

# INNOVATION-BASED APPROACH IN RECONSTRUCTION OF REDUCED JAW ALVEOLAR RIDGE BONE USING CELL REGENERATION TECHNOLOGIES

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**ABSTRACT** — AIM. This study was performed to assess the size, structure and functional features of the reconstructed alveolar ridge using the autologous stromal and vascular fraction of adipose tissue (SVF-AT) up to 10 years.

**MATERIALS AND METHODS.** This study evaluates 141 patients (61 males, 80 females) aged range 45 to 78 years (Moda 57 years) with jaw alveolar ridge regression. 112 osteoplastic surgical procedures in the test (TG) were performed using SVF-AT with subsequent placement of 297 dental implants in the reconstructed ridge; in the control (CG) — 117 osteoplastic surgical procedures were performed using generally approach with subsequent placement of 323 dental implants. The alveolar ridge size and reconstructed bone features was evaluated in terms of up to 10 years. Histologic and histomorphometric study of 27 reconstructed bonecor-biopsies was conducted. The data were statistically analyzed.

**RESULTS.** The results of this comparative study confirm the advantages of the proposed cell-potentiated approach over the current generally accepted methods for the reconstruction of the alveolar jaw ridge. The use of SVF-AT with osteoplastic material allows to achieve new and sufficient bone growth with an insignificant risk of complications and reoperations (8% and 21% of cases in TG and CG, respectively,  $p = 0,231$ ), optimal morphological properties of the regenerate ( $40,14 \pm 3,36$  and  $24,23 \pm 2,63\%$  of bone tissue in TG and KG respectively,  $p = 0,001$ ), that provides reliable fixture integration in the reconstructed alveolar ridge and high efficiency of the implant-supported restorations (97% and 88% in TG and KG, respectively, up to 10 years,  $p < 0,001$ ).

**CONCLUSION.** The proposed innovative approach can be recommended as a basis for a valid surgical protocol with a pronounced of the jaw bones regression transformation. This will allow more successful and predictable use the implant-supported prosthetic restorations in reconstructed ridges in this category of patients.

**KEYWORDS** — regression bone transformation, stromal and vascular fraction of adipose tissue, reconstruction of the alveolar ridge, dental implantation

## INTRODUCTION

Given the current stage of research and technology progress, dental implants installation is the best option to support dentures with partial or complete loss of teeth. However, it requires a certain amount of supporting tissues, which is often lacking due to the regressed transformation of the alveolar ridge bone, which is an inevitable effect of missing teeth in the natural bite along with disappearing endosseous mechanical stimulus [1]. The progressing over time bone atrophy leads to osseogenic failure accompanied with low activity in osseoinductive factors of the systemic and/or local level, small number of cambial cells, reduced density of hemomicrocirculatory bed functioning vessels [2]. A significant limitation to the three-dimensional increase in the lost structures over a considerable length of the alveolar ridge bone is the lack of vertical walls, which requires additional use of osteoplastic materials featuring frame functions.

Autologous bone transplant is known to be the gold standard for the recovery of maxillofacial area defects [3]. This procedure, however, has certain drawbacks — an additional area of operational trauma in the oral cavity or extraoral localization; a limited amount of graft; a two-stage operation protocol with an increase in the intervention time and morbidity. Patients often suffer from neurosensory disorders in the donor area. The probability of bone autograft resorption is high [3, 4]. The results can be improved through combinations of auto-bone with various animal- or synthetic-origin bone substitutes that do not possess osteoinductive and angiogenic potential; they can be used to replace minor defects yet prove ineffective in case of larger or lengthy issues [5, 6]. Besides, the remaining non-resorbed bone substitute granules impair significantly the quality of the regenerate.

Given the above, there is a significant clinical need for full-function osteoplastic materials that can be used to eliminate the jaw bones regression transformation followed with dental implantation.

### *Aim of study*

To evaluate the size, the structure and capacity for chewing load in the bone reconstructed with autog-

enous stromal-vascular fraction of adipose tissue (SVF-AT) through a long-term study (up to 10 years).

## MATERIALS AND METHODS

The study involved 141 patients (61 males and 80 females) aged 45–78 (median age — 57) with regressed transformation of the alveolar ridge bone of various degree and duration. All the patients needed an increase in the bone tissue prior to dental implantation. The study did not include patients with uncontrolled general somatic diseases.

The treatment and further clinical observation included the period of 2006–2018. All the patients signed a voluntary informed consent to undergo a comprehensive rehabilitation of the dental system, including the use of the SVF-AT. A positive conclusion was obtained from the Ethical committee of the Stavropol State Medical Academy (Protocol # 6 of December 17, 2010). The observation went on until all the stages of dental prosthetics were completed including the evaluation of the reconstructed alveolar ridge bone parameters, as well as the status of the artificial supports in the reconstructed ridge bone through later observation periods — up to 10 years.

In the test group (TG, 68 patients, 112 surgeries) vitalized SVF-AT heterogeneous osteoplastic material was used for osteoplastics; in the control group (CG, 73 patients, 117 surgeries) the same material was used yet with no cellular component. The studied groups had a comparable distribution by gender and age ( $p > 0.05$ ). The preoperative computed tomography results showed that the average residual height of the alveolar ridge in the proposed implantation area was  $5.04 \pm 0.16$  mm and  $5.49 \pm 0.17$  mm; the residual width was  $3.13 \pm 0.06$  mm and  $3.46 \pm 0.01$  mm in the TG and CG, respectively. Table 1 shows the distribution of the clinical material based on the type of surgical intervention, on the number of patients, and on the installed osseointegrable dental implants in the groups in question.

Prior to the reconstructive surgery, the patients underwent teeth sanitation, removal of insolvent orthopedic constructions, professional oral hygiene with monitoring of the patient's sustained skills in individual dental and gingival control, periodontal inflammatory and destructive disease treatment, preliminary correction of prosthetics, and received temporary orthopedic structures not relying on the bone augmentation areas.

The planning stage involved diagnostic wax modeling of the final orthopedic work in an articulator; identified the required locus of surgical interventions; made navigation patterns for placing artificial supports in the right spots and with correct angulation.

Lipoaspiration was performed in the plastic surgery department. The isolation of SVF-AT was carried out in a research institute laboratory (Prof. Ochapovsky Krasnodar Regional Hospital #1; Krasnodar, Russia) following the method into which we introduced certain improvements [7, 8].

### *Autogenous SVF-AT separation Protocol*

Autogenous blood serum (ABS; volume — 10 ml) was prepared following the standard procedure. Local infiltration with tumescent anesthesia, Sol. Lidocaini 0.3%, 500 ml, with adrenaline (1: 500000) through punctures with a #11 scalpel using a 12 G cannula (Khuori Harvesting Cannula), a 50 ml LuerLock syringe and a locking device (ByronMedical) were used to aspirate 40–50 ml of subcutaneous fat tissue from the anterior abdominal wall. The punctures were covered with aseptic drapes with a compression bandage applied. For one hour, the patient stayed in the hospital under observation. The lipoaspirate in syringes was washed with sterile saline with a wide activity spectrum antibiotic and further delivered to the laboratory in a thermal container. The volume of the lipoaspirate was brought to 60 ml with physiological saline containing lyophilized enzyme collagenase (50 mg), to be further put in a sterile plastic bag. After a 20-minute exposure at (37 C) (ELMI thermostat, Laboratory Equipment) the suspension was distributed in test tubes with its volume taken up to 10 ml with physiological saline, and centrifuged for 20 min (CLMN-P10-02 centrifuge). The upper layer of liquid lipids and flotizing adipocytes was aspirated, and the supernatant drained. The SVF-AT was collected from the tubes bottom, while the washing cycle was performed by resuspending in the ABS and centrifuging. Next, SVF-AT was resuspended in 5 ml of ABS and kept like that (until it was used) at a temperature of +4°C. When delivered to the clinic, the material was supplied with a cell counting protocol indicating the viability.

Cell viability was assessed using the vital trypan blue stain, whereas the count was performed on a Countess cell counter (Invitrogen, USA). After SVF-AT was isolated, a sample was taken for immunophenotyping. Since SVF was represented with different types of cells, while mesenchymal stromal cells were one of the main types, we decided to cultivate the cells until the first passage with further identification of the immunophenotype of adherent cells using the immunofluorescent method, which would allow confirming the quality of the stromal vascular fraction. The following monoclonal antibodies were used: CD13 (Serotec), CD31 (BD, Pharmingen), CD34 (BD, Pharmingen), CD44 (Abcam), CD90 (Calbiochem), CD105 (Serotec), Pro-collagen Type I (Taka-

Table 1. Characteristics of clinical material

Type of surgery	Number of operations, localization, study group	Number of patients	Number of dental implants installed
Sinus lifting open	107 TG – 55 CG – 52	67 TG – 35 CG – 32	301 TG – 154 CG – 147
The operation of horizontal, vertical and three-dimensional augmentation of the alveolar ridge	122 UJ TG – 26 UJ CG – 28 LJ TG – 31 LJ CG – 37	74 UJ TG – 15 UJ CG – 20 LJ TG – 18 LJ CG – 21	319 UJ TG – 80 UJ CG – 96 LJ TG – 68 LJ CG – 75
Total	229 TG – 112 CG – 117	141 TG – 68 CG – 73	620 TG – 297 CG – 323

Note. TG – test group; CG – control group; UJ – upper jaw; LJ – lower jaw.

ra), Fibronectin (Abcam), SMA (Sigma), Desmin (Sigma) and C-kit (BD, Pharmingen). To identify the nuclei, the cells were stained with fluorescent DAPI (Dako). The preparations were placed into a medium for fluorescent preparations (AquaPoly/Mount; Polysciences, Inc). Staining visualization and image analysis were performed using an Axiovert 200M fluorescence microscope (Zeiss, Germany) equipped with a digital camera (20× and 40× obj.).

*Methodology for preparing vitalized (activated) SVF-AT osteoplastic material.*

Granulated and (or) bioresorbable osteo-substituting material presented as a block was introduced into a tube with the SVF-AT, resuspended in 10 ml of autogenous blood serum. The following proportions were observed: 1 part of the concentrated fraction (the sediment volume on the bottom of the tube after centrifugation) to 2–4 parts of the osteo-substituting material. Within the 15–30 minute exposure with regular shaking of the tube, the SVF-AT got adsorbed on the surface and inside the pores of the cancellous bone substitute, and the serum became transparent. Immediately before use, the block-type osteo-substituting material was removed. The granulated material was removed from the tube bottom after centrifugation at a speed of 1000 rpm for 4 minutes and the supernatant was drained. A morphological study of 5 samples of vitalized SVF-AT granulated osteoplastic material included making monolayer cytological preparations (Cytospin-4 cytocentrifuge; Shandon, United Kingdom), their fixation in May-Grunwald solution, staining subject to the Romanovsky method. The examination and photography of the preparations was carried out using an AxioStar microscope (Zeiss, Germany) (magnification ×100 and ×200).

In case of three-dimension regression transformation of the jaws alveolar ridge, autogenous cortical blocks up to 1.5 mm thick, taken from the area of the external oblique mandible line, or xenogenous cortical blocks and bone plates (200, 400 and 600 micron thick) were used as a frame (Lamina, OsteoBiol; OsteoplastFlexCortical, BioTech, Italy), fixed with screws to the cortical plate of the recipient bed. The gaps were filled in the TG with a mixture of SVF-AT with osteoconductor granules (BioGen, BioTech, Italy) and autogenous cancellous bone taken by trepan from the donor retromolar area, maxillary tubercles or a toothless alveolar ridge, crushed in a bone mill. In the control group, the same osteoconductors were used, yet with no cellular material. A membrane (Heart, BioTech, Italy; Evolution, OsteoBiol, Italy; Bio-Gide, Geistlich, Switzerland; e-PTFE, GoreTex, USA; Ecoflon, St. Petersburg, Russia) was placed under a soft tissue flap, and then the wound was sutured without tension by mattress and cross stitches. Prior to suturing the donor area the soft tissues at the bone wound were covered with collagen (Osteoplast, Vitaform, Russia).

To increase the size of the subantral area at an open sinus elevation, ordinary (in CG) or vitalized SVF-AT (in TG) granules of osteoconductive material were placed under the cranially displaced Schneider membrane, after which the wound was sutured with 4-0 Teflon without tension.

Postoperative instructions included liquid and soft food, oral cavity antiseptic treatment with a 0.05% chlorhexidine solution until the stitches were removed, brushing the teeth twice a day with a sonic brush, except the surgery site. Non-steroidal anti-inflammatory drugs were administered. Preventive antibiotic therapy was performed. Painkillers were prescribed as needed. Examinations and dressings were performed on the

day following the surgery, and then once every 3 days. The stitches were removed 2 weeks after the surgery.

6–8 months later, a second cone-beam computed tomography was performed to evaluate the reconstructed bone status, after which 620 osseointegrable dental implants (DI) were installed followed by preparing non-removable or hybrid orthopedic structures. Artificial supports with different immersion degree into tissues were used — 346 implants were installed into the gum level; while 274 titanium alloy, Grade 4 implants were installed into the bone level (or subcrestal). The healing caps were installed following the standard load protocol — 2–3 months after the implants installation.

Histological examination: 27 trepan biopsies from the reconstructed bone (14 from TG and 13 from CG) obtained during the development of dental implant wells were fixed in 10% neutral formalin, washed, decalcified in trilon-B, and further, according to the standard technique, 5  $\mu$ m sections were prepared with hematoxylin and eosin staining. The study was performed with a light-optical microscope at standard magnifications (7 $\times$ , 10 $\times$  oc.; 10 $\times$ , 40 $\times$ , 60 $\times$ , 90 $\times$  obj.). Not less than in four sections of each sample, the relative area of vital mineralized tissue, non-vital mineralized tissue and non-mineralized tissue, were identified and calculated. The tissue structures area analysis in digital micro-images was performed with the 3D-doctor program (Able Software Corp., USA).

The data obtained through the study underwent statistical processing using the methods of parametric and non-parametric analysis following the compared sets testing results for distribution normality. The statistical analysis was performed using IBM SPSS Statistics 23 software.

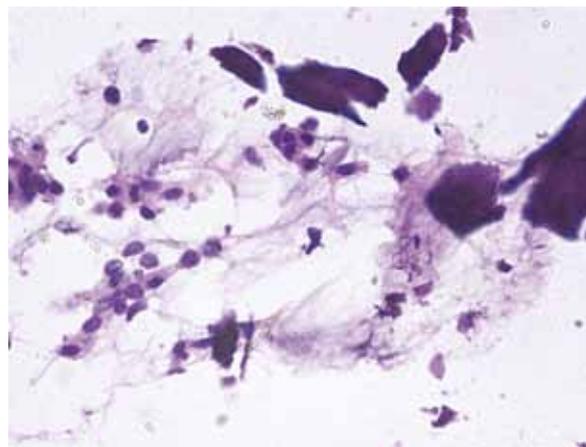
## RESULTS AND DISCUSSION

Patients easily went through the lipoaspiration procedure, with no complaints and complications registered. The number of viable nucleated cells in the portions of SVF-AT used for each individual patient ranged from 25 to 60 mln. The immunofluorescence data revealed that at the end of the first passage, 95–100% of the SVF-AT culture cells expressed mesenchymal stromal cell (MSC) markers: CD13, CD44, CD90, CD105. A small number of cells (15–25%) in the samples expressed CD31 (endothelial cell marker), C-kit (receptor for stem cell factor SCF and some other progenitor cells), desmin and smooth muscle actin (muscle cell markers). No CD34-positive cells were detected in the culture (hematopoietic stem cell marker).

Cultured cells are synthetically active in relation to the extracellular matrix components, such as fi-

bronectin and collagen I (the cells revealed expression of Pro-collagen I, the precursor of type I collagen). The identified phenotype corresponds to the known features of MSC [9, 10].

Fig. 1 shows a micro-image of an SVF-AT vitalized osteoplastic material smear.



**Fig. 1.** Cell smear of test group osteoplastic material: microfragments of xenogenic bone matrix Bio-Oss are surrounded with a cell-rich fibrous substance — autogenic SVF-FT. Romanovskystaining. Magnification  $\times 200$

In most cases, the postoperative period of intraoral intervention entailed no complication and was accompanied with mild general discomfort and moderate local pain. The number of cases and the types of complications in the study groups can be seen from Table 2.

An analysis of the sinus elevation complications frequency in the studied groups revealed no statistically significant difference ( $p > 0.05$ ). In cases where augmentation was performed on significantly reduced areas of the alveolar ridge with a relative deficit of epithelial soft tissues, some cases of seam divergence and exposure of osteoplastic material were observed. This condition poses a significant risk of the graft loss. Nary Filho et al. (2014) performed an analysis of autologous bone blocks exposed to the oral cavity after the alveolar ridge reconstruction, and detected bacterial colonization, similar in microflora species and their distribution to chronic jaw purulent osteomyelitis [11]. Our study confirms a higher resistance level of the graft containing SVF-AT to infection, even if it comes to a direct contact with the oral environment. A statistical analysis using the relative risk index (RR) shows that after the seams diverged with osteoplastic material exposure, the

**Table 2.** Characteristics of early complications

Type of surgery	Type of complication	Test group		Control group		p
		abs.	%	abs.	%	
Sinus lifting open	Acute maxillary sinusitis	0	0	1	1,9	0,486
	Encapsulation of osteoplastic material	0	0	2	3,8	0,234
	Total	0	0	3	5,7	0,111
The operation of horizontal, vertical and three-dimensional augmentation of the alveolar ridge	Early suture failure with secondary healing	8	14,0	4	6,2	0,231
	Early failure of sutures with infection and loss of the graft	1	1,8	14	21,5	<0,001*
	Total	9	15,8	18	27,7	0,131
Total		9	8,0	21	18,0	0,027*

\* — statistical significance of differences in  $p < 0,05$

probability of further infection and loss of graft was 7 times lower in the TG than in the CG (RR 0.143; 95% CI 0.022–0.922;  $p < 0.05$ ). The potential mechanisms of this outcome include a direct antibacterial effect of mesenchymal stromal cells that are part of SVF-AT [12, 13, 14], immunomodulation with an increase in the phagocytic M2-macrophages proportion [15, 16, 17], and accelerated graft reperfusion [18, 19, 20].

Table 3 shows the measurements results obtained from the study groups for the alveolar ridge height and width before the surgery and 6–8 months after it (prior to dental implantation).

available bone, including the reconstructed area, in TG exceeded a similar value in the CG by 20.3% ( $p < 0.001$ ); the width — by 7.6% ( $p < 0.001$ ) (see example in Fig. 2).

Note to be made that the estimated indicators depend on the volume set for the future reconstructed bone due to the size of the cortical frameworks.

In both groups' samples obtained 6 months after the osteoplasty, staining with hematoxylin and eosin allowed identifying mineralized bone, osteoid and residual particles of osteoplastic material (Fig. 2). However, the ratio of the main regenerate tissue components, their morphological features and distribution

**Table 3.** Measurements of alveolar ridge, (mm), ( $M \pm m$ )

Parameter	Deadline	Test group		Control group		p
		$M \pm m$	n	$M \pm m$	n	
Height	Before surgery	5,04±0,16	112	5,49±0,17	117	0,055
	After operation	9,37±0,15	112	7,79±0,14	117	<0,001*
	p1	<0,001*		<0,001*		
Width	Before surgery	3,13±0,06	112	3,46±0,01	117	<0,001*
	After operation	8,21±0,16	112	7,63±0,05	117	<0,001*
	p1	<0,001*		<0,001*		

p — significance of differences between study groups; p1 — significance of differences before and after surgery; \* — statistical significance of differences ( $p < 0,001$ )

As follows from the table, after augmentation osteoplasty, both groups revealed a statistically significant increase in the respective parameters of the reconstructed area ( $p < 0.001$ ); a sufficient amount of supporting bone was obtained for dental implantation. When comparing the groups, the height of the

indicated a more active and productive osteogenic process in the TG if compared with the CG. Table 4 offers a view on the results of histomorphometric analysis of trepan biopsy specimens of the groups.

As follows from the table, the relative area of the vital mineralized, i.e. regenerated, bone tissue



**Fig. 2.** CT of the patient B-va following maxilla alveolar ridge CD-reconstruction in 6 months: in the test group (second sector) there is a double and uniform increase in the mineralized bone volume, a homogeneous trabecular structure, the recipient zone border are not visualized; in the control group (the first sector), due to premature removal of fixing screws and partial graft loss, there was no formation of the expected volume of supporting tissues, the formed structures heterogeneity is determined due to the presence of unresorbed granules osteoplastic material, the recipient zone border of the reduced alveolar ridge are clearly visualized

earlier placement of artificial supports following the osteoplasty using SVF-AT.

2–3 months after the installation of the intraosseous part, an initial assessment of the implant stability was carried out. In the TG, there were no cases of artificial supports non-survival registered. In the CG, osteointegration was missing in 5 dental implants (in 3 patients) including 2 implants in the upper jaw and 3 in the lower jaw; 3 out of 5 unstable implants were installed subcrestally, 2 — to the gum level. These patients underwent reimplantation after preliminary surgical interventions. The dependence between the frequency of intraosseous implant primary non-integration and the osteoplasty method was statistically significant ( $p = 0.032$ ). Given the fact that the implants were identical, just like the technology for their installation and the postoperative loading conditions, the differences can be explained by optimal biological specifics of the bone tissue restored by the plastic material including SVF-AT.

SVF-AT, as part of the graft composition, has been found to contribute to the development of a better periimplant soft tissue profile with an attached keratinized gum of 2 mm or more. Due to the lack of a similar effect in the CG, at the stage of gum healing caps installation, an extra surgical intervention was often performed — apical reposition of the flaps

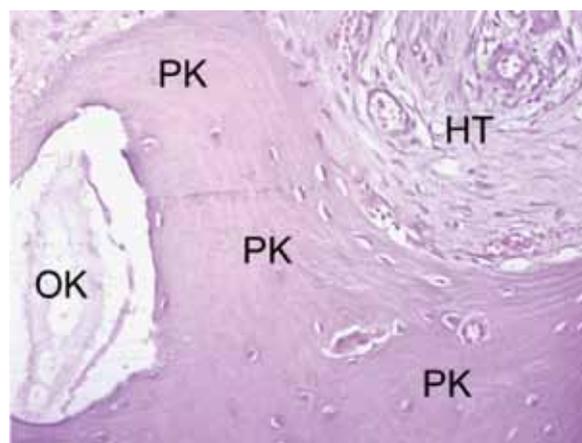
**Table 4.** Histomorphometric evaluation results (area of tissue structures, in %)

Bone Regenerate Components	Test group	Control group	p
Vital mineralized tissue	40,14±3,36	24,23±2,63	0,001*
Nevital Mineralized Tissue	13,31±1,59	24,98±1,97	<0,001*
Non-mineralized fabric	47,11±2,07	50,79±2,10	>0,05

\* – statistical significance of differences ( $p < 0,01$ )

in the trepan biopsy specimens sections of the TG was 1.7 times larger than that of the CG ( $p < 0.01$ ). The relative area of non-vital mineralized tissue, reflecting the level of residual non-resorbed osteo-substituting material in the TG, on the contrary, was 1.9 times as small as that in the CG ( $p < 0.01$ ). There we observed a tendency towards a decrease in the number of non-mineralized tissues, including bone-marrow and fibrous tissues, in the TG samples if compared with the CG; however, the difference of 7.3% cannot be viewed as statistically significant ( $p > 0.05$ ) (Fig. 3).

The fact that the bone tissue content in the TG biopsy material was significantly higher than in the CG material allows us considering the possibility of



**Fig. 3.** Histological section of reconstructed bone trepan-biopsy material of test group: vital mineralized tissue of regenerated bone (PK), nonvital mineralized tissue of residual osteoconductive bone substitutes (OK) and intertrabecular non-mineralized tissue (HT) are identified. 6 months after osteoplasty. Hematoxylin & eosin staining. Magnification  $\times 200$

to shape the required width of the attached gingiva around the artificial supports. It is a well-known fact, that keratinized gums around the implant, apart from the aesthetic value, is an important factor helping prevent gingival recession, periimplantitis and delayed disintegration [21, 22, 23]. SVF-AT contributes to an increase in the width of the attached/keratinized gingiva, increasing significantly the oral cavity vestibule size in the surgical area and reducing the risk of complications. A similar effect was described by Gjerde et al. (2018) after alveolar ridge augmentation with bioceramic scaffolds, seeded with a mesenchymal stromal cells culture isolated from the bone marrow [24]. The authors came to a conclusion that the cells have a positive effect not only on the biomaterial osteogenic remodeling yet also on the adjacent soft tissues, promoting their healing and regeneration [24]. The phenomenon in question can be explained by paracrine activity of the cells that are part of SVF-AT, and that synthesize a number of angiogenic and trophic factors [25, 26].

In the initial loading period, the indicator of peri-implant marginal bone loss (PMBL) reflects indirectly the response of the supporting structures in the proximal bone-implant contact area to chewing loads. As Table 5 shows, at any level of implant installation and through all control periods, bone loss was significantly lower in the TG if compared to the CG ( $p < 0.001$ ).

ment, and a significant proportion of regenerated bone tissue on histomorphograms.

Within a period of up to 5 years, the rate of successfully functioning dental implants in the TG was 98.3%, in the CG — 93.5%; within the observation period of up to 10 years — 97% and 88%, respectively. A statistical analysis confirmed the relationship between the augmentation osteoplasty method at the atrophied alveolar ridge and long-term outcome of prosthetics relaying on intraosseous implants at  $p < 0.006$ . The relative risk calculation indicates that within a period of up to 5 years, the risk of losing a dental implant due to the supporting bone resorption was 11 times as low after augmentation osteoplasty using SVF-AT if compared with osteoplasty using ordinary osteoplastic materials (RR 0.099; 95% CI 0.013–0.761;  $p < 0.05$ ).

An example of a long-term outcome of the reconstruction of the atrophied alveolar part of the mandible and the subsequent dental implantation in a patient of the main group is presented in Fig. 4.

At later stages of observation, the sizes of the alveolar ridge area reconstructed with SVF-AT were more stable compared to the outcomes of reconstruction using conventional methods, which reveals indirectly the use of permanent bone tissue remodeling around the osteointegrated dental implants against the regular chewing load background. Within terms of up to 5 years, the horizontal size of the ridge in the TG

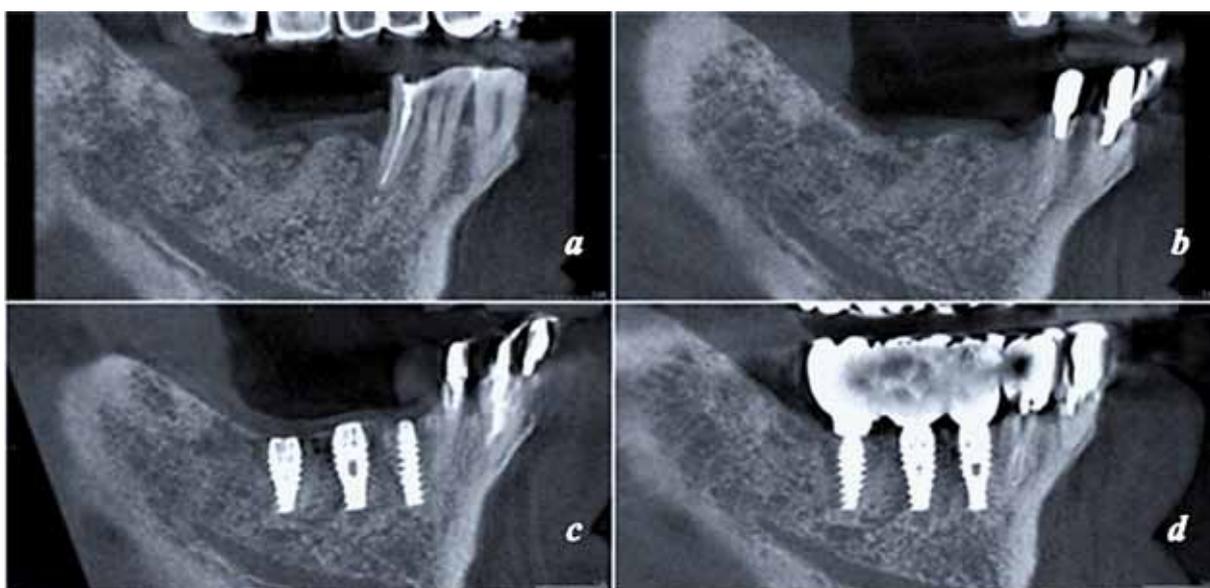
**Table 5.** Peri-implant marginal bone loss, (mm)

Implant installation level	Deadline after dental prosthetics	Bone loss, mm				p
		Test group		Control group		
		M±m	n	M±m	n	
Subcrestal	6 months	1,13±0,04	109	1,64±0,03	152	<0,001*
	12 months	1,47±0,08	109	2,88±0,09	152	<0,001*
p1		<0,001*		<0,001*		
To gum level	6 months	0,27±0,01	188	0,98±0,08	171	<0,001*
	12 months	0,33±0,03	188	1,69±0,08	171	<0,001*
p1		0,059		<0,001*		

p — significance of differences between study groups; p1 — significance of differences of indicators in terms of 6 months and 12 months; \* — statistical significance of differences ( $p < 0,05$ )

The differences between the groups by this indicator point at functional advantages of the supporting bone reconstructed with SVF-AT. Perhaps this is also due to the principle of structure and function interrelation, as well as to the described above macro- and microstructural features of the reconstructed alveolar ridge in TG patients, including the optimal size of the implant bearing bone, high-quality soft tissue environ-

was reduced statistically insignificantly (by an average of 0.37 mm ( $p = 0.103$ ), which turned out to be 4 times as low compared to the control, where the width decreased by an average of 1.6 mm (in the CG the differences of indicators within terms of 6–8 months and up to 5 years were significant at  $p < 0.001$ ). The alveolar ridge height during these periods decreased statistically significantly in both groups ( $p < 0.004$ ),



**Figure 4.** Long-term clinical result of the replacement of the end defect of the alveolar crest and the restoration of the dentition of the fourth jaw segment. Computed tomography, the main research group: a) before treatment — uneven atrophy of the alveolar ridge after extraction of 45, 46 and 47 teeth; b) 6 months after the reconstruction — a sufficient amount of bone tissue was obtained to accommodate dental implants; c) 3 months after the installation of dental implants — the structure and density of the near-implant bone tissue indicate successful osseointegration; d) after 24 months of functioning of the denture structures — there is no near-implant marginal bone loss, there is no reduction in the height of the reconstructed alveolar crest, a high degree of bone-implant contact

although the loss rate for the bone vertical dimensions in the CG was 2.8 times as high as in the TG: the average decrease was 1.67 mm and 0.59 mm in the CG and the TG respectively. Within the period of 5 to 10 years, the reduction rate for the reconstructed bone volume dropped significantly, averaging 0.08 mm in the height and 0.06 mm in the width in the TG, and 0.09 mm in the height and 0.2 mm in the width in the CG (the indicators changes for this period were not statistically significant,  $p > 0.05$ ).

## CONCLUSION

The comparative study confirmed the advantage of using the proposed innovation-based approach rather than the currently common methods for reconstructing the jaw alveolar ridge. The use of autogenous stromal vascular fraction of adipose tissue (SVF-AT) as a source of regenerative cells and stimuli in the osteoplastic material composition increases the efficiency of preimplantation osteoplasty of the reduced alveolar ridge — it allows a sufficient increase in the bone size with minimal risk of complication development; proper morphological features in the reconstructed tissue; reliable osseointegration, and long-term functioning for the dental implants. This approach can be recommended as a basis for a valid surgical protocol in case of significant regression transformation of the

jaw bones. This would allow a more successful and predictable use of the most advanced methods for rehabilitation of patients suffering from non-heritable edentulism using dental prosthetics on intraosseous supports.

## REFERENCES

1. **ESPOSITO M., WORTHINGTON H.V., THOMSEN P., COULTHARD P.** Interventions for replacing missing teeth: different times for loading dental implants. *Cochrane Database Syst Rev.* 2004;(3):CD003878. doi: 10.1002/14651858.CD003878.pub2
2. **DEEV R.V., DROBYSHEV A.YU., BOZO I.YA.** Ordinarnye I aktivirovannye osteoplasticheskie materialy. *Vestnik travmatologii I ortopedii N.N. Priorova.* 2015; 1: 51–65. (inRuss.).
3. **KHOJASTEH A., MORAD G., BEHNIA H.** Clinical importance of recipient site characteristics for vertical ridge augmentation: A systematic review of literature and proposal of a classification. *J Oral Implantol.* 2013; 39(3): 386–98. doi: 10.1563/AAID-JOI-D-11-00210
4. **FELICE P., PISTILLI R., LIZIO G., PELLEGRINO G., NISII A., MARCHETTI C.** Inlay versus onlay iliac bone grafting in atrophic posterior mandible: a prospective controlled clinical trial for the comparison of two techniques. *Clin Implant Dent Relat Res.* 2009; 11(1): 69–82. doi: 10.1111/j.1708-8208.2009.00212.x.
5. **PEROVA MD, KOZLOV V.A., MELNIK E.A., KARPYUK V.B.** New possibilities for replacing large defects

- of the jaws in the treatment of odontogenic cysts with the help of the vascular-stromal cell fraction. *Institute of Dentistry*. 2011; 1 (50): 52–54.
6. CALORI G.M., MAZZA E., COLOMBO M., RIPAMONTI C. The use of bone-graft substitutes in large bone defects: any specific needs? *Injury*. 2011; 42(2): 56–63. doi: 10.1016/j.injury.2011.06.011.
  7. KARPYUK V.B., PEROVA M.D., SHUBICH M.G. To the study of newly isolated autologous stromal cells of the subcutaneous fatty tissue for the regeneration of biological tissues. *Institute of Dentistry*. 2009; 3 (44): 74–76.
  8. KARPYUK VB, LAVRESHIN P.M., SHUBICH MG, PONKINA ON, PAVLYUK K.S., BEREZHNOY D.V. Study of the efficacy of the use of the vascular-stromal cell fraction in contour plastics of the facial soft tissues (MRI data using the example of lipofilling of the lips). *Annals seam., Reconstructive. and esthete. surgery*. 2014; 3: 57–63.
  9. DOMINICI M., LE BLANC K., MUELLER I., SLAPER-CORTENBACH I., MARINI F., KRAUSE D., DEANS R., KEATING A., PROCKOP DJ., HORWITZ E. Minimal criteria for defining multipotent mesenchymal stromal cells. *The International Society for Cellular Therapy position statement*. *Cytotherapy*. 2006; 8(4): 315–317. DOI: 10.1080/14653240600855905.
  10. MILD MAY-WHITE A., KHAN W. Cell Surface Markers on Adipose-Derived Stem Cells: A Systematic Review-*Curr. Stem Cell Res. Ther.* 2017; 12(6): 484–492. doi: 10.2174/1574888X11666160429122133
  11. NARY FILHO H., PINTO T.F., DE FREITAS C.P., RIBEIRO-JUNIOR P.D., DOS SANTOS P.L., MATSUMOTO M.A. Autogenous bone grafts contamination after exposure to the oral cavity. *J Craniofac Surg*. 2014; 25(2): 412–414. doi: 10.1097/SCS.0000000000000682.
  12. GUPTA N., KRASNODEMBSKAYA A., KAPETANAKI M., MOUDED M., TAN X., SERIKOV V., MATTHAY M.A. Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia. *Thorax*. 2012; 67(6): 533–539. doi: 10.1136/thoraxjnl-2011-201176.
  13. MEZEY E., NEMETH K. Mesenchymal stem cells and infectious diseases: Smarter than drugs. *Immunol Lett*. 2015; 168(2): 208–214. doi: 10.1016/j.imlet.2015.05.020.
  14. SUNG D.K., CHANG Y.S., SUNG S.I., YOO H.S., AHN S.Y., PARK W.S. Antibacterial effect of mesenchymal stem cells against *Escherichia coli* is mediated by secretion of beta-defensin-2 via toll-like receptor 4 signalling. *Cell Microbiol*. 2016; 18(3): 424–436. doi: 10.1111/cmi.12522.
  15. BADYLAK S.F., VALENTIN J.E., RAVINDRA A.K., MCCABE G.P., STEWART-AKERS A.M. Macrophage phenotype as a determinant of biologic scaffold remodeling. *Tissue Eng. Part A*. 2008; 14(11): 1835–1842.
  16. DONG Z., PENG Z., CHANG Q., AND LU F. The Survival Condition and Immunoregulatory Function of Adipose Stromal Vascular Fraction (SVF) in the Early Stage of Nonvascularized Adipose Transplantation. *PLoS One*. 2013; 8(11): e80364.
  17. BOWLES A.C., WISE R.M., GERSTEIN B.Y., THOMAS R.C., OGELMAN R., FEBBO I., BUNNELL B.A. Immunomodulatory Effects of Adipose Stromal Vascular Fraction Cells Promote Alternative Activation Macrophages to Repair Tissue Damage. *Stem Cells*. 2017; 35(10): 2198–2207. doi: 10.1002/stem.2689.
  18. PARFENOVA E.V., TKACHUK V.A., KALININA N.I., TRACTUEV D.O., RATNER E.I., TALITSKY K.A., MARCH KL, JOHNSTON B., RAKHMAT-ZADE T.M., ZOKOLAYEVA Z.I. Search for new “tools” for therapeutic angiogenesis. *Molecular medicine*. 2006; 2: 10–23.
  19. KOH Y.J., KOH B.I., KIM H., JOO H.J., JIN H.K., JEON J. ET AL. Stromal vascular fraction from adipose tissue forms profound vascular network through the dynamic reassembly of blood endothelial cells. *Arterioscler Thromb Vasc Biol*. 2011; 31(5): 1141–50. doi: 10.1161/ATVBAHA.110.218206.
  20. ZAKHARI J.S., ZABONICK J., GETTLER B., WILLIAMS S.K. Vasculogenic and angiogenic potential of adipose stromal vascular fraction cell populations in vitro. *In Vitro Cell Dev. Biol. Anim*. 2018; 54(1): 32–40.
  21. ZERNITSKY A.YU., MEDVEDEVA E.YU. The role of the volume of soft tissues around dental implants in the development of periimplantitis. *Institute of Dentistry*. 2012; 1: 80–81.
  22. BASSETTI R.G., STÄHLI A., BASSETTI M.A., SCULEAN A. Soft tissue augmentation procedures at second-stage surgery: a systematic review. *Clin Oral Investig*. 2016; 20(7): 1369–1387. doi: 10.1007/s00784-016-1815-2.
  23. LIN G.H., CHAN H.L., WANG H.L. The significance of keratinized mucosa on implant health: a systematic review. *J Periodontol*. 2013; 84(12): 1755–1767. doi: 10.1902/jop.2013.120688.
  24. GJERDE C., MUSTAFA K., HELLEM S., ROJEWSKI M. ET AL. Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial. *Stem Cell Res Ther*. 2018; 9(1): 213. doi: 10.1186/s13287-018-0951-9.
  25. OSUGI M., KATAGIRI W., YOSHIMI R., INUKAI T., HIBI H., UEDA M. Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. *Tissue Eng Part A*. 2012; 18(13–14): 1479–89. doi: 10.1089/ten.TEA.2011.0325.
  26. HIROSE Y., FUNAHASHI Y., MATSUKAWA Y., MAJIMA T., YAMAGUCHI M. ET AL. Comparison of trophic factors secreted from human adipose-derived stromal vascular fraction with those from adipose-derived stromal/stem cells in the same individuals. *Cytotherapy*. 2018; 20(4): 589–591. doi: 10.1016/j.jcyt.2018.02.001.