Clinical Study of the Efficiency of Combined Cell Transplant on the Basis of Multipotent Mesenchymal Stromal Adipose Tissue Cells in Patients with Pronounced Deficit of the Maxillary and Mandibulary Bone Tissue

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The use of synthetic osteoplastic materials not always provides the required amount of the bone tissue. Transplantation of tissue-engineering constructs containing osteogenic precursor cells can be an alternative high-technology implantation method. Here we present the results of a pilot clinical study demonstrating safety of this method, accelerated healing of the operation wound, formation of young bone tissue after transplantation, and the possibility of mounting implants after 3 months in case of sufficient amount of the bone for primary fixation.

Key Words: *oral surgery; multipotent mesenchymal stromal cells from the adipose tissue; tissue engineering; sinus lifting; osteoplastics*

Reconstruction of the bone tissue is an actual problem of modern surgical dentistry and oral surgery. The search for new and improvement of known materials for replacement of bone tissue defects and stimulation of bone growth is now in progress [1,2, 12]. The use of bone materials or bone substitutes is based on the assumption that they promote the formation of a new bone at the site of implantation. Autogenous bone transplants are considered as golden standard for reconstructive craniomaxillofacial surgery [4]. However, obtaining a sufficient amount of automaterial and the presence of an additional operation field considerably limit the use of these methods [7]. The existing methods for bone tissue reconstruction do not provide complete recovery and stimulation of bone regeneration. In most cases they perform an osteoconductive function [3,9].

Regeneration of the bone tissue is the most studied field in tissue engineering. Equivalents of the bone tissue can be obtained by targeted osteogenic differentiation of multipotent mesenchymal stromal cells (MMSC) of the bone marrow (BM) or adipose tissue [6,11,13]. MMSC predifferentiated towards osteogenic lineage are applied on biocompatible materials maintaining osteoinduction and possessing sufficient osteoconductive properties [6]. The resultant tissue-engineering construct is transplanted into the bone defect area.

Creation of tissue equivalents of the bone tissue is now beyond the scope of experimental studies. Numerous experimental and clinical studies demonstrated the possibility of effective reconstruction of the bone tissue using various biodegradable materials and MMSC [5,8,14].

A clinical study of reconstruction of bone defects and maxillary and mandibulary bone deficit

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with tissue-engineering constructs carrying MMSC from the adipose tissue was performed at Research Institute of Dentistry and Oral Surgery in cooperation with ReMeTeks Company in accordance with decision of Federal Agency for Health Care and Social Development (2006) and in compliance with Helsinki Declaration (2000). Here we present the results of this study.

MATERIALS AND METHODS

For isolation of primary human adipose tissue MSC culture we used adipose tissue samples from the anterior abdominal wall obtained during lipoaspiration. The material was delivered to the laboratory within 1 h in a special transporting container. The adipose tissue was repeatedly washed with Versen solution (PanEko), then with trypsin—Versen 1:1 (PanEko), and incubated at 37°C for 2-3 h. The isolated cells were pelleted by centrifugation (1100 rpm for 10 min) and transferred into NUNC culture flasks with complete growth medium (DMEM/F12, 1:1, PanEko) supplemented with FCS (HyClone-Perbio) to 10%, L-glutamine (584 mg/liter, PanEko), and amikacin (500 mg/liter, Sintez AKO). Cultured adipose tissue MSC attained confluence after 1.5-2 weeks, then were subcultured 5-7 times; experiments were performed on cells before the 3rd passage. Adipose tissue MSC cultures were immunophenotyped using FACS Calibur flow cytofluorometer (BD Biosciences).

Biomatrix (Konnektbiofarm) in the form of crumbs or blocks was used as the matrix carrier. For creation of the construct, adipose tissue MSC were harvested with trypsin—Versen solution, centrifuged, resuspended in a small volume of medium, and carefully applied on the carrier (5-7 mln. cells per 1 cm^3).

Targeted osteogenic differentiation of adipose tissue MSC. After the cells completely filled pores of the carrier, the growth medium was replaced with osteogenic one (growth medium containing 50 mg/liter L-ascorbic acid, 10-2 M P-glycerophosphate, and 10-8 M 1,25-dihydroxy vitamin D_3). Predifferentiation was carried out for 7 days.

Preparation of PRP (platelet-rich plasma). The blood was taken using Vacuette® vacuum system with sodium citrate. The blood was centrifuged at 1000 rpm for 10 min, the top layer (not containing erythrocytes) was collected and centrifuged at 3600 rpm for 10 min. The greater part of the supernatant was discharged and the platelet pellet was resuspended in the remaining plasma.

Preparation of the tissue engineering construct for transplantation. PRP was carefully layered on blocks washed with Hanks solution (PanEko) containing cephazolin (1 g/liter, Sintez), thrombin solution was added drop by drop (P.Z. Cormay, 50 U/ml in 10% $CaCl_2$, Dal'khimfarm) until polymerization.

Clinical study. Stage I of clinical study (started in July 2006) included 8 patients (4 women and 4 men, age 29-55 years). All patients had pronounced atrophy of the bone tissue at the site of lost teeth (reduced height and width of the alveolar process). This state makes impossible intraosseous implantation without sufficient enlargement of the lost bone volume. The bone tissue defects were corrected by transplantation of the tissue-engineering construct on the basis of adipose tissue MMSC in all patients. Complex biochemical, clinical, and serological blood tests (HIV, HCV, HBV, Wasserman reaction) were performed, the major oncomarkers (total prostate-specific antigen, PSA, free PSA, carcinoembryonic antigen Ca 19-9 for men, and carcinoembryonic antigen Ca 15-3, Ca 19-9, and Ca-125 for women). The patients signed informed concern for participation in the study.

The adipose tissue was obtained by lipoaspiration in the umbilical area of the anterior abdominal wall. Computer-assisted tomography and panoramic X-ray imaging of the upper and lower jaws before and 1, 3, and 6 months after transplantation were performed. Intraosseous implants were mounted 3 months after transplantation. The bone shaving obtained after drilling with a pilot borer was fixed in 10% formalin after Lilly and histological sections were prepared after decalcination with EDTA and standard histological processing. The sections were stained with hematoxylin and eosin and with picrosirius red. The results were evaluated after 1, 3, and 6 months by the data of clinical examination, CT, and histologically documented formation of young bone tissue.

RESULTS

Here we present our clinical observations.

Patient Yu., 47-year-old women. State after implantation of BioOss osteogenic material into alveolar socket 47 and intraosseous implantation of a screw implant, which was removed 1 month after implantation because of resorption of the adjacent bone tissue and mobility. The height and width of the alveolar crest at the site of lost tooth 47 were markedly reduced before transplantation of the tissue-engineering construct. CT revealed loosening of the bone tissue with high-density inclusions at the site of the removed implant. In November 2006, a tissue engineering construct containing adipose

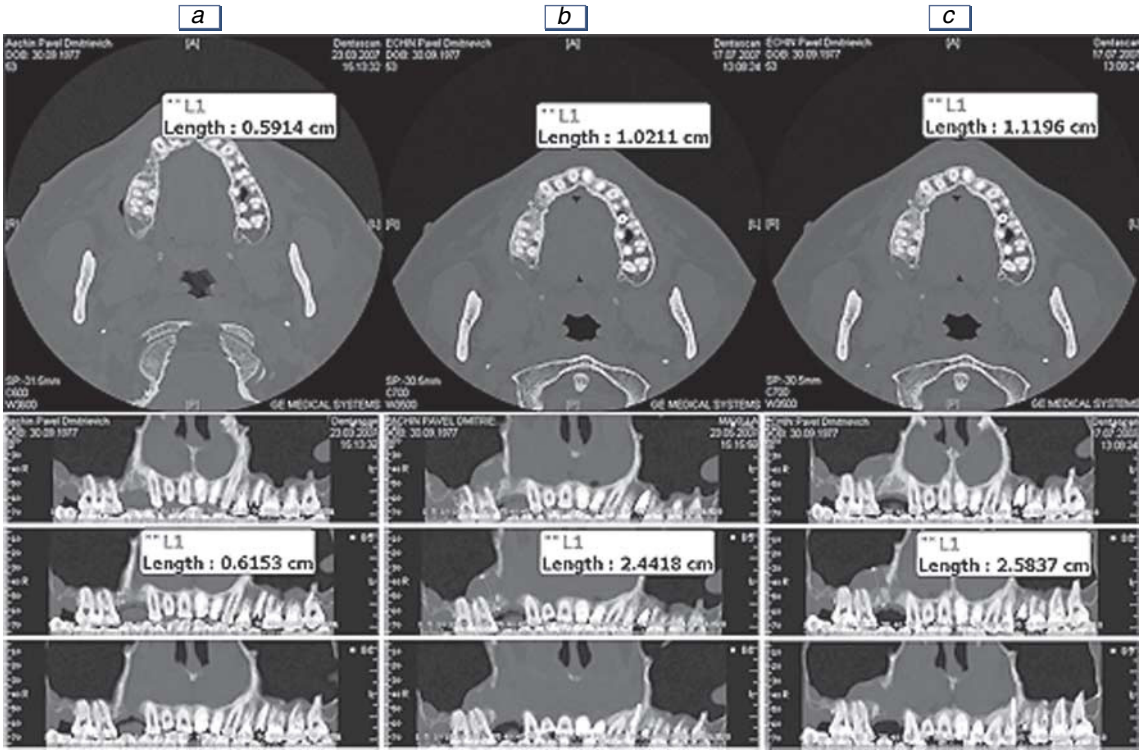

Fig. 1. Patient E., 29-year-old men. CT of facial bone skeleton. *a*) before treatment; *b*) 1 month after transplantation of tissue-engineering construct; *c*) 3 months after transplantation.

tissue MMSC was transplanted after removal of the remaining BioOss material. X-ray examination 1 and 3 months after transplantation revealed increased height and width of the alveolar crest and evenly increased density of the bone tissue. The sutures were removed on day 5, the oral mucosa looked rose, mild postoperation edema was observed. In February 2007, a Nobiel Bioker implant was mounted at the site of lost tooth 47.

Patient E., 29-year-old men. In January 2007, extraction of tooth 14 and tooth 15 roots was performed. Revision of the wound revealed perforation of the maxillary sinus and the absence of bone walls on the vestibular surface of the alveolar sockets of the extracted teeth. The wound was tightly sutured using a displaced mucoperiosteal flap. No inflammation in the maxillary sinus was observed. One month after extraction, the height and width of the alveolar crest were 0.61 and 0.59 cm, respectively (CT data). In April 2007, sinus lifting surgery with transplantation of the tissue-engineering construct in the area of sinus bottom was performed and the width of the alveolar crest at the site of extracted teeth was increased. The wound healed by primary intention on day 5. CT after 1 month revealed a volume formation in the region of the sinus bottom and increased width of the alveolar crest (height 2.44 cm, width 1.0 cm). After 3 months, parameters of the artificial bone in the area of sinus bottom and alveolar crest remained unchanged: we observed ossificating formation on the bottom of the right sinus and sufficient width of the alveolar crest for intraosseous implantation (Fig. 1). In July 2007, two Bicon implants were mounted. Histological examination of the bone shaving obtained during the formation of the implant bed revealed young bone tissue (Fig. 2).

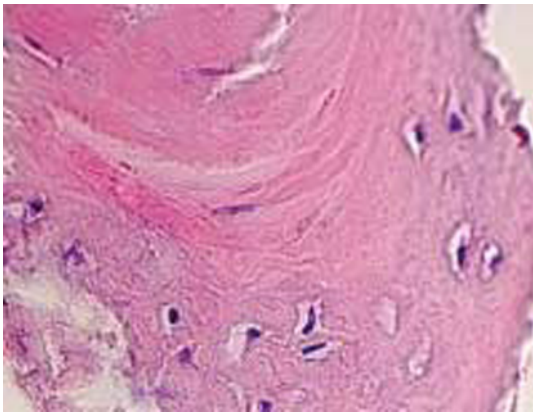

Fig. 2. Patient E., 29-year-old men. Histological picture of young bone regenerate 3 months after transplantation of tissue-engineering construct (obtained during drilling with a pilot borer). Hematoxylin and eosin staining, ×400.

Patient P., 35-year-old women. Chronic mild generalized parodontitis at the stage of remission.

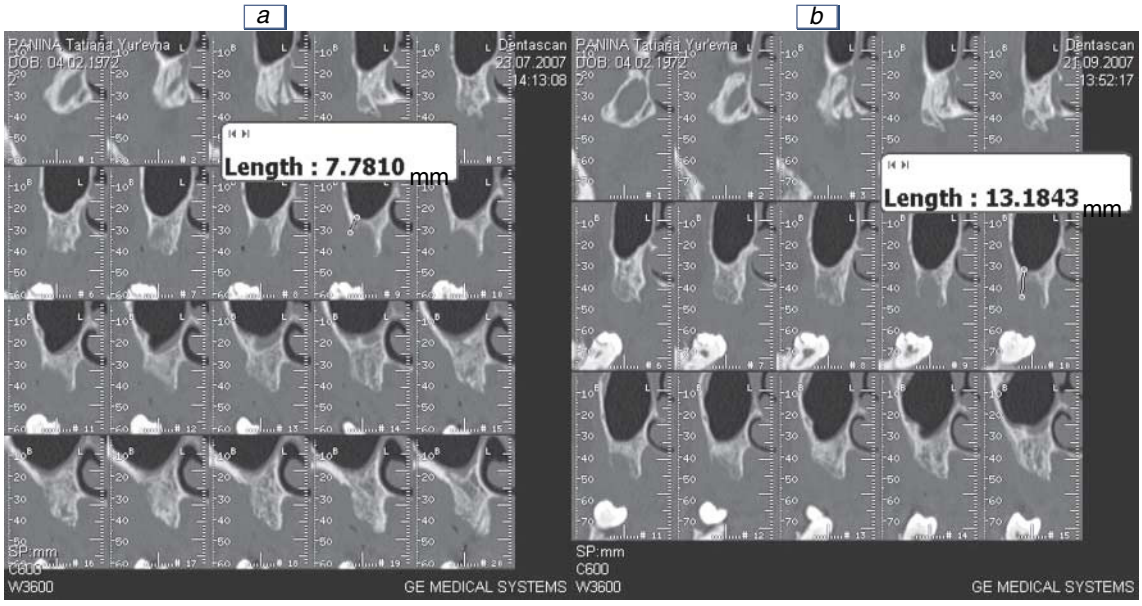

Fig. 3. Patient P., 35-year-old women. Implantation of tissue-engineering construct into alveolar socket after tooth extraction. *a*) before tooth extraction; *b*) 3 months after tooth extraction: recovery of vestibular bone lamina.

Secondary adentia of tooth 17, destruction of the crown of tooth 18. In June 2007, a tissue-engineering construct into the alveolar socket of tooth 18 was transplanted during surgery. According to CT data, the cortical lamina in the zone of extracted tooth 18 was completely restored (Fig. 3). In September 2007, two Bicon implants were mounted in the zone of tooth 17 and 18. Histological examination of regenerate tissue revealed forming young bone tissue.

The presented case reports demonstrate the absence of the reaction of the transplant bed and oral mucosa to the transplanted tissue-engineering construct. We observed rapid healing of the operation wound without mucosa ingrowth into the transplant, which eliminates the need in separating membranes. None patients had microbial inflammatory complications, exposure of the implanted material, or flap necrosis. X-ray examination 3 month after transplantation revealed ossified tissue similar to cortical lamina at the boundary of the transplants and oral mucosa. Histological examination of tissue samples from the center of the regenerate revealed the development of young low-mineralized bone tissue.

Thus, the results of our pilot study suggest that transplantation of tissue-engineering constructs for bone defect reconstruction is a safe procedure allowing to solve some complex clinical problems of oral surgery. The transplanted tissue-engineering construct ensured rapid organotypic recovery of the lost tissue in all patients. We hope that the use of tissue-engineering constructs on the basis of adipose tissue MMSC will improve the results and shorten the terms of treatment in patients with pronounced maxillary and mandibulary bone deficit.

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