



Therapeutic efficacy of connective tissue autotransplants with periosteum and platelet rich plasma in the management of gingival recession

Terapijski efekat plazme obogaćene trombocitima i autotransplantata vezivnog tkiva sa periostom u zbrinjavanju gingivalnih recesija

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Abstract

Background/Aim. Gingival recession progression in clinical practice has influenced the development of various surgical procedures and techniques for solving esthetic imperfections and subjective difficulties caused by gingival recession. The aim of this study was to verify efficacy of surgical procedures and to compare both of surgical procedures through the keratinized tissue width. **Methods.** The study included 20 teeth with gingival recession, Müller class I and II. Ten teeth with gingival recession were treated with connective tissue autotransplants with periosteum in combination with coronary guided surgical flap (CTG group). On the contralateral side 10 teeth with gingival recession were treated with the same surgical procedures but in combination with platelet-rich plasma (CTG-PRP group). We measured the keratinized tissue width. For statistical significance we used the Student's *t*-test. **Results.** The study revealed a statistical significance in reducing vertical deepness of recession by both used treatments. Root deepness in CTG and CTG-PRP group was 90% and 93.5%, respectively. With both surgical techniques we achieved larger zone of keratinized gingiva but with a wide zone of keratinized tissue in CTG – the PRP group. **Conclusion.** The concept regeneration technique with PRP and with the stimulating influence of platelets activated growth factors results in the regeneration of deep periodontal tissue as an important prerequisite for the successful treatment of gingival recession.

Key words:

gingival recession; dentistry, operative; transplantation, autologous; platelet-rich plasma; treatment outcome.

Apstrakt

Uvod/Cilj. Sve veća zastupljenost gingivalnih recesija u svakodnevnoj kliničkoj praksi uslovlila je zahteve za što efikasnijim rekonstruktivnim hirurškim procedurama, u cilju rešavanja estetskih nedostataka i subjektivnih tegoba usled povlačenja gingive. Cilj rada bio je da se uporedi efikasnost lečenja autotransplantatom vezivnog tkiva (ATVT) sa periostom i plazme obogaćene trombocitima (PRP) sa ATVT sa periostom, ali bez PRP, na širinu keratinizirane gingive. **Metode.** Studijama je bilo obuhvaćeno 20 zuba sa recesijama gingive klase I i II prema Mülleru. U toku terapije 10 recesija lečeno je ATVT sa periostom u kombinaciji sa koronarno pomerenim režnjima (ATVT grupa). Na kontralateralnoj strani, isti broj recesija lečen je ATVT uz koronarno pomeren režanj i uz primenu PRP (PRP + ATVT grupa). Od kliničkih parametara praćena je širina keratinizovane gingive kao pokazatelj pokrenutih regenerativnih procesa. U statističkoj obradi rezultata korišćen je Studentov *t*-test. **Rezultati.** Analizom kliničkih parametara ustanovljeno je statistički značajno proširenje zone keratinizirane gingive, što je od izuzetnog značaja za regeneraciju s tim što je proširenje bilo izraženije u ATVT + PRP grupi. Procentualno gledano, prekrivenost korena u ATVT grupi iznosila je 90%, a u ATVT + PRP grupi 93,5%. **Zaključak.** Primenom koncepta aktivne regeneracije odnosno primenom PRP i stimulativnim dejstvom aktiviranih faktora rasta iz trombocita, postiže se regeneracija dubljeg periodontalnog tkiva što je bitan preduslov za uspešno zbrinjavanje recesije gingive.

Ključne reči:

gingiva, povlačenje; stomatologija, operativna; transplantacija, autologa; plazma bogata trombocitima; lečenje, ishod.

Introduction

Gingival recession is defined as a condition when the edge of the gingival margin is located apically to the cement-enamel border, and the tooth root surface exposed towards the external surface of the tooth¹. Such clinical conditions are commonly seen in a daily clinical practice in population of different ages, and their prevalence increases with age². Most frequent etiological factors related to and responsible for the occurrence of gingival recession include mucogingival anomalies, poor oral hygiene, inadequate tooth brushing techniques, a high frenum attachment and blisters as well as various orthodontic deformities³. From the pathogenetic aspect, gingival recession is closely associated with tissue inflammation caused by dental plaque.

In addition to esthetic defects leading to gingival recession, unpleasant sensitivity to mechanical, chemical and technical stimuli are also the most common reasons for patients to visit dentists⁴. Elimination of pain sensation and aesthetic correction of gingival recession along with the creation of conditions for adequate maintenance of oral hygiene are only some of the aims of mucogingival plastic surgery⁵.

There are numerous surgical procedures that allow for creation of a stable zone of the attached gingiva, coverage of the exposed tooth root surface and prevention of the gum from receding further⁶. The gold standard among surgical procedures for the root coverage has for long been connective tissue autograft (CTG) used with the periosteum in various combinations with coronally and laterally positioned grafts and double papilla graft, as well⁷. Apart from those methods, more advanced methods developed by modification of the existing surgical procedures were introduced into clinical practice. Some of those methods include guided tissue regeneration (GTR) where various resorbable and non-resorbable membranes are used⁸, and procedures in which free gingival and pedicle grafts are used most commonly in combination with grafts in coronal position.

A method that has recently been singled out as a special one is the concept of tissue engineering, *ie* the process in which the growth factor is applied to promote the process of regeneration. A question is why there are so many diverse surgical procedures for the management of gingival recession-related problems. The answer lies in the fact that the regenerative capacity of periodontium is limited, and that surgical therapy provides only a partial regeneration in terms of long junctional epithelium, not a complete reconstruction in terms of '*restitution ad integrum*', something the modern periodontology tends to⁹. To achieve complete periodontal tissue regeneration, an additional stimulation of the regeneration process is required. Due to that the agents in blood that increase the possibility of bone and soft tissue regeneration have used¹⁰. Activated platelet preparations have been shown to be able to induce angiogenesis, synthesize collagen and inhibit monocytes¹¹. It refers to local application of platelet-rich plasma (PRP) that proved to be very suitable for release of platelet-derived growth factors (PDGF), enabling better tissue regeneration and faster healing process. The es-

sence of PRP are the potent bioactive growth factors, the components of platelet α -granules. Therefore, 7 specific polypeptide molecules, *ie* growth factors were included: 3 isomers of PDGF (PDGF-AA, PDGF-BB and PDGF-AB), 2 transforming growth factors beta (TGF- β), TGF- β 1 and TGF- β 2, the vascular endothelial growth factor (VEGF) and the epithelial growth factor (EGF)¹². Secreted growth factors facilitate mitosis in cells, proliferation and migration of periodontal ligament cells, stimulate their replication and matrix synthesis, initiate the vascular ingrowth, and induce cell differentiation at the site of injury when they arrive and become the predominant cells in wound¹³. The essence of PRP application is that the high concentration of platelets increases concentration of growth factors and enhances the periodontal tissue response¹⁴.

The main parameter that testifies preservation of healthy structure of periodontium is the keratinized gingival width. The width of attached gingiva is a variable in human population, and ranges from minimal 0.5 mm – 1 mm to maximally measured 9 mm. Its main role is to prevent free gingiva from moving under the effect of muscle force produced by closer implanted muscles. It is thought that an adequate width of attached gingiva is the one that prevents movement of the soft oral tissue onto the gingival margin. The trials conducted so far have shown that the 2-mm keratinized gingiva is a minimum that ensures healthy paradontium¹⁵. In case of less than 2 mm width of keratinized tissue, or a total loss of gingival margin, the marginal gingiva comes into direct contact with the movable alveolar mucosa¹⁵. Such a condition causes pulling of the marginal gingival away from the alveolar mucosa when speaking or laughing¹⁶. Continuous pulling is transferred into the area of the epithelial attachment causing thus the damage and separation of the attached epithelium from the tooth. This newly created relation allows for the accumulation of dental plaque which due to its pathogenic properties causes inflammation and damage to the periodontium. All that leads to etiopathogenetic *circulus vitiosus* and in the end to the development of gingival recession^{17,18}.

The aim of the study was to compare the clinical effects of CTG and the periosteum in combination with the coronally positioned graft application and CTG and the periosteum in combination with PRP application through their effects on keratinized gingiva width.

Methods

This comparative prospective study included 20 patients of both genders in the age between 18 and 35 years. All of them were non-smokers. The criteria for their selection was the presence of bilateral gingival recession with the visible cement-enamel border at the canine and premolar sites, that corresponded to the Müller Class I and II gingival recession. All the patients were treated at the Clinic of Dentistry, Military Medical Academy in Belgrade. The mouths were divided by methodological concept, so the recession was, on one side, treated with CTG and the periosteum in combination with the coronally positioned graft and the ap-

plication of PRP (the CTG-PRP group) and, on the other side, recession was reconstructed with the CTG and the periosteum but without PRP (the CTG group).

PRP preparation was carried out following the methodology described in the works of Anitua¹⁹ and Sonnleitner et al.²⁰. So, 4.5 mL of patient's blood mixed with 0.5 mL of sodium nitrate was put in a test tube for centrifugation-based separation of blood cells. During the first spin cycle, the tube rotated at the speed of 1,200 rpm (160 g) for 20 min separating blood into the lower blood cell component layer and the upper plasma layer. The second 15-min spin cycle at the speed of 2,000 rpm enacted further blood cell separation into the upper fraction containing plasma and a small number of platelets, and the lower one containing PRP. The number of platelets in such a preparation was $1,340 \times 10^9/L - 1,670 \times 10^9/L$, and was utilized within the next 60 minutes.

The surgical procedure involving CTG application in combination with PRP implies, prior to its application, the autograft conditioned with activated platelet concentration obtained by the special technological procedure immediately before surgical intervention. The method of CTG use includes the following procedures: after anesthesia administration, and the initial beveled incisions originating from the cement-enamel border made at the surgical site to raise the mucoperiosteal flap, the root of the tooth is completely exposed. The root surface is then mechanically cleaned with sharp curettes to remove the necrotic layer of cement. By

ated factors in the same sample of the observed subjects, and by using the Student'-*t* test for independent samples.

As for the non-parametric prediction intervals, the chi-square test was used as a tool to determine the frequency and the significance of the observed sample differences. The differences at the level of 0.05 considered to be significant.

Results

The results for the width of keratinized gingiva were significant indicating the efficiency of the applied surgical procedures. The mean value assessed prior to the surgical intervention in the CTG-PRP test group (Figures 1–5) was 1.00 ± 0.23 mm. Six months after the surgery, the obtained value clearly indicated a significant augmenting of the zone of keratinized gingiva of 3.70 ± 0.55 mm ($t = 7.56$; $p < 0.01$). In the CTG group (Figures 7 and 8) the value of this parameter was 1.20 ± 0.28 mm. Following six months of the surgery, the value of the obtained widening of keratinized zone was found to be 3.50 ± 0.50 mm, what was statistically very significant ($t = 5.89$; $p < 0.01$). The differences in values of those parameters ranging from 2.3 mm in the CTG group to 2.7 mm in the CTG-PRP group clearly indicated a statistically significant difference observed in both test groups particularly in the CTG PRP group, and also confirmed that regeneration processes occurred in periodontium (Table 1).

Table 1

Study group	Keratinized gingival width (mm)		<i>t</i>	<i>p</i>
	before surgery ($\bar{x} \pm SD$)	6 months after surgery ($\bar{x} \pm SD$)		
CTG – PRP	1.00 ± 0.23	3.70 ± 0.55	7.56	< 0.01
CTG	1.20 ± 0.28	3.50 ± 0.50	5.89	< 0.01

CTG – connective tissue graft; PRP – platelet rich plasma.

cutting the periosteum in the apical area of the flap, the preparation of the recipient site to receive the graft is completed. The autograft is harvested from the premolar side of the palate using the technique known as 'trapdoor' technique. The sharp dissection is then completed to elevate the palate flap and ensure the access to the palatum for taking the autograft. After that, the donor site is closed with individual sutures. The autograft is positioned over the exposed surface of the root with the periosteum angled towards the root. The autograft is fixed using an individual suture, and is completely covered with the coronally positioned flap fixed with the individual silk sutures. During the recovery period, the treatment includes the use of analgesics, special diet regime and oral hygiene.

Before the surgical intervention and 6 months after the surgery, we evaluated the width of keratinized gingiva of the periodontal tissue and the effects of the applied therapy. The width of keratinized gingiva was expressed in mm.

The arithmetic means and standard deviations of all observations were calculated by using parametric prediction intervals. The differences between those parameters were calculated by using the differentiation test for small associ-

Discussion

Since the 50s of the last century when the first pioneer efforts were made in the area of the treatment of gingival recession up to nowadays, there has been a lot of work done to improve surgical procedures used for managing gingival recession-related problems. Those procedures ranging in complexity from coronally and laterally positioned grafts through free gingival autografts, connective tissue grafts with periosteum to GTR along with the use of various membranes as an inductor of regeneration have given satisfactory results in terms of satisfying aesthetic criteria but without adequate functional support. The main problem with the majority of those surgical procedures was a long attached epithelium as it had histologically been confirmed in a great number of previously conducted and published studies, so the long-term preservation of the achieved outcomes could not be guaranteed^{9,21}. It was one of the crucial reasons for the introduction of the active regeneration concept into the field of paradontology. The efficacy of this concept lies in the local and continuing application of numerous bioactive growth factors and proteins that enhance the reparatory-tissue processes in the body²².

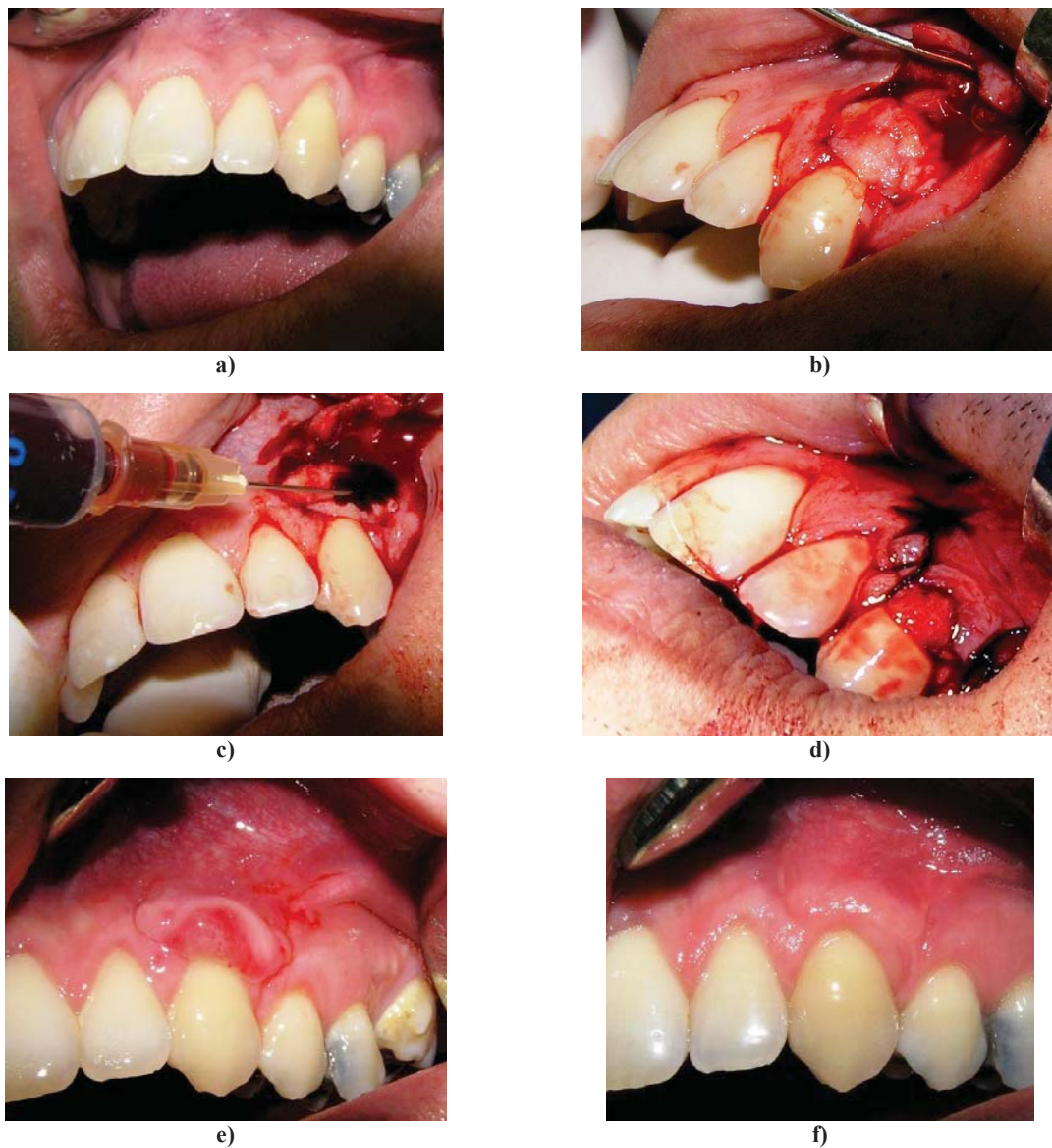


Fig. 1 – Gingival recession in tooth #23 treated with connective tissue graft (CTG) in combination with platelet rich plasma (PRP).
 a) gingival recession before the treatment; b) CTG placed on the recipient site; c) application of activated PRP; d) a view on gingival recession after suture; e) two weeks after the surgery; f) six months postoperatively.



Fig. 2 – Gingival recession in tooth # 24 treated with connective tissue graft (CTG) without platelet rich plasma (PRP).
 a) gingival recession before the treatment; b) six months after the surgery.

The results of our study confirm the role of the 'gold standard' that CTG plays in the treatment of recession, particularly in widening of the zone of keratinized gingiva²³. The presence of periosteum as a biological membrane increases the biological capacity of an autograft to a great extent²⁴. Periosteum as a highly differential tissue of mesenchymal origin has a very important role in bone biology, and, thus, enables the regeneration of a part of the alveolar bone. All that ensure better therapeutic effects and a stable remission of the disease, what was proved by Harris²⁵ in his research work.

On the basis of data analyses, we could observe that widening of the zone of keratinized gingiva showed to be much more successful in the studied subjects treated with the autograft in combination with PRP as compared to those in which PRP was not applied²⁶. It is presumed that, by using PRP, we stimulate the proliferative capacity in undifferentiated mesenchymal cells, their growth, migration and adhesion, thus enhancing the regenerative processes in deeper periodontal tissue structures²⁷.

It is obvious that most responsible for that are the PDGF and the TGF β present in a PRP fraction, as polypeptide growth factors involved in the cell proliferation, differentiation and morphogenesis of tissue²⁸. Growth factors may stimulate either a mitogenic response since they enhance the proliferation of certain types of cells, or respond mitogenically because they change the target cell phenotype²⁹. More efficient results observed in the CTG-PRP test group can be explained by the fact that lies in the use of PRP, *ie* the high concentration of platelets increases the local concentration of secreted growth factors what thus enhances the initial response to the healing process of the soft and bone tissue. Upon the activation of the platelet fraction, the release of the growth factors important in the wound healing is enacted³⁰. Thus the role of growth factors in the chemotaxis of the stem cells and differentiation into

corresponding cell groups involved in the regeneration of periodontium is expressed. The fact concerning the effect of the growth factor on the type-I collagen synthesis should not be neglected because it is the base for the formation of a new extracellular matrix of the connective and bone tissue as well as the formation of a new attachment³¹.

One of the very important facts for the survival of an autograft is a good postoperative vascularization and fibrovascular invasion from the surrounding tissue combined with proper diet regime by diffusion, in which the activated plasma plays a crucial role, particularly in the first 48 hours. All that indicates that the PDGF, TGF and GF growth factors have the positive effects on the early tissue and bone healing process^{32,33}.

By the elimination of subjective problems and achievement of satisfactory aesthetic results as well as the establishment of a favorable anatomic-morphological ratio, conditions for adequate oral hygiene are created, since it have shown to be one of the crucial factor in maintaining the achieved outcomes.

The obtain results of CTG application with periosteum and PRP justify the use of growth factors. Taking into account the augmentation of the zone of keratinized gingiva indicating the stimulated regeneration of periodontium, what was achieved particularly in the CTG-PRP test group, we can conclude to get more closer to our aim, the one the modern periodontology tend to, and that is restitution *ad integrum*.

Conclusion

The concept regeneration technique with PRP and with the stimulating influence of platelet activated growth factors results in the regeneration of deep periodontal tissue as an important prerequisite for the successful treatment of gingival recession.

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