiv National Medical University of June 5, 2013. The animals were implanted with gold alloy clips in the soft tissues of the tongue and cheek. To analyze the changes in the piercing channel over time, histological examinations were performed on days 7, 14, 28, and 60 after implantation. Morphological analysis of the soft tissues of the tongue and cheek was performed at the Central Research Laboratory and the Department of Pathological Anatomy of Kharkiv National Medical University. This study was based on the morphological study of the development of the inflammatory process when using gold alloy piercing clips. In animals withdrawn from the experiment, samples of the tongue and cheek were taken from the places where the clips were installed. The collected material was preserved in a 10% aqueous solution of neutral formalin. After the alcohol treatment, the material was subjected to paraffin embedding, and then sections of 4-5 μm thickness were prepared. Hematoxylin and eosin staining was used to assess the general condition of the tissues studied, and the Van Gieson method was used to identify and distinguish connective tissue structures [6]. The analysis and description of microdissections, as well as microphotography were performed using an Olympus BX-41 microscope (Japan).

Results of the study and their discussion. On day 7, when examining the longitudinal section of the wound canal of the tongue, epithelialization was observed, where the newly formed epithelium on the dorsal side covered less than a third of the total length of the canal. The restored multilayered squamous epithelium was thin and contained a small number of epithelial cells [6] (Fig. 1).

In the deep parts of the wound canal without epithelialization and in the adjacent tissues, mild inflammation and moderate microcirculatory disorders were noted. The granulation tissue was represented by loops of thin-walled capillaries, between which there were undifferentiated cells, neutrophilic granulocytes, plasma cells and labrocytes.

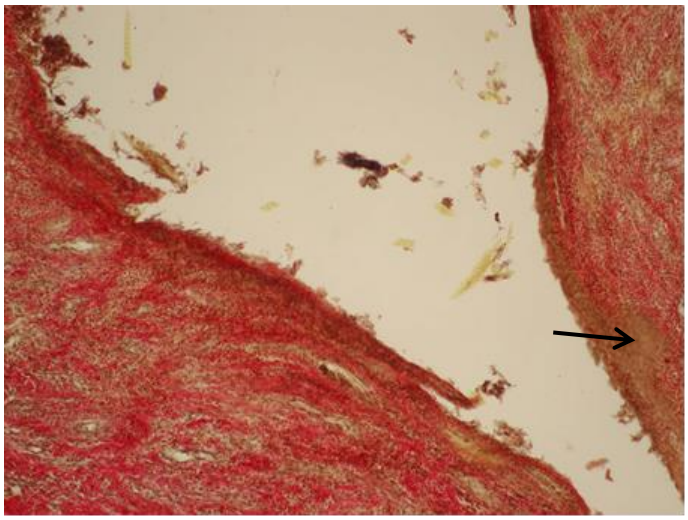

Fig. 1 Wound canal of the tongue with its partial epithelialization (arrow). Gold clip. 7th day. Van Gieson's stain. ×10

The fibrous layer was not detected. In the subepithelial areas of the granulation tissue, there was an increase in collagen fibers that formed bundles, with a simultaneous decrease in the number of vessels and their differentiation into small arteries and veins. Collagen fibers showed increased fuchsinophilia, indicating maturation, with neutrophilic granulocytes predominating among the cells. Sarcoplasm of muscle cells in the area of the wound canal with foci of basophilia, many cells are nucleated.

On the 7th day, in most of the studied samples of the transverse section of the cheek wound canal, an epithelialized wound canal was observed. The multilayered squamous epithelium was formed by numerous rows of epithelial cells, with the surface layer showing signs of keratinization (Fig. 2).

In the granulation tissue, collagen fibers form bundles and show increased fuchsinophilia, indicating their maturation. Among the cellular components there are lymphocyte-like cells, plasma cells, labrocytes, and single neutrophilic granulocytes (Fig. 3).

On the 14th day, epithelialization was observed on the longitudinal section of the wound canal of the tongue, and the newly formed epithelium on the dorsal surface covered more than half of the length of the canal. The regenerated multilayered squamous epithelium consisted of squamous cells, granular cells with keratohyaline granules in the cytoplasm, as well as spinous and basal cells. In some small areas, the epithelium formed "recessed" acanthotic outgrowths (Fig. 4).

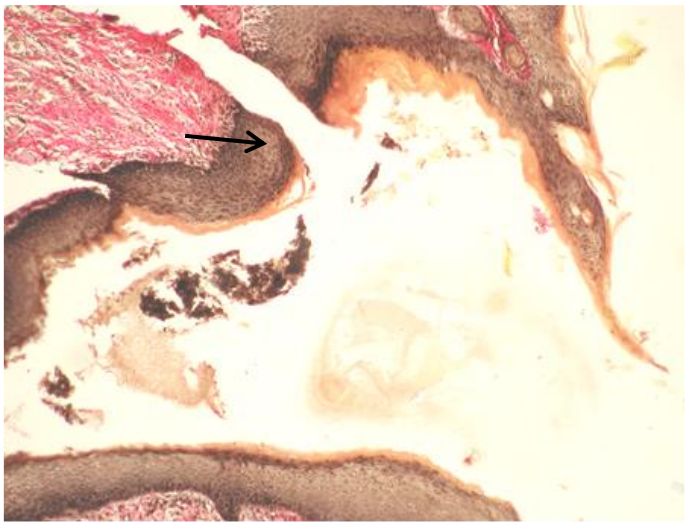

Fig. 2 Transverse section of the cheek wound canal with its epithelialization around the entire circumference (arrow). Gold clip. 7th day. Van Gieson's stain. ×100

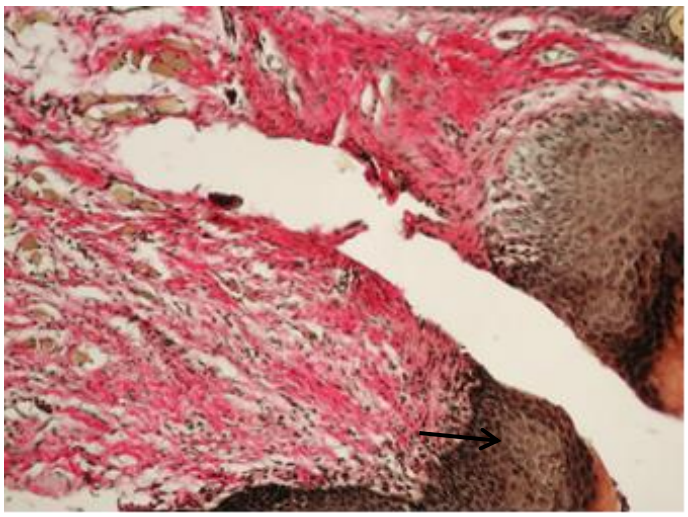

Fig. 3 Subepithelial fuchsinophilic fibers forming a bundle structure (arrow) are identified. Gold clip. 7th day. Van Gieson staining. ×200

Proliferating squamous epithelium cells show polymorphism and hyperchromatic nuclei, with basophilic cytoplasm indicating high RNA content and regenerative potential. The epidermal basal membrane is uniformly thick and firmly attached to granulation tissue. Mild subepithelial inflammation includes lymphocytes, neutrophils, plasma cells, and labrocytes. Granulation tissue has increased collagen fiber bundles with enhanced fuchsinophilia, reflecting maturation, alongside reduced vessels differentiated into small arteries and veins. Neutrophilic granulocytes are also present.

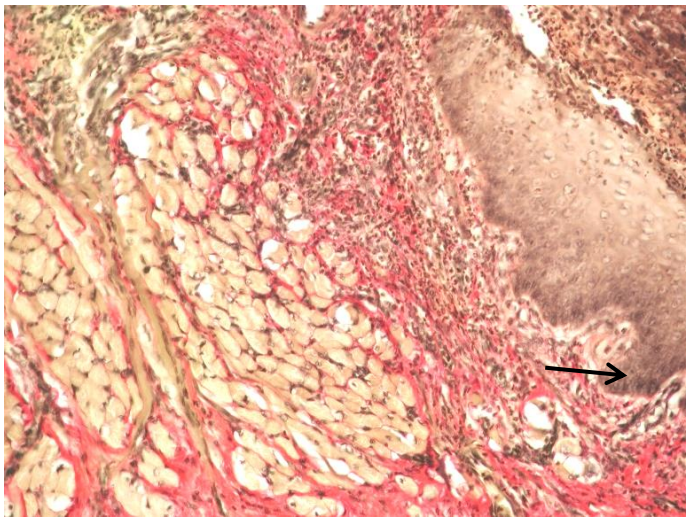

Fig. 4. The longitudinal section of the tongue wound canal shows its epithelialization. Epithelium with "submerged" acanthotic growths (arrow). Gold clip. Day 14. Van Gieson stain. ×200

On the 14th day, most samples showed complete epithelialization of the cheek wound canal with a multilayered squamous epithelium featuring a wide plate, acanthotic outgrowths, and surface keratinization. Subepithelial granulation tissue exhibited collagen fibers with increased fuchsinophilia, indicating maturation, alongside single fibroblast-like cells, lymphocytes, plasma cells, labrocytes, and neutrophilic granulocytes (Fig. 5).

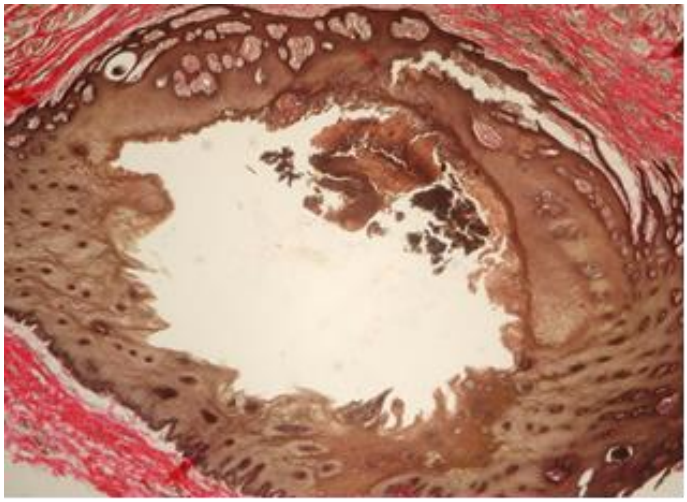

Fig. 5 Epithelialized wound channel of the cheek. Gold clip. 14 days. Van Gieson's stain. ×40

On day 28, the longitudinal section of the tongue wound canal wall was covered with squamous epithelium over most of its length. The epithelial layer was thickened, with acanthotic outgrowths and signs of hyperkeratosis. The subepithelial tissue is represented by maturing granulation tissue, in which collagen fibers show high fuchsinophilia, are ordered

and form bundles. Fibroblasts and fibrocytes, lymphoplasmacytic cells, and single neutrophilic granulocytes were noted among the cellular components of the granulation tissue (Fig. 6).

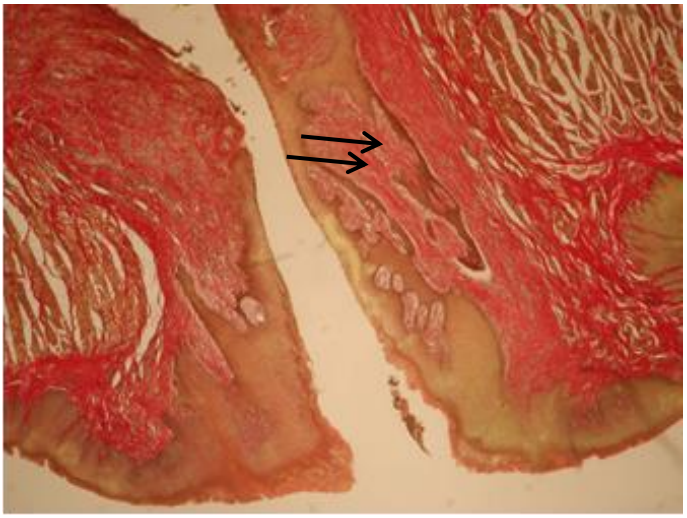

Fig. 6 Epithelialized wound canal of the tongue. The epithelial layer shows hyperkeratosis and acanthosis (arrow). The subepithelial tissue is represented by maturing granulation tissue (two arrows). Gold clip. Day 28. Van Gieson's staining. ×40

On the 28th day, complete epithelialization was observed on the transverse section of the cheek wound channel. The multilayered squamous epithelium consisted of numerous rows of cells, and the surface layer showed signs of keratinization. The epithelial layer was tightly integrated with the underlying tissue.

The subepithelial granulation tissue was transformed into connective tissue, as evidenced by the presence of intensely fuchsinophilic collagen fibers grouped in bundles. There were no signs of inflammation and microcirculatory disorders (Fig. 7).

On the 60th day, complete epithelialization was noted on the longitudinal section of the tongue wound canal. The epithelial layer was thickened, with acanthotic outgrowths and signs of hyperkeratosis. The subepithelial tissue consisted of ordered bundles of collagen fibers. The cellular elements were single, represented by fibroblasts, fibrocytes and rare lymphoplasmacytic cells (Fig. 8)

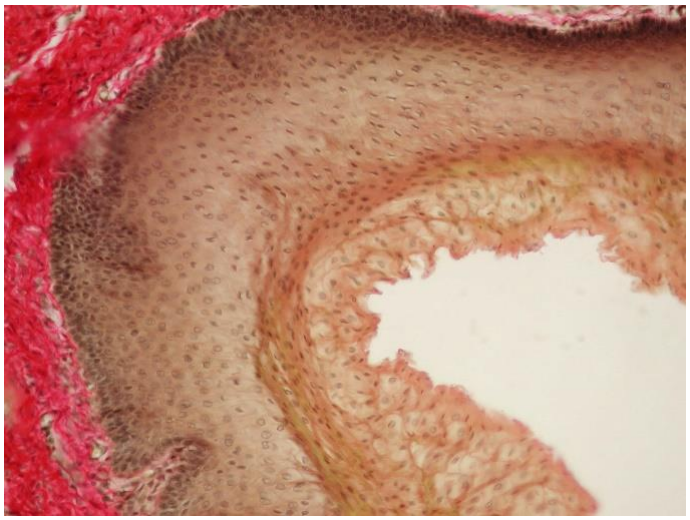

Fig. 7 Transverse section of the cheek wound canal with its complete epithelialization. The epithelial layer is thickened, tightly connected to the underlying tissue. Subepithelially, intensely fuchsinophilic collagen fibers are grouped in bundles. Gold clip. 28th day. Van Gieson's coloration. ×200

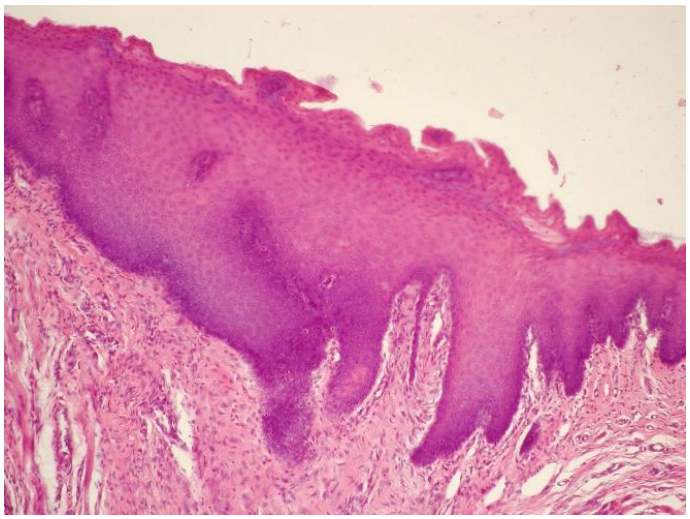

Fig. 8 Wound canal of the tongue. Thickened epithelium with hyperkeratosis and acanthosis. Subepithelial tissue - collagen bundles. Single cells: fibroblasts, fibrocytes, rare lymphoplasmacytic elements. Gold clip. 60 days. Hematoxylin-eosin, ×100.

On the 60th day, the cheek wound canal was completely epithelialized around the circumference and along its entire length. The multilayered squamous epithelium showed signs of keratinization and acanthosis. The basal membrane of the subepithelial tissue was thin, well-defined, with a tight connection of the epithelial layer with the underlying tissue. Mature connective tissue was observed subepithelially (Fig. 9)

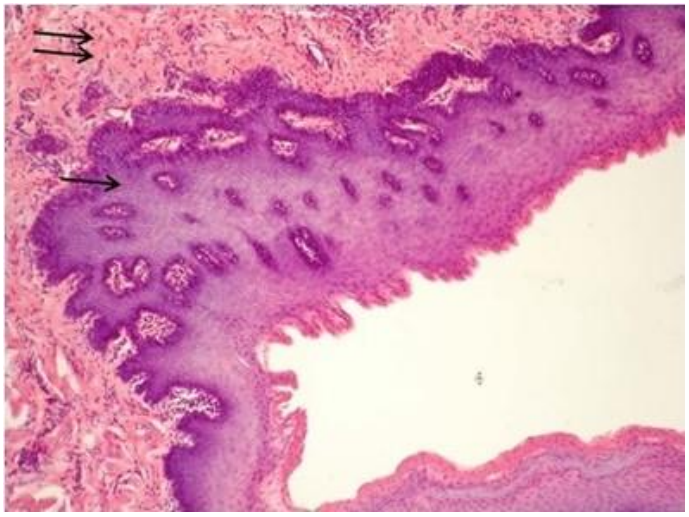

Fig. 9 Transverse section of the cheek wound channel with its complete epithelialization. Signs of hyperkeratosis and acanthosis of the epithelium (arrow). Mature connective tissue is found subepithelially (two arrows). Gold clip. Day 60. Hematoxylin and eosin staining. ×100.

Thus, our study of the morphological changes in the wound canal of the tongue and cheek showed that the healing process is gradual and completed within 60 days. In the early stages (7-14 days), initial and progressive epithelialization is observed with the development of granulation tissue containing mature collagen fiber bundles and a limited number of cells. In the later stages (28-60 days), the wound channels are completely epithelialized, the epithelium is thickened with signs of acanthosis and hyperkeratosis, and the subepithelial tissue is formed into a mature connective tissue with ordered collagen bundles and single cellular elements. The absence of signs of inflammation and microcirculatory disorders indicates the effectiveness of regenerative processes and the high regenerative potential of the oral tissues.

Conclusions. The process of epithelialization of the wound canal of the tongue and cheek occurs in stages and is completed by the 60th day after the injury, with a gradual thickening of the epithelial layer, the formation of acanthotic outgrowths and signs of hyperkeratosis. The subepithelial granulation tissue is transformed into mature connective tissue, as evidenced by the formation of ordered collagen fiber bundles, a decrease in the number of vessels, and a single arrangement of cellular elements. Morphological changes indicate effective reparative regeneration, absence of inflammation and microcirculatory disorders in the late stages of healing. The data obtained confirm the high ability of oral tissues to recover and can serve as a basis for evaluating the effectiveness of treatment and regenerative strategies in dental practice.

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