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Efficacy of the use of a therapeutic composition containing stem cells in the augmentation of the alveolar ridge according to the indicators of bone remodeling markers in the saliva

Andrii Bambuliak[1], Yurii Bandrivsky[2], Nataliia Kuzniak[1], Stepan Tkachyk[1], Pavlo Perebyinis[1], Lesia Lopushniak[3]

[1]Department of Surgical Dentistry and Maxillofacial Surgery, Bukovinian State Medical University, Chernivtsi, Ukraine
[2]Department of Pediatric Dentistry I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
[3]Department of Human Anatomy, Bukovinian State Medical University, Chernivtsi, Ukraine

ABSTRACT

Maxillofacial bone defects may result from various pathological conditions, such as traumatic tooth extraction, aggressive periodontitis, jaw osteomyelitis, neoplasms, cysts, congenital malformations, and gunshot injuries. These circumstances can pose difficulties in addressing both (primary and secondary) dental adentia, especially when considering dental implantation. One of the current challenges in dentistry is to give more attention to the advancement of innovative osteoplastic materials.

The purpose of this study was to assess the effectiveness of a therapeutic composition that includes stem cells in enhancing the alveolar ridge, as suggested by bone remodeling markers in oral fluid. The research revealed that all study groups showed favorable changes in oral fluid markers of bone metabolism during the extended follow-up period (6-12 months), with statistically significant differences ($p2<0.01$, 0.05). However, in group A, acid phosphatase activity decreased by 12.98% ($p>0.05$) and 32.49% ($p<0.01$, $p1<0.05$), while alkaline phosphatase increased by 8.73% ($p>0.05$) and 15.16% ($p>0.05$), respectively, after one year of observation compared to groups B and C. Thus, it can be inferred that a reduction in acid phosphatase activity, along with a rise in alkaline phosphatase levels in oral fluid, suggests enhanced regenerative processes and the initiation of bone remodeling in the patients belonging to the study group. However, it seems that the procedures were particularly effective in patients belonging to group A, where we used the combination of "Bio-Oss"+MSCs-AT+PRP to fill bone defects. This suggests the best effectiveness of the composition we developed for alveolar ridge augmentation.

Keywords: stromal cells, augmentation, saliva, markers of bone remodeling, bone mineralization index

INTRODUCTION

The development of the implant industry has contributed to the highly effective rehabilitation of dental patients with partial and complete edentulousness. This has significantly reduced the range of contraindications to implantation, especially in cases of bone deficiency in the alveolar ridge area [1,2]. This trend, along with the parallel progressive expansion of the possibilities of directed bone reconstruction using various osteoplastic and auxiliary materials, provides a predictable regeneration of bone tissue with the restoration of not only its height and width, but also its quality indicators [3,4].

The use of biological stimulants is regarded as a promising approach to advancing regenerative therapy [5,6]. It has the potential to enhance the pre-

Corresponding authors:
Yurii Bandrivsky
E-mail: bandrivsky@tdmu.edu.ua

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dictability and reliability of augmentation outcomes. The issue of quantification of pluripotent cells in specific tissues of the body is still relevant for modern cell engineering. The presence of such cells is recorded by indirect signs, by the type of expression of relevant stimulants, cell cycle characteristics, or clonogenicity [7,8].

An article has been released that discusses the classification of the use of stem cells in dental practice, taking into account their origin and potential applications. The article discusses the capacity of mesenchymal stem cells to differentiate into the mesodermal lineage and their growing potential for bone formation. [9].

There has been a proposal that adipose tissue (AT) may be considered as an optimal source for multipotent mesenchymal stem cells (MMSc) [10]. The accessibility of the cell extraction method, its minimally invasive nature, and the capacity to procure an ample quantity of cellular material at the appropriate time are the primary reasons for this. As stated by reference [11], it has been observed that adipose tissue stromal cells possess the capability to differentiate into various pathways such as adipogenic, osteogenic, chondrogenic, endothelial, myogenic, hepatogenic, epithelial, and neurogenic pathways. That is why the use of MMSCs as a population of pluripotent precursors is justified by the prospect of their differentiation into specific cells with histological development of the surrounding structure [12]. Enhancing our comprehension of cell specialization and morphogenesis mechanisms could aid in the creation of successful approaches for using cell engineering, particularly in areas such as maxillofacial surgery and clinical dentistry.

Maxillofacial bone defects may result from various pathological conditions, such as traumatic tooth extraction, aggressive periodontitis, jaw osteomyelitis, neoplasms, cysts, congenital malformations, and gunshot injuries. [13,14]. These circumstances can pose difficulties in addressing both (primary and secondary) dental adentia, especially when considering dental implantation. One of the current challenges in dentistry is to give more attention to the advancement of innovative osteoplastic materials.

The purpose of this study was to assess the effectiveness of a therapeutic composition that includes stem cells in enhancing the alveolar ridge, as suggested by bone remodeling markers in oral fluid.

MATERIALS AND METHODS

The study was carried out at the Department of Surgical Dentistry and Maxillofacial Surgery of Bukovinian State Medical University in Chernivtsi, Ukraine. In order to examine bone tissue remodeling processes, a study was carried out on a group of 97 patients. The patients were divided into three groups: Group A, consisting of 34 patients who underwent augmentation using an therapeutic composition of “Bio-Oss”+MSCs-AT+PRP; Group B, consisting of 32 patients who received “Bio-Oss” – for bone defect replacement; and Group C, which included 31 subjects with bone defect healing occurring spontaneously or under a blood clot. The assessment involved the measurement of acid phosphatase (AP) and alkaline phosphatase (ALP) levels in the saliva, as well as the calculation of the bone mineralization index (BMI). Evaluations were conducted post-surgery within 3-5 days of observation, and at 3, 6, and 12 months after the surgery.

Methods for the preparation of the osteoplastic composition “Bio-Oss”+MSCs-AT+PRP: after washing the “Bio-Oss” granules with Hanks’ solution with cefazolin (1 g/l), a mixture of cell culture after osteogenic differentiation and platelet-rich plasma was carefully layered on them, after which a thrombin solution was added dropwise for polymerization.

The bone defects were filled with “Bio-Oss” material, which consists of granules ranging in size from 1mm to 2mm. Multipotent mesenchymal stromal cells of adipose tissue (MSCs-AT) were obtained according to the method described [15].

Platelet-rich plasma was obtained from the patients' cubital vein [16]. The sample was processed using centrifugation at a speed of 1000 revolutions per minute for a duration of 10 minutes. After centrifugation, the top layer, which was free of red blood cells, was separated and then subjected to another centrifugation at 3000 revolutions per minute for 10 minutes. The liquid portion was mostly removed, and the platelets that had settled were subsequently re-suspended in the remaining plasma.

For fixation of the osteoplastic material in the bone defect, the collagen membrane Alpha - Bio's Graft (Israel) was used.

The determination of acid phosphatase (AP) activity in the oral fluid was performed using reagents from «Filicit-Diagnostics» (Ukraine), a unified method by the "endpoint" method. The principle of the method is that the amount of k-nitrophenol formed per unit time is proportional to the enzyme activity and is determined by the optical density of the sample on a spectrophotometer at a wavelength of 405 nm. The activity of alkaline phosphatase (ALP) in the oral fluid was determined using a set of reagents from «Filicit-Diagnostics» (Ukraine). The determination was performed on a spectrophotometer at a wavelength of 500 - 560 nm. Bone mineralization index (BMI) in the oral fluid was calculated by the ratio of the activity of the enzymes ALP and AP and was calculated by the formula: BMI = ALP/AP [17].

The results were analyzed using licensed software programs, including “Microsoft Excel 2021” and “Statistica”, on a personal computer.

RESULTS AND DISCUSSION

According to the research outcomes presented in Table 1, it was observed that during the postoperative period, patients from group C had the highest activity of acid phosphatase (AP) in oral fluid, measuring 195,12±12,21 nmol/s.l. It is worth noting that this value was 10.25% higher than that of group B (p>0.05) and 16.15% higher than that of group A (p1<0.05). Within this timeframe, the alkaline phosphatase activity in oral fluid ranged from 827,77±66,96 nmol/s.l. in group A patients to 728,14±66,09 nmol/s.l. in group C patients. The obtained values did not show a statistically significant difference between the two groups (p, p1>0.05). In the postoperative phase, there were no significant differences in bone mineralization index (BMI) values among the study groups. Group A patients had the highest BMI values of 5.19±0.82 (p<0.05), while group C patients had the lowest values of 3.81±0.61 (p, p1>0.05).

After a 3-month observation period and administration of various therapeutic medications, a slight decrease in acid phosphatase activity was observed in the saliva of the individuals examined. The reduction was 18.49% in group A, 17.27% in group B, and 15.43% in group C compared to the postoperative data, with no statistically significant difference (p>0.05). Simultaneously, it was observed that there was no statistically significant difference (p>0.05) in acid phosphatase (AP) activity values in the oral fluid of patients in groups A and B. However, it was noted that group C exhibited a 19.19% higher AP activity in the saliva than group A (p<0.05).

The study found a marginal increase in alkaline phosphatase (ALP) activity in the oral fluid of patients in the study groups compared to the preceding observation period (p2>0.05). Group A had an ALP activity of 856,91±67,31 nmol/s.l. while group C had an ALP activity of 758,29±64,56 nmol/s.l. (p, p1>0.05). At the same time, after 3 months of observation, the value of bone BMI was characterized by minimal values in group C – 4,52±0,72 and maximum values – 6,24±1,01 in group A, p, p1, p2>0.05.

After 6 months of investigation, a statistically significant decrease in oral fluid acid phosphatase (AP) activity was observed compared to postoperative data. Both group A and group B showed a reduction in AP activity, with group B showing a reduction of 23.55% and group A showing a reduction of 25.22%, with p2<0.01. Simultaneously, it was observed that there was a decrease of 16.50% in acid phosphatase (AP) activity in group C, and this change was statistically significant (p2<0.05). It is worth noting that the acid phosphatase (AP) activity in the oral fluid of individuals from groups A and B showed a difference of 24.94% and 17.83%, respectively, compared to the data in group C. It is important to mention, that the

TABLE 1. Changes in the values of bone metabolism markers in the saliva of patients

Terms observation	Biochemical indicators	Group A («Bio-Oss»+MSCs-AT +PRP), n=34	Group B («Bio-Oss»), n=32	Group C (spontaneous healing of a bone defect under a blood clot), n=31
Postoperative period (3-5 days)	Acid phosphatase (AP), nmol/s.l.	163.61±9.29	175.21±10.38	195.12±12.21*
	Alkaline phosphatase (ALP), nmol/s.l.	827.77±66.96	783.97±66.18	728.14±66.09
	BMI	5.19±0.82	4.59±0.72	3.81±0.61
After 3 months	Acid phosphatase (AP), nmol/s.l.	133.36±7.37	144.95±8.46	165.02±9.58*
	Alkaline phosphatase (ALP), nmol/s.l.	856.91±67.31	796.26±66.59	758.29±64.56
	BMI	6.24±1.01	5.46±0.88	4.52±0.72
After 6 months	Acid phosphatase (AP), nmol/s.l.	122.35±7.42 ΔΔ	133.94±7.89 ΔΔ	163.01±8.28 Δ, **, •
	Alkaline phosphatase (ALP), nmol/s.l.	1069,04±74,12	955,17±70,37	900,69±69,40
	BMI	8.41±1.23 Δ	6.47±1.01	5.37±0.88 *
After 12 months	Acid phosphatase (AP), nmol/s.l.	115.65±6.84 ΔΔ	130.66±7.12 ΔΔ	153.22±7.53 **, •, Δ
	Alkaline phosphatase (ALP), nmol/s.l.	1038.43±75.81	947.82±71.23 Δ	881.06±69.76
	BMI	9.27±1.46 Δ	7.47±1.28	5.93±1.16

Notes:

* p<0,05, **p<0,01 – significant difference in values in relation to the data of group A.

• p1<0,05 – significant difference in values in relation to the data of group B.

Δ p2<0,05, ΔΔ p2<0,01 – a significant difference in values in relation to the data of the postoperative period

difference in group B was statistically significant (p<0.05), and in group A it was (p<0.01).

During the observation period, it was noted that the alkaline phosphatase (ALP) activity in the oral fluid of patients increased. The lowest values were observed in group C at 900,69±69,40 nmol/s.l., while the highest values were observed in group A at 1069,04±74,12 nmol/s.l. It is worth noting that the differences between the groups were not statistically significant (p, p1, p2>0.05). At the same time, bone BMI was the highest in patients of group A – 8,41±1,23, p, p1>0.05 p2<0.05, and 1.5 times less in patients of group C – 5,37±0,88, p<0.05, p1, p2>0.0.

After 1 year of observation, a significant decrease in acid phosphatase (AP) activity was observed in the oral fluid compared to the postoperative data. Group A showed a reduction of 29,31% with p1>0.05, p2<0.01; group B showed a reduction of 25.43% with p2<0.01; and group C showed a reduction of 21.47% with p<0.01, p1, p2<0.05. Although groups B and C showed a slight increase in oral fluid alkaline phosphatase (ALP) activity (20.90% and 21.00%, respectively with p, p1>0.05), there was no statistically significant difference from the postoperative values, (p2>0.05). A notable rise in alkaline phosphatase (ALP) activity was observed in the oral fluid of patients in group A (25.45%, p2<0.05) when compared to the initial data. After a 12-month study, the bone mineralization index (BMI) of the subjects showed an increase. Group A exhibited an increase of 1.8 times (p2<0.05), while group B and C exhibited increases of 1.6 times respectively. The differences in p-values were not statistically significant (p, p1, p2>0.05).

Thus, our results are consistent with those obtained by [18], who managed to restore a significant cranial defect using mesenchymal stem cells, and at the same time developed an appropriate prognostic module for such reconstructions with possible promising use of the potential of undifferentiated progenitor cells.

Yamada et al. (2018) reported findings that suggest the potential benefits of using autologous stromal cells in sinus lift procedures. Biological scaffolds were used for surgical procedures involving the application of PRP clots [19]. Following a two-year post-augmentation period, the average vertical bone gain was approximately (8.8±1.6) mm compared to the initial values. During the monitoring period of 2-6 years, no progressive resorptive changes were observed in the residual ridge in the area of the implants placed after augmentation, in the area of any of the 46 infraconstructions. This injection method, according to the authors, provides predictable results of augmentation and reduces the volume of surgical procedures to a reasonable minimum. Further laboratory investigations have provided evidence that the use of mesenchymal stem cells appears to stimulate the expression of osteogenic markers, such as STRO-1, CD13, CD29, CD44, CD73, alkaline phosphatase, and osteocalcin [20, 21].

CONCLUSION

Thus, it can be inferred that the reduction in acid phosphatase activity and elevation in alkaline phosphatase levels in the oral fluid may suggest a positive trend towards regenerative processes and activation of bone remodeling among the patients in the study groups. Nevertheless, in group A patients, where the combination of "Bio-Oss"+MSCs-AT+PRP was employed for bone defect filling, these processes were more pronounced compared to groups B and C. This suggests the best effectiveness of the composition we developed for alveolar ridge augmentation.

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