CYTOLOGICAL CHANGES IN THE EPITHELIZATION OF THE WOUND AFTER MUCOGINGIVAL SURGERY

Selma Durmishi
Phu Sul-dental, North Macedonia, Resident in Oral surgery in University Dental Clinical center "St. Pantelejmon", Department of oral surgery and implantology at UKIM, Skopje North Macedonia, selma.566@student.ugd.edu.mk
Mirjana Markovska Arsovska
Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia, University Dental Clinical center "St. Pantelejmon", Department of oral surgery and implantology, Skopje, North Macedonia, mirjana.arsovska@ugd.edu.mk
Zaklina Mencheva
Faculty of Dental Medicine, MIT University, North Macedonia, University Dental Clinical center "St. Pantelejmon", Department of oral surgery and implantology, Skopje, North Macedonia, menceva@yahoo.com
Spiro Spasovski
Faculty of Dentistry "St. Cyril and Methodius"- Skopje, North Macedonia, spasovskispiro@gmail.com
Simona Simjanovska
PHU Zabna ordinacija dr. Snezana Temelkova, Veles, North Macedonia, sssimjanovska@gmail.com

Abstract: The main therapeutic goals of mucogingival surgery that every therapist strives for, are: achieving satisfactory aesthetics, reducing dental hypersensitivity, creating conditions to prevent the occurrence of caries on the root surface and reducing the possibility of progression of periodontal disease. In our country, a clinically widely applied technique is periosteal separation, which is still insufficiently exploited scientifically. The mucosal epithelial cells of the gingiva plays an important role in the innate immune defense system, the transmission of sensory signals from the surround environment, maintaining the balance between health and disease
The aim of this paper is to show the epithelization in the operated area, through cytological analysis followed by parameters of an indicative character in the healing process.
Matherial and method. For the realization of this study, a total of 60 patients with an insufficient area of attached gingiva were followed. The patients included in the study underwent surgical intervention - periosteal separation. They were divided in 2 groups. In the first group after intervention a Solcodental adhesive pasta and protective packaging were applied, and in the other group tin foil and protective packaging were applied
Results showed that on the fourteenth day after the intervention in the second group, the presence of epithelial cells in 100.0% of the patients is satisfactory. The registration of the satisfactory epithelial cell presence in the second group at 7 and 14 days is statistically significant versus the satisfactory epithelial cell presence in the first group for $p<0.05$ ($p=0.0072$; $p=0.0027$).
Conclusion. Cytological analyzes show good tissue epithelization after mucogingival intervention/periosteal separation, and better epithelial healing in patients where Solcodental adhesive pasta was applied.
Keywords: attached gingiva, periosteal separation, epithelialization

1. INTRODUCTION

The main therapeutic goals of mucogingival surgery that every therapist strives for, are: achieving satisfactory aesthetics, reducing dental hypersensitivity, creating conditions to prevent the occurrence of caries on the root surface and reducing the possibility of progression of periodontal disease. (Diana Mostafa, Nikhat Fatima, 2021)
1) In this segment of periodontal surgery, several techniques appeared and developed, with purpose to obtain satisfactory coverage of the root, to expand the attached gingiva, and to get a deep vestibulum. The success of the implemented intervention depends: on the initial condition, on the biological capacity of the tissue, on the choice of the technique, but it is largely conditioned by the good blood supply and the solid regenerative potential of the periodontal tissue. (Mehdi Ebrahimi, Waruna L Dissanayaka, 2022)
After a satisfactory surgical treatment in addition to good vascularization, a solid regenerative potential of the lost periodontal structures is needed.
The bone periosteum in adults is well vascularized, contains fibroblasts and progenitor cells (ie, osteoblasts) and stem cells. In all age groups, periosteal cells retain the ability to differentiate into fibroblasts, osteoblasts,

chondrocytes, adipocytes, and skeletal myocytes. The tissues produced by these cells are fibrous cementum, periodontium and bone (Chunyi Li , Peter Fennessy, 2021). The presence of the periosteum in close proximity to the gingival recession makes it suitable for grafting.

The choice of technique to increase the height or correct the inadequate width of the gingiva depends on: the defect, size and localization. In recent years, the most preferred intervention is the free mucogingival connective tissue autograft. But in our country, a clinically widely applied technique is periosteal separation, which is still insufficiently exploited scientifically (Saini, Amanpreet Kaur, Tewari Shikha & Arora Ritika, 2020). Vestibular extension techniques have been used mainly to increase vestibular depth (Andry Elvandari, neira N. Sakinah et al. 2022). However, the postoperative course during these interventions regularly results with: a limited functional gingival zone, and the exposed alveolar bone, followed by intense bone resorption (Hench LL, 2006 & Cohen ES, 2017). Of course, in such and similar situations, an important point is epithelization of the tissue. The mucosal epithelial cells of the gingiva plays an important role in the innate immune defense system, the transmission of sensory signals from the surround environment, maintaining the balance between health and disease (Zuhr O, et al. 2007). Regenerative periodontal surgery using the two techniques "tissue engineering" and "in situ tissue healing" are constantly being remodeled and changed (Nunley JA, 2008).

The aim of this paper is to show the epithelization in the operated area, through cytological analysis followed by parameters of an indicative character in the healing process.

2. MATERIAL AND METHOD

The examinations for this study were carried out at the University Dental Clinical center “St. Pantelejmon”, Department of oral surgery and implantology, Skopje, North Macedonia, Faculty of Dentistry “St. Cyril and Methodius”- Skopje and the Private Cytological Laboratory "St. Mina" also from Skopje.

For the realization of this study, a total of 60 patients with an insufficient area of attached gingiva were followed. The patients included in the study underwent surgical intervention - periosteal separation. They were divided in 2 groups. In the first group after intervention a Solcodental adhesive pasta and protective packaging were applied, and in the other group tin foil and protective packaging were applied.

The inclusion criteria were: Initial periodontal affection, clinically and X-ray verified; Absent or insufficient zone of attached gingiva below 2 mm; Presence of shallow periodontal pockets

Exclusion criteria: Patients with chronic and systemic diseases

The healing process of the oral mucosa from the aspect of cytomorphological changes was carried out through the following cytological parameters: presence of granulocytes, presence of erythrocytes, epithelial cell presence, presence and type of cytologically visible microbiological flora, graduation index, karyopyknotic index, eosinophilic index and presence of stromal cells.

For this purpose, swabs were taken from all subjects before the intervention, on the third, seventh and fourteenth day after the intervention.

As a control, a swab was taken from all 60 subjects before the intervention.

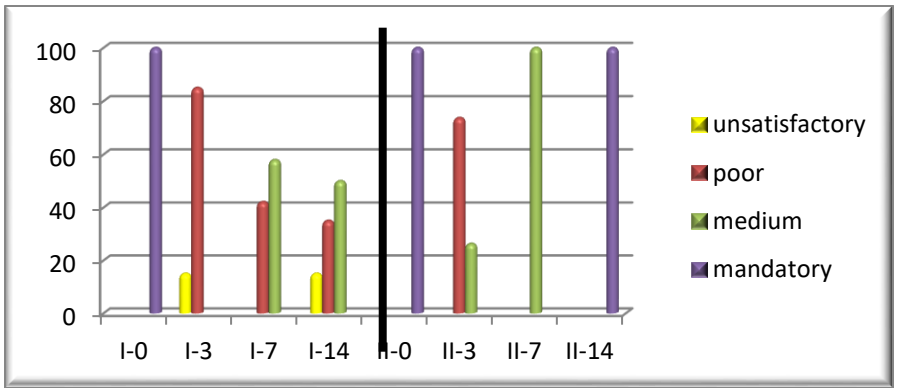
The swab for cytological analysis is taken with appropriate instruments (spatula, plastic instrument, brush). The sampled material was applied to the subject glass. The examples are fixed with 96% alcohol for at least 15 minutes and at most 30 minutes. After that, the example was preserved in tinfoil and stained according to Papanicolaou, in the next 10 days and was followed microscopically. For Papanikolaou staining, a solution composed of three reagents was used: Papanikolaou I-hemtoxylin, Papanikolaou II-orange, Papanikolaou III-polichrom.

3. RESULTS

Based on the statistical tests, the following results were obtained

On the fourteenth day after the intervention in the second group, the presence of epithelial cells in 100.0% of the patients is satisfactory. The registration of the satisfactory epithelial cell presence in the second group at 7 and 14 days is statistically significant versus the satisfactory epithelial cell presence in the first group for $p<0.05$ ($p=0.0072$; $p=0.0027$).

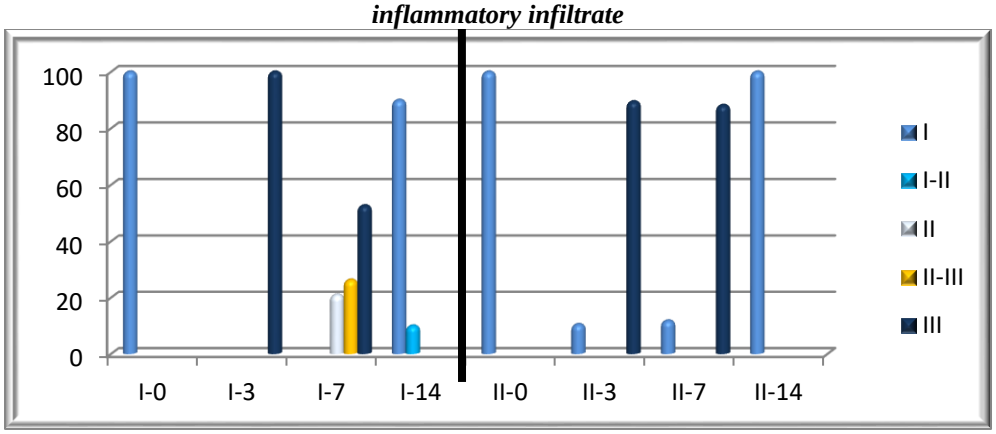
Chart 1. Graphic representation of the distribution of patients from both groups according to epithelial cell presence



Source: Author's research

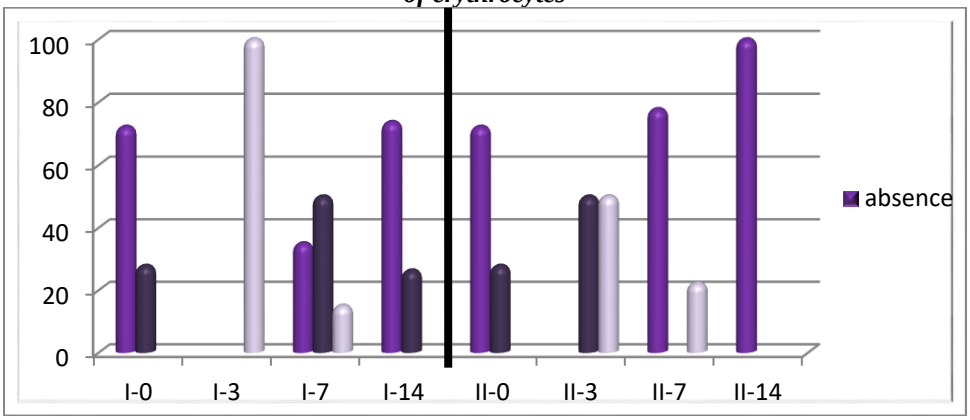
The results show the presence of an inflammatory infiltrate of mixed cellularity. The mononuclear infiltrate is marked separately. The labeling does not go into the etiology of the inflammatory infiltrate, which may be of an infectious cause or reactive in the context of reparative regenerative processes as a manifestation of the stages of inflammation.

Graph 2. Graphic representation of the distribution of patients from both groups according to the presence of inflammatory infiltrate



Source: Author's research

Chart No. 3 Graphical representation of the distribution of patients from both groups according to the presence of erythrocytes



Source: Author's research

The registration of the absence of erythrocytes in the second group on day 7 and 14 is statistically significant versus the absence of erythrocytes in the first group for $p<0.05$ ($p=0.0119$; $p=0.0337$).

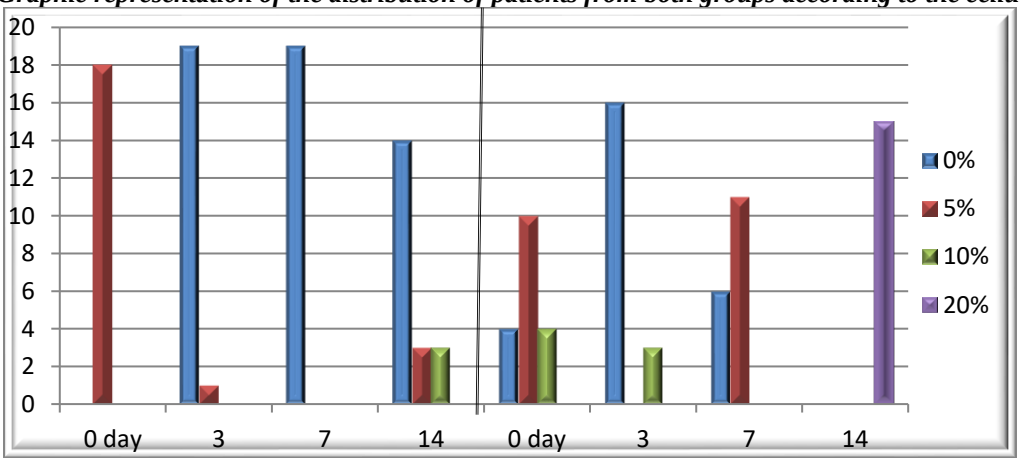
Table 1. Distribution of patients from both groups according to the graduation index

day	I group		II group	
		number		number
zeros	0:0:80:20	4	0:20:80:0	4
	0:0:100:0	4	0:0:60:40	4
	0:0:90:10	10	0:0:70:30	4
			0:0:90:10	6
3 day	0:50:50:0	4	0:30:70:0	7
	0:20:80:20	4	0:20:80:20	4
	0:20:80:0	4	0:10:90:0	4
	0:10:90:0	4		
	0:30:70:0	4		
7 day	0:50:50:0	5	0:5:80:5	6
	0:30:70:0	8	0:20:80:20	7
	0:20:80:0	5	0:10:90:0	6
14 day	0:20:80:0	4	0:0:70:30	15
	0:0:100:0	4		
	0:0:80:20	4		
	0:0:95:5	4		
	0:0:90:10	4		

Source: Author's research

The cellular index indicates the percentage presence of cells with a pyknotically altered nucleus.
It can also be used as an indicator of healing, of course with internal control of a smear from another location of the oral mucosa.
The cellular index on day zero was 5.0% in 18 patients in the first group, 5.0% in 10 patients in the second group, 0.0% and 20.0% in four.
The cell index on day 3 after the intervention was 0.0% in 19 patients and 5.0% in one patient in the first group, 0.0% in 16 patients in the second group and 10.0% in three.
The cellular index on day 7 after the intervention was 0.0% in 19 patients in the first group, 0.0% in 6 patients in the second group and 5.0% in 11 patients.
The cell index on the 14th day after the intervention was 0.0% in 149 patients, 5% and 10% in three patients in the first group, 20.0% in 15 patients and 5.0% in 11 in the second group.

Chart 4. Graphic representation of the distribution of patients from both groups according to the cellular index



Source: Author's research

4. DISCUSSION

Recently the opinion was that the oral mucosa is a passive covering, but now it`s known that oral mucosa plays an active role in host defense.

Epithelial cells react interactively in several ways: they produce antimicrobial peptides, such as chemokines, they attract monocytes and neutrophilic granulocytes, they activate the immune system.

Interventions, which were performed during deepening of the vestibulum, can cause damage to the superficial regions of the gingiva (Young-Dan et al. 2021). In fact, in these conditions, periodontal microsurgery has found as a solution in certain cases and get solid results. Clinical observations have shown good epithelial healing when performing microsurgical techniques (Robinson RE & Agnew RG, 1963). There are studies that attempt to obtain scientific evidence by determining MMP9, TGF-1beta and TNF-alpha emphasizing their role in the healing process (Robinson RE, 1963 & Nevins M, 2005).

By performing the periosteal separation, there is direct effect on the essential factor that affected the gingival or periodontal health. Although the method is a kind of modification of the oldest techniques, it is widely used in our country to solve the insufficient zone of attached gingiva with quite satisfactory results. It`s simple in its performance and it is definitely a satisfactory solution for the timely extension of the insufficient size and width of the vestibular area.

In the literature, we found a data that proteins play the role of glue and the type of intervention together with the extracellular protein matrix participate in the regeneration process. It is assumed that the structures laminin, lamin gamma, integrin and integrin alpha participate in the healing process (Salo, 1994).

The author indicates that the secretion of laminin 5 in the connective tissue can induce epithelial cell migration. Also, binding of laminin 5 to integrin alpha and beta can cause cell adhesion and cell migration, which participates in epithelial regeneration (1Wyrebek B, 2016).

In contrast, other authors followed the regenerative phases in subjects with furcation defects applied a purified recombinant human growth factor (rhPDGF-BB) that can be a potent factor in healing, by increasing and reorganizing periodontal and bone cells. The same results were shown in the Camelo study (Camelo M, 2003). They demonstrated for the first time that complete periodontal regeneration was achieved in advanced class II furcation defects using purified recombinant growth factor (Nevins M, 2005).

5. CONCLUSION

Cytological analyzes show good tissue epithelization after mucogingival intervention/periosteal separation, and better epithelial healing in patients where Solcodental adhesive pasta was applied.

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