

СРПСКО ЛЕКАРСКО ДРУШТВО
ОКРУЖНА ПОДРУЖНИЦА КРАГУЈЕВАЦ

Med. čas.
ISSN 0350-1221
COBISS.SR-ID 81751559
UDK 61

МЕДИЦИНСКИ ч а с о п и с

Основао 1961.

Годиште 58

Свеска 3

Стране 83-132.

Founded 1961.

Volume 58

Issue 3

Pages 83-132.



MEDICAL JOURNAL
Serbian Medical Society
Section Kragujevac

Крагујевац 2024.

www.medicinskicasopis.org

Крагујевац 2024.

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Okružna podružnica Kragujevac

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Serbian Medical Society
Section Kragujevac

IZLAZI ČETIRI PUTA GODIŠNJE**ISSUED QUARTERLY****TIRAŽ - 500**

Štampa se latinicom i ćirilicom

CIRCULATION - 500

Printed in Latin and Cyrillic

Adresa uredništva

SLD, Okružna podružnica Kragujevac
Univerzitetski Klinički centar "Kragujevac"
Zmaj Jovina 30, 34000 Kragujevac
Tel. 034-372-169
Tel/faks 034 -337-583
www.medicinskicasopis.org
e-mail: medicinskicasopis@gmail.com

Address of Editorial Office

Serbian Medical Society, Section Kragujevac
Univeristy Clinical Centre Kragujevac
Zmaj Jovina 30, 34000 Kragujevac, Serbia
tel. +38134-372-169
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ISSN 0350-1221 = Medicinski časopis / Medical Journal / Med. čas.
COBISS.SR-ID 81751559 / UDK 61

Lektor za srpski jezik - Serbian Language Editing: Katarina Pišteljić

Lektor za engleski jezik - English Language Editing: Marko Banković

Tehnički sekretar - Technical Secretary: Jasmina Jovanović

Sekretar – coordinator: Doc.dr Dalibor Jovanović

Kompjuterski slog - Computer Layout: "Spektar 7" d.o.o., Kragujevac

Naslovna strana - Cover Page: Jovana Anić

Štampa - Printed by: "Spektar 7" d.o.o., Kragujevac

GODIŠNJA PRETPLATA: 1.200,00 dinara za pojedince (500,00 dinara za studente), 3.500,00 za ustanove.
Pretplatu vršiti na: Tekući račun 150-12509-53 SLD „Medicinski časopis” Kragujevac (kopije uplatnica slati na adresu časopisa).

ANNUAL SUBSCRIPTION: 25 Euros for Individuals (10 Euros for Students), 70 Euros for Institutions.
Bank transfer instructions. Beneficiary customer: RS35150007180000049637 SLD OKRUZNA PODRUZ
KRAG ZMAJ JOVINA 30 KRAGUJEVAC. Account with institution: Direktna Banka AD Kragujevac SWIFT:
KRAGRS22. Intermediary bank: BKAUATWW UNICREDIT BANK AUSTRIA or PIRBGRAA PIRAEUS BANK SA,
ATHENS or VTBRUMM VTB BANK (PJSC) MOSCOW. (send a copy of payment on Journal address).

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SUBEPITHELIAL CONNECTIVE TISSUE GRAFT FOR ROOT COVERAGE: CLINICAL CASE REPORTS AND HISTOLOGIC EVALUATION

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СУБЕПИТЕЛНО ПРЕСАЋИВАЊЕ ВЕЗИВНОГ ТКИВА ЗА ПОКРИВАЊЕ КОРЕНА: ПРИКАЗ СЛУЧАЈЕВА И ХИСТОЛОШКА ЕВАЛУАЦИЈА

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ABSTRACT

Objective. The goal of periodontal surgery is to cover the exposed root completely or partially in teeth with gingival recession. This correction is important from an aesthetic and a functional point of view. Periodontal regeneration is visible only through histologic analysis. Histologic analysis can reveal the arrangement of the cells embedded in the periodontal-tissue attachment. Micro punch gum technique (2 mm) was used for biopsy.

Method. Three patients with gingival recessions were treated with subepithelial connective tissue graft (CTG) and clinical parameters and histological results were recorded.

Results. Complete coverage of the exposed root part was obtained in the three cases, and the histologic analysis revealed a selective settlement with ligament cells that are typical for the formation of connective tissue in periodontal regeneration. Histologic analysis showed keratinized sulcular epithelium with thin and tall extensions into the gingival connective tissue and a junctional epithelium which extended to new cementum. Also increased number of blood vessels and new insertion of the connective tissue was observed. If the clinical goal of periodontal surgery is complete coverage of gingival recessions, then the goal at the microscopic level is to obtain true periodontal regeneration.

Conclusion. CTG is the gold standard in the selection of grafts to resolve recessions. From the choice of gingival flaps, CAF (coronally advanced flap) was and still is number 1, but also the laterally displaced flap gives quite good results in single recessions.

Key words: gingival recession; elastic tissue; collagen; connective tissue.

САЖЕТАК

Увод. Циљ пародонталне хирургије јесте да се откријени корен потпуно или делимично покрије у зубима с рецесијом гингиве. Ова корекција је важна са естетске и функционалне тачке гледишта. Пародонтална регенерација видљива је само хистолошким анализом. Хистолошка анализа може открити распоред ћелија уграђених у прилог пародонталног ткива. За биопсију је коришћена техника микробушења гуме (2 мм).

Метод. Три пацијента са рецесијом гингиве лечена су субепителним графтом везивног ткива (CTG) и забележени су клинички параметри и хистолошки резултати.

Резултати. У три случаја добијена је потпуна покривеност експонираног дела корена, а хистолошким анализом утврђено је селективно насељавање лигаментних ћелија које су типичне за формирање везивног ткива у пародонталној регенерацији. Хистолошка анализа је показала кератинизовани сукуларни епител са танким и високим продужецима у везивно ткиво гингиве и спојни епител који се проширио до новог цемента. Уочен је и повећан број крвних судова и ново уметање везивног ткива. Ако је клинички циљ пародонталне хирургије потпуна покривеност рецесије гингиве, онда је циљ на микроскопском нивоу да се добије права пародонтална регенерација.

Закључак. CTG је златни стандард у избору графтова за решавање рецесије. По избору гингивалних режњева, CAF (коронарно померени флап) био је и остао број 1, али и латерално померени режањ даје прилично добре резултате у појединачним рецесијама.

Кључне речи: рецесија гингиве; еластична влакна; колаген; везивно ткиво.

INTRODUCTION

It is common knowledge among clinicians and scientists that parodology is the science of the periodontium. It is about the supporting tissues of the teeth that provide all the disorders that occur in: the gingiva, the periodontium, the alveolar bone and the cementum of the tooth. The name comes from the Latin word (para) – which means: beside, end and what is the word (odus) – which means: tooth. The basic and main role of the periodontal is to secure the tooth in the alveolus, which is why it is important in maintaining the function of the masticatory apparatus. Developmentally and embryologically monitored, it has been proven that the beginnings of the periodontal tissue are parallel to the formation of the teeth.

As an integral part of the oral cavity, deviations that occur at different levels have repercussions on this very important organ. Thus, the changes in the oral flora, in which the central place belongs to anaerobes, are reflected by the appearance of certain inflammatory, destructive or resorptive changes in all tissues of the periodontium. A special place from this point of view belongs to the dental plaque. With its mechanisms (enzymes, toxins, etc.), it strongly affects changes in the periodontium. Pathogenetic developments are reflected in the clinical manifestation of the periodontium, which is reflected by subjective symptoms and objective findings. Hence the need for therapy is imminent and imperative.

In certain conditions, conservative therapy is quite sufficient to achieve an adequate effect, but sometimes conservative therapy is insufficient, and the only real therapy is surgical treatment. Relying on the fact that regeneration cannot be achieved with conventionally applied therapy, the application of certain techniques to achieve the goals of each therapist is fully justified. The results in most of the interventions are not absent, and are evident through the assessment and monitoring of certain parameters and criteria, which are essential for determining the diagnosis and prognosis of the disease (1).

The same degree of destruction does not always occur under the same circumstances, nor under different circumstances. Somewhere the resorptive and destructive processes progress, and somewhere they stagnate. The answer to this dilemma lies in the existence of various factors that influence the periodontal status. The body's weakened immunity in the presence of many other risk factors, including pro-inflammatory mediators and bacteria from dental plaque, leads to inflammation of the periodontium. The inflammation of the gingiva, which is not treated at all or is treated inappropriately, progresses further and affects the remaining structures of the periodontium and causes damage to the periodontium, which is the most common cause of recession. Reversible

inflammation of the gingiva progresses to the destruction of the periodontal ligament, resorption of cement and destruction of alveolar bone, which is essentially a response to the dental plaque tissue that has accumulated on the teeth and in its immediate surroundings.

Under the influence of plaque, disintegration of collagen and breakdown of alveolar bone occurs, while the epithelium of the attached gingiva passes into the epithelium of the pocket, which proliferates apically and laterally. This is how the periodontal pocket is created, which is a pathognomonic sign of periodontal disease, and also a predilection site and a kind of reservoir of opportunistic pathogenic bacteria that potentiate inflammation and encourage the progression of the disease. The process progresses, and in inappropriate circumstances ends with the loss of the tooth. Because of this, continuous and timely therapy is the basic procedure to be taken when dealing with an unwanted progressive condition. In essence, periodontal therapy aims at regeneration of periodontal tissues, restoring their initial condition and function, i.e. establishment of normal and physiological activity in terms of function and aesthetics. In order to achieve this goal in periodontal surgery, especially when addressing recessions, a correctly set indication and the right choice of surgical technique are required. In this area there are many possibilities, quite a large number of techniques that can be the choice of any therapist in periodontal surgery. In addition to the various solutions in periodontal and regenerative surgery, the materials that can be selected as bone substitutes are important for the periodontist, in order to use regenerative periodontal surgery, whose effects would have repercussions on the patient's clinical condition. Knowing the properties of materials, their advantages and disadvantages is necessary, for the reasons that the right choice should be made, which can enable their maximum use in the interest of patients (2). The etiology of gingival recession is multifactorial and the factors that cause recessions are mostly heterogeneous and diverse and according to Zucchelli they are divided into the following three groups: anatomical, physiological and pathological.

Anatomic factors associated with gingival recession include the presence of fenestration and dehiscence of alveolar bone, abnormal position of the tooth in the dental arch, inappropriate path of eruption of the tooth, and the shape of the tooth that has recession (3).

Physiological factors include the orthodontic movements of the teeth outside the alveolar arch, which leads to the formation of dehiscence (4). and which act as a "locus minoris resistentiae" for the development of the recession.

Pathological factors include: improper brushing caused by various factors of potentially confounding variables such as: pressure, time, strength and the cleaning

agent used (5), then the use of dental floss, perioral and intraoral piercings, direct trauma due to malocclusion, partial restorative therapy, diseases such as herpes simplex virus (6), as well as the presence of dental plaque.

From an etiological aspect, mucogingival anomalies according to Carranza (7) are classified as: developmental, congenital and acquired.

Developmental anomalies are related to the period of replacement of milk teeth with permanent ones, and the problems arise due to the beginnings of permanent teeth that occupy a more vestibular position in relation to the milk ones, thus reducing the width of the keratinized gingiva, which is key to the occurrence of recessions.

Congenital anomalies of the gingiva are conditioned by the genotypic information that is written in the DNA of each individual.

Acquired anomalies occur as a result of damage to the gingiva for various reasons.

From a prospective study in India that included 710 patients (8), it was concluded that recession occurs in a higher percentage in the male population than in the female population. Recession is most common in the area of lower frontal incisors and it occurs mostly in Miller I classification, while according to the etiological cause, dental plaque takes the leading role, and incorrect brushing technique comes second. In another study, the authors looked at the epidemiological causes of recession, as well as its prevalence in certain groups of patients: recession acquired by trauma, the association of recession with gender, individuals with malocclusion of teeth, inflammation as a cause of recession, as well as the use of tobacco (9).

The influence of tobacco, apart from the appearance of recessions as one of the etiological factors, according to Anisha P. Yadav, significantly affects the postoperative course of recessions and achieving the best possible results (10).

The results showed that 88% of people aged 65 and older have the most recession teeth. A second important point in this research shows that 50% of people between the ages of 18 and 64 present with one or more areas of recession, which means that the presence and degree of gingival recession increases with age.

The group of 50% of the subjects who have one or more teeth with recession have a tooth neck exposure of 1 mm or more. Recession monitoring was conducted in patients with good and poor oral hygiene. Hence, it is confirmed that the recession is multifactorial. One type of recession is associated with anatomical factors and another type with physiological or pathological factors. However, it was observed that it is more often found on the buccal surfaces of the teeth than on the rest.

The esthetics of the gingiva is becoming an important concern in dentistry as well as the esthetics of the teeth in

the mouth. Just as teeth with a beautiful shape, size and color are necessary for a beautiful aesthetic smile, the morphology of the gingiva is also important (11-15). Apart from being important from an aesthetic point of view, recession is also associated with hypersensitivity of the exposed root part as well as patients' fear of tooth loss. However, from all aspects, the treatment of recessions is a rather complex procedure where the success of the intervention depends on many reasons. Among those that are worth pointing out are the following: the initial condition, the biological capacity of the tissue, the choice of the technique, the blood supply and the regenerative potential of the periodontal tissue (16). The result of periodontal surgical treatment depends to a large extent on the choice of the technique and guided tissue regeneration (GTR) is recommended in the literature, the main benefit of which is the creation of a new periodontal-tissue attachment (17). Otherwise, there are several methods for the treatment of recessions: surgical flap methods and methods using a graft in combination with different types of flaps. According to Chambrone (18-20) the use of CTG histologically confirms the finding of increased keratinized tissue that protects against marginal inflammation and trauma. The application of enamel matrix derivatives Emdogain as well as growth factors derived from PRF platelet-enriched fibrin membrane (21) also lead to the creation of new cementum with functional collagen fibers on the exposed part of the tooth root (22).

Acellular cementum signals the development of the periodontal ligament, followed by the formation of new alveolar bone and a new periodontium of the tooth. Emdogain acts as a tissue healing modulator, mimics the events that occur during root development and helps stimulate periodontal regeneration (23).

Although the clinical results are quite predictable, with the application of different types of periodontal flaps in combination with auto and xenograft, the histological results can only be compared with a biopsy sample of the transplanted tissue of the patients. Taking material for biopsy is a difficulty encountered by many foreign authors in convincing patients to consent to biopsy despite the fact that the technique is minimally invasive. The comparisons are more from studies of animal origin as well as biopsies after extraction of teeth that have already been transplanted with auto or xenograft. In these biopsies, periodontal regeneration is recorded, which is histologically confirmed. Adhesion between the root and graft surface indicates that healing occurs primarily by the formation of a new periodontal tissue attachment between the root and the graft (24). The aim of this study was to make a histological verification in the intervened segment and a comparison of the clinical results between the coronary displaced flap and the laterally displaced flap after 6 months of treatment.

PATIENTS***Case No. 1, patient description***

24-year-old female visited the Oral Surgery Clinic at UDCC "St. Panteleimon" in Skopje with the chief complaint, hypersensitivity of the tooth to thermal stimulus and the fear of losing it. The patient had a Miller II recession of the lower right central incisor measuring 3 mm, PPD 0.5 mm and KTW 1.5 mm (Figure 1).

The patient had excellent oral hygiene, was a non-smoker, orthodontic treatment was etiological factor for recession. The surgical and histological protocols have been approved by the Ethics Committee of the Faculty of Dentistry at the University "St. Cyril and Methodius" in Skopje, the Republic of North Macedonia (09-599/10.05.2016) Informed consent form was obtained from the patient after a careful explanation of the surgical procedure, prognosis, and possible complications. A treatment plan was made to cover the root of the tooth with a coronally advanced flap and a free connective tissue graft. Initiate treatment included conventional therapeutic methods for the treatment of periodontal diseases: treatment of pockets and cleaning of tartar, including instructions for maintaining the correct method of teeth brushing. The surgical procedures were performed by the same surgeon and the histological analysis was performed at the Institute of Pathological Anatomy at the Faculty of Medicine, Skopje.

Surgical procedure

Each oral-surgical intervention was performed after the application of local anesthetic 3% Scandonest in the form of plexus anesthesia using a carpal syringe for n. maxillaris and mandibularis. With the periodontal surgery instrumentation of Carl Martin GmbH Solingen from Germany, single and multiple gingival recessions have been treated. With scalpel No. 15, 2-3 mm from the marginal gingiva on the mesial side of the first molar, an incision was made up to the periosteum, directing it parallel to the position of the teeth, to the distal side of the canine. During the intervention, rugae palatinae were not included to ensure good aesthetic results. By placing the scalpel parallel to the longitudinal axis of the teeth an injury of a. palatina was avoided, and at the same time, the connective tissue was separated from the periosteum and from the lamina propria in the length required for the grafting site. After the operative intervention, sutures were placed on the palatum, and primary hemostasis was performed with 5 minutes of digital compression. The graft was sutured to the appropriate place (recipient site) with resorbable Vicryl sutures with 5-0. The rest of gingiva was sutured with non-absorbable sutures (12). The patient was instructed to stop brushing teeth for 3 weeks and to avoid trauma to the surgical site and to rinse the wound with 0.2% chlorhexidine digluconate with hyaluronic acid 1% (Curasept) three times a day. Sutures were removed after 3 weeks. Clinical measurements were recorded at baseline before the operative intervention, after 3, 6, 9 and 12 months using an ASA Dental periodontal graduated probe.

Table 1. Case no. 1, clinical parameters preoperative, 3, 6, and 12 months postoperatively.

Measured mid-buccal parameters in mm	Preoperative	3 months postoperative	6 months postoperative	12 months postoperative
Recession - R	3	1.5	0	0
Periodontal pocket - PD	0.5	0	0	0
Keratinized tissue - KW	1.5	3	3	5
Clinical attachment level - CAL	4	4.5	4.5	4.5

Table 2. Case no. 2, clinical parameters preoperative, 3, 6, and 12 months postoperatively.

Measured mid-buccal parameters in mm	Preoperative	3 months postoperative	6 months postoperative	12 months postoperative
Recession -R	5.5	0	0	0
Periodontal pocket - PD	2	1.5	0	0
Keratinized tissue -KW	0.5	7	7	7
Clinical attachment level - CAL	7.5	1.5	1	1

Table 3 – Case no. 3, clinical parameters preoperative, 3, 6, and 12 months postoperatively.

Measured mid-buccal parameters in mm	Preoperative	3 months postoperative	6 months postoperative	12 months postoperative
Recession - R	5	1	1	2
Periodontal pocket - PD	0.5	0	0	0
Keratinized tissue - KW	1	3.5	3.5	3
Clinical attachment level - CAL	6	1.5	3	3

Preparation for biopsy

For histological examination, a biopsy was taken from the transplanted site with the micropunch gum technique in a size of 2 mm after previous application of 3% local anesthetic Scandonest. The biopsy was performed 6 months after the intervention. The biopsy was taken from the lower parts away from the grafting site, thus avoiding disturbing the achieved goal of the grafting site by holding the micropunch with the right hand and with a little pressure, we gently rotated to the bone. Then the micropunch was removed, and the material that did not come out with the micropunch was lifted from the substrate with the help of a plastic instrument. The biopsy material was 2 mm in diameter and 3-4 mm deep. The collected material was fixed in neutral formalin in Eppendorf tubes for 6 to 18 hours and processed by paraffin embedding procedure. 4-6 micron sections were stained with hemalaon eosin on a LEICA automatic stainer. Furthermore, the material was subjected to histological analysis to determine the structure of the tissue in terms of collagens and elastic fibers.

For all subjects included in the research, a histological verification of the tissue sample was done, graded into 4 categories: a) immature collagen tissue; b) mature (normal) collagen tissue; c) fragmented collagen and d) edematous tissue.

In terms of the structure of the elastic fibers, in all subjects from both groups, findings were registered in the tissue samples, which are distributed in three groups: a) with a normal structure; b) fragmented rare and c) fragmented multiplied.

The images of the histological analysis were taken on a transmission electron microscope (TEM) at the Institute of Pathological Anatomy magnified 100 times, and the measurement of the thickness of the epithelium was performed with an orthodontic caliper.

Clinical analysis

Wound healing proceeded smoothly. The defect resulted in 100% coverage of the root surface after 12 months (Figure 2). The amount of keratinized tissue was 5 mm.

Histological analysis

The epithelium was 1 mm thick and parakeratized. The connective tissue was composed of collagen fragmented fibers of varying thickness and the presence of fusiform cells accompanied by rare blood vessels. Absence of inflammatory infiltrate was recorded (Figure 3).



Figure 1. Case No. 1, recession Miller II 3.5mm lower central incisor preoperative root coverage.



Figure 2. Case No. 1, recession Miller II on lower central incisor with 100% after 12 months postoperatively.

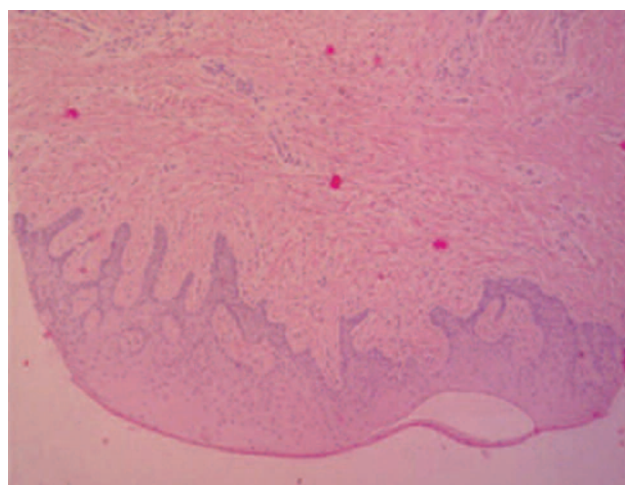


Figure 3. Case No. 1, recession Miller II on a lower central incisor collagen and elastic fibers after 6 months postoperatively.



Figure 4. Case No. 2, recession Miller II 7.5 mm lower central incisor preoperative root coverage.



Figure 7. Case No. 3, recession Miller II on left upper canine of 5.5 mm preoperatively.



Figure 5. Case No. 2, recession Miller II on lower central incisor with 100% after 12 months postoperatively.



Figure 8. Case No. 3, recessions Miller II on left upper canine with 100% root coverage after 12 months postoperatively.

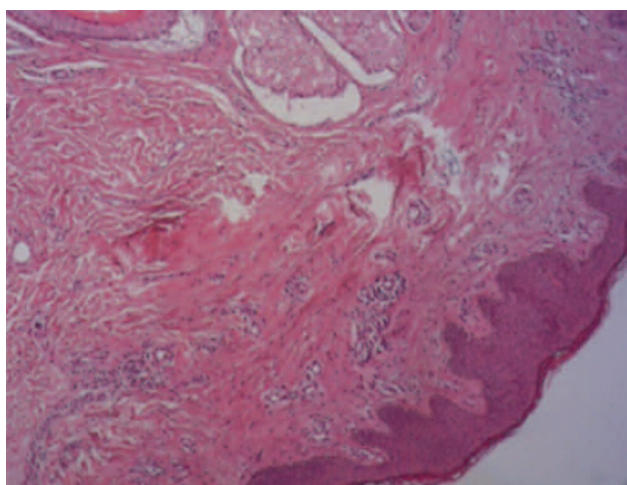


Figure 6. Case No. 2, recession Miller II on a lower central incisor collagen and elastic fibers after 6 months postoperatively.

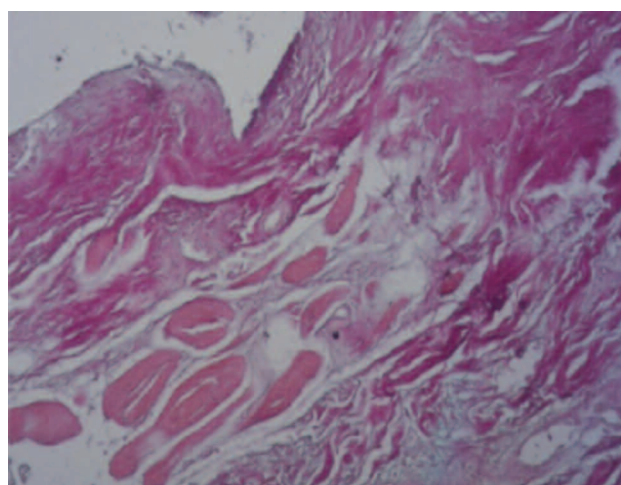


Figure 9. Case No. 3, Miller II recession on upper left canine Mature collagen and elastic fibers after 6 months postoperatively.

Case No. 2, description of the patient

20-year-old man visited the Oral Surgery Clinic at UDCC "St. Panteleimon" in Skopje with the chief complaint, hypersensitivity of the tooth to thermal stimulus and the fear of losing it. This patient, like the previous, was in good general condition, a non-smoker and had a great fear of losing the lower right central incisor with a Miller II class recession measuring 5.5 mm (Figure 4). The patient underwent resurfacing of the root surface with a laterally displaced flap and a free connective tissue graft. The surgical procedure and biopsy material collection were the same as in the previous case and the observation regarding the clinical results showed 100% root coverage after 12 months. An increase in the keratinized tissue was 7 mm in both cases and the periodontal pocket probing was reduced to 0 mm in both interventions.

Histological analysis

The epithelium was 1 mm thick and parakeratized. The connective tissue was composed of collagen fragmented fibers of varying thickness and the presence of fusiform cells accompanied by rare blood vessels. Absence of inflammatory infiltrate was recorded with the presence of elastic fibers and fragmented collagen tissue (Figure 6).

Case no. 3, description of the patient

23-year-old male patient visited the Oral Surgery Clinic at UDCC "St. Panteleimon" in Skopje with the chief complaint, hypersensitivity of the tooth to thermal stimuli. The patient, like the previous patients, was in good general condition, a non-smoker with a recession of the Miller II upper left canine measuring 5 mm (Figure 7). The patient underwent root surface resurfacing with a laterally displaced flap as in the previous patient and a free connective tissue graft. In this patient too, we have 100% root coverage after 12 months postoperatively (Figure 8).

The surgical procedure and biopsy material collection were the same as in the previous case and the observation of clinical results showed 100% root coverage after 12 months (Figure 9). An increase in the keratinized tissue was 3 mm in size and the probing of the periodontal pocket was also reduced to 0 mm in this intervention.

DISCUSSION

The free gingival graft is the oldest surgical technique used in periodontal surgery, where the graft can be taken from the palatum or maxillary tubercle. Clinically, the choice of the surgical technique as well as the first postoperative week is decisive for therapeutic success (25). The clinical results of the patients were excellent

with 100% root coverage in all three cases after 12 months of observation. A large percentage of orthodontic patients are indicated for periodontal surgery and these are mostly young patients who have irregular habits in terms of diet and home hygiene. In addition, orthodontic therapy is also a long-term treatment that increases the risk of bone dehiscence or fenestration due to vestibular placement of permanent teeth, and thus it represents an important factor for the development of gingival recession and our results are in accordance with Gebistorf (26).

The clinical results are not surprising considering that operative interventions in Miller class I and II give a 100% result and that the use of connective tissue graft is the gold standard. An increase in keratinized gingiva is a major factor against marginal inflammation or trauma. Individual recommendations and a protocol for maintaining oral hygiene were given to the patients after the surgical therapy. Postoperative brushing of the teeth at the grafting site should be done using a Curaprox Surgical Brush. The use of softer foods and avoidance of solid food contamination is advised to be avoided. The use of means for planning the oral cavity 3 times a day after every meal is inevitable. We recommended Chlorhexidinet digluconat 0.20% with hyaluronic acid 1%. The follow-up of the findings was carried out after 6 months, but unfortunately the assessment of the healing process and histological verification of the tissue has not been sufficiently processed and included in the literature available to us, and hence there is no possibility of confrontation of the results obtained from this study with other findings.

In conclusion, in this study, histological analysis showed that the sulcus epithelium is keratinized with thin and tall extensions, which project into the gingival connective tissue and a junctional epithelium that extends over the new cementum, which is in agreement with our results, where on the histological preparations, the border with the periosteum is observed unevenly elongated and thin papillae, in others uniform papillomatosis with high extensions and an increased number of blood vessels. New insertion of connective tissue including ligamentous epithelium was also observed. If the clinical goal of periodontal surgery is complete coverage of gingival recessions, then the goal at the microscopic level is to obtain true periodontal regeneration with periodontal tissue reattachment that is only proven by histological examination of the transplanted site.

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Medicinski časopis - Info

Basics:

ISSN	0350-1221
eISSN	2406-0380
variant title	Medical Journal
publisher(s)	Serbian Medical Society - Section Kragujevac, Kragujevac
web address	www.medicinskicasopis.org/arhiva.php
frequency	quarterly
starting year	1961
editor-in-chief	Radiša Vojinović, University of Kragujevac, Faculty of Medical Sciences, Department of Radiology, Serbia Dragan Milovanović, University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacology and toxicology, Serbia

Contact:

publisher address	Zmaj Jovina 30, 34000 Kragujevac
contact person	Radiša Vojinović
e-mail	medicinskicasopis@gmail.com
phone	034/372-169, 034/337-583

Status:

journal tier/category	A2: Journal of global importance and high publishing quality in research area: Medical and health sciences
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CEON WoS IF5	0.019
CEON IF5	0.078
indexed by	SCIndeks, EMBASE, SCOPUS, EBSCO
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