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Reconstruction of alveolar defects with regenerative properties of adipose-derived stem cells

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ABSTRACT

Introduction: Loss of tissue and dysfunction of body organs due to injuries and diseases have caused serious health problems despite the vast advances in Medicien. Bone repair through tissue engineering is one of the most important areas of attention for specialists in a wide range of departments, including orthopedics and maxillofacial surgeries.

Materials and Methods: This case-series describes efficient clinical applications of adipose-derived stem cells (AdSCs) in combination with conventional guided bone regeneration as protected healing space for reconstruction of large alveolar defects after hemi-mandibulectomy.

Results: As the result, this approach represented a considerable amount of three-dimensional bone formation in presented cases. Regenerating this amount of resected bone tissue rehabilitates the patients and gives clinicians a sufficient amount of bone and opportunity to insert dental implants to retrieve patients' function and aesthetic.

Conclusion: The application of AdSCs can be considered as an efficient treatment for bone regeneration in large alveolar bone defects.

Keywords: Alveolar bone reconstruction; Mesenchymal stem cell; Adipose-derived stem cells; Natural bovine bone mineral.

Introduction

Stem cell-based treatment aiming for bone regeneration requires a consistent source of osteoprogenitor cells [1]. Although the bone marrow (BM) aspirates considered as the most well-known origin for harvesting stem cells for bone tissue engineering, hence, the invasive procedure and decreasing osteogenic capability of BM

mesenchymal stem cells (BMMSCs) after certain age challenge its beneficial application. Thus, a multitude of alternative sources including dental tissues, skeletal muscle, and adipose tissue has been proposed [2,3]. Among these recent sources, adipose- derived stem cells (AdSCs) were successfully utilized in several in vitro, in vivo, and clinical investigations for bone repair [4]. In comparison to BMMSCs, AdSCs revealed longer conservation in culture and maintained their proliferation and osteogenic differentiation capabilities. Moreover, these multipotent cells in adipose tissues are easily accessible. Also, their harvesting procedure is less invasive, and they are abundant in the human body [4-6].

In order to reconstruct large bone defects in craniofacial region which caused by congenital factors, trauma and various pathologies [7,8], cell-based approach can be beneficial due to overcome some shortcomings of conventional methods such as obtaining iliac crest autograft, which causes a considerable morbidity in donor site [1,9]. In this regard, it seems application of AdSCs can simplify surgical procedures and diminish clinical risks compared to large autograft harvesting [10,11]. This case series aimed to evaluate and describe the potential clinical application of adipose-derived stem cells (AdSCs) in combination with conventional guided bone regeneration (GBR) as protected healing space for reconstruction of large alveolar defects after multi-teeth extractions.

Materials and Methods

Five patients were admitted to the hospital with a portion of their mandibular bone removed (hemi-mandibularctomy) and a desire to rebuild the bone and then place the implant in that area. Defects classified as:

1. Horizontal alveolar ridge deficiency: Alveolar crestal width of less than 4mm.

2. Vertical deficiency defined alveolar: Bone height of less than 8mm.

Inclusion criteria:

- Age over 30 and under 70 years.
- Patient's ability and agreement to surgical fat removal.

Exclusion criteria:

- Present active infectious diseases and blood-borne diseases.
- The background disease causing weakness such as malignancies.
- History of drug addiction or mental illness.
- Any proven bone disease.

After confirming the lesions, a three-dimensional computed tomography (CT) scan of the patient's head was performed, and an ideal virtual alternative to the missing parts of mandible simulated and designed with CAD module. After confirmation of the studied lesions, 20 cc of fat was obtained from the patient's subcutaneous area using suction or surgical approach. The amount of adipose tissue extracted was between 20 and 30 cc depending on the injection dose. Adipose tissue was transferred to the cell processing unit immediately (less than 3 hours). The processing of adipose tissue was performed inside the controlled space of clean room using GMP/Clinical grade materials and observing aseptic principles. In order to extract the stromal vascular fraction (SVF) cells, first the extracted adipose tissue was minified and washed with sufficient amounts of injectable sterile normal saline solution. The adipose tissue was then incubated in a solution containing saline buffer and 0.3% collagenase enzyme GMP grand for 45 to 60 minutes at 37 C to fully digest. After digestion, the enzyme was neutralized by adding a solution of Phosphate-buffered saline and then centrifugation was performed. By discarding the superior solution containing debris and fat droplets, the resulting cellular pellet was diluted again with a normal saline solution and passed through a 70µm filter. After filtering, two washing was performed with a buffer solution and the SVF was used to continue the work.

Stem cells transfer to scaffold

At the beginning of the cell processing, the scaffolding was removed from its packaging by aseptic and inside the clinic room and placed inside the incubator until the cell was ready. After preparation and initial evaluation of the stem cells, the scaffold was transferred to a suitable plate and 500,000 live cells were used to connect per unit volume of scaffolding. The required number of cells was taken to the scaffold according to the size of the scaffold in the volume of 1 to 3mm. After one night, the scaffold was removed and washed with PBS solution and injected into a sterile container containing normal saline serum for patient use and transferred to the operating room with a container containing ice.

The fluid collected from the scaffold was collected to count the cells, and based on the initial number, the percentage of cell binding to the scaffold was calculated and values were recorded for each patient.

Quality control of prepared cells

Considering the main parameter studied in this study is fat stem cells, quantitative and qualitative evaluation of these cells before and after transplantation were critical. This assessment included the evaluation of the safety and function of the cells, some of which were performed before and the rest after transplantation. After preparing the final cell suspension, a sample was taken in a volume of 50µl and the total counting and determination of the percentage of living cells with very high speed and accuracy was performed using the cell nucleus counting device (Nucleocounter, Chemometec, Denmark). According to existing standards, the number of living cells in the final suspension was more than 70%, and samples with less viability were considered unusable.

Evaluation of sterilization of products

Due to the fact that cellular products are consumed in a short time, it is not possible to stay for normal microbial and fungal culture results, so compliance with GMP standards along with observing aseptic principles in fat harvesting in the operating room in reducing side effects is vital. In addition to these standards and according to the existing criteria, a sample was taken from the adipose tissue imported before any washing to study microbial and fungal. Normal aerobic, anaerobic, and fungal and mycoplasma cultures were also taken from the final sample. Due to time limitation prior to direct smear injection, endotoxin testing performed and negative results considered as unusable samples.

Surface Marker Evaluation

Due to the importance of SVF cell composition and its differences in each individual, a sample of the final product obtained and studied for surface marker by flowcytometry. The markers surveyed were CD34, CD45, CD13, CD37, CD90, CD44, and CD31. Efficiency evaluation performed using colony creation capacity (CFU-F assay).

Surgical intervention & clinical evaluation

A crestal incision was made molar to molar regions in the mandible, with two releasing incisions. Sub-periosteal dissection of buccal and lingual mucosal flap was performed gently. A cortical autogenous bone block was harvested from both sides of lateral ramus of mandible. The obtained bone tissue cut into 3-4 pieces. Thin labia plate and 1.2-mm micro-screws with lateral ramus cortical plates (LRCP) have been fixed together in order to create a protected healing space. This space filled with natural bovine bone mineral (NBBM) loaded with adipose-derived stem cells (AdSCs). The whole construct covered with collagen membrane and sutured. Subsequently retracted flap replaced to its position and closed by continuous horizontal mattress sutures (Figure 1).

All procedures were done in a hospital setting by an experienced oral & maxillofacial surgeon. Patients were all given 500mg Amoxicillin three times a day for a week, analgesics (Ibuprofen 400mg with or without Acetaminophen codeine 300/10mg depends on the severity of the pain), and one intramuscular injection of 8mg Dexamethasone (Alborzdarou, Tehran, Iran) and Chlorhexidine mouthwash 0.2% (Behsaco, Tehran, Iran) for seven days postoperatively. In order to evaluate clinical healing, soft tissue and healing process of grafted tissue were checked biweekly for the first month post operation and then monthly for 6 months. Patients had 2 cone beam computed tomography (CBCT) scans taken on the NewTom VG 179 9000 CBCT device (Quantitative Radiology SRL Co., Verona, Italy) before and 6 months after graft surgery.

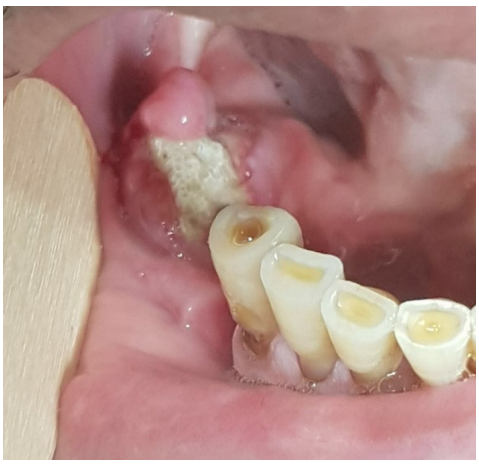

Figure 1. Space filled with natural bovine bone mineral (NBBM) loaded with adipose-derived stem cells (AdSCs).

Histological assessment

The specimens were collected from the 2-mm trephine biopsies obtained during implant surgery after six months from space between bone blocks that had been filled with NBBM filler. Tissues were fixed in 10% buffered formalin and further prepared for fixation in paraffin blocks. Serial sections were obtained and stained with Hematoxylin and Eosin (H&E). Using a light microscope (CX31-OLYMPUS, Japan) and a magnification field of 40×, specimens were investigated. Percentage of new bone formation was analyzed by computerized image analysis software (ImagePro Plus; Media Cybernetics, Silver Spring, MD, USA).

Clinical and radiological evaluation

To evaluate clinical healing, soft tissue and healing process of grafted tissue were checked weekly for the first month postoperatively and then monthly for 6 months. CBCT was taken preoperatively and again at 6 months postoperatively, by NewTom VG 9000 CBCT device (Quantitative Radiology SRL Co, Verona, Italy) at 0.2 mm voxel size, 110 kVp, 5.4 seconds, and 0.7mA. The images were reconstructed and cross sectional viewed were exported into Image Pro software (National Institutes of Health [NIH], Bethesda, MD).

Ethical considerations

All the procedures described for the patients and informed written consent were obtained from each individual. The protocol of this study reviewed and confirmed by the ethic committee of the Tehran University of Medical Sciences, Tehran, Iran, under the registration code of IR.tums.vcr.rec.1397.228.

Results and Discussion

Clinical and radiological findings

Five patients, including 3 males and 2 females, with mean age of 49±12.02 (34-65 years old) were included in current study. No post-surgical infections reported in the study cases and there were no serious post-operational complications. Hence, after 1-month follow-ups 3 cases had moderate inflammations. There were no signs of inflammation after 6-months follow-ups. The mean of defect size was 173.84±7.3 mm^2 ranging from 167.45 up to 184.4 mm^2. Two patients had horizontal defects (alveolar crestal width of less than 4mm) while three suffers from vertical defects (alveolar bone height of less than 8mm). Over 6-months follow-ups of the patients two patients (Case 3 and 5) reveal more than 10% new bone formation (NBF) (10%< <50%) and three patients, with regard to their improving situation, gain <10% new NBF (Case 1, 2, & 4).

Microscopic findings:

Mild inflation was found in scaffold with (AdSCs). The foreign body reaction did not report. The woven and lamellar bones were observed, as lamellar bone was greater (Figures 2).

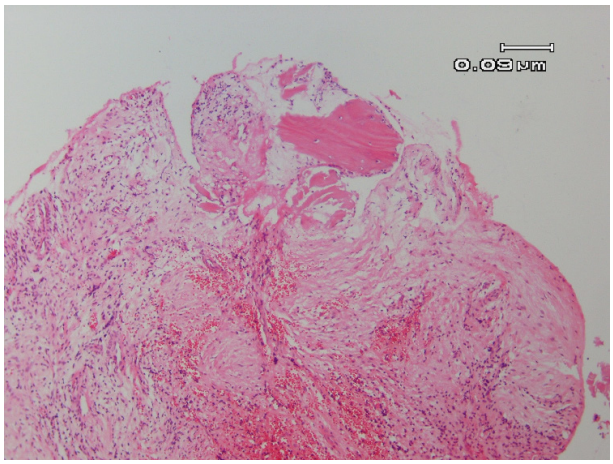

Figure 2. Microscopy images show the amount of bone formation in defect.

Autogenous iliac crest bone graft is the golden standard for rehabilitation of large bone defects due to its rapid incorporation to the host, the presence of a multitude of growth factors, and its osteogenic properties [12]. However, there are some major drawbacks in its clinical application such as the need for a second site of surgery, its limited tissue volume, morbidity of donor site, infection and scar creation [12-14]. In addition, it is estimated that up to 80 percent of the osteocytes do not survive the transplantation process between the donor site and the surgical site and they showed a resorption rate between 65%-85% [15]. Moreover, from a biological perspective, the origin of iliac crest harvested bone is the mesoderm germinal layer which generates bone by endochondral ossification process, whereas bone grafts that are obtained intraorally from the mandible turn into bone much faster, since they generate bone by intraosseous bone formation. Thus, emerging of the regenerative medicine in order to address these shortcomings of iliac crest harvested bone is pivotal. One of the most imperative purposes of bone tissue engineering is to seek a reliable source of stem cells that provide a proper number of stem cells for clinical administration with minimal invasiveness and morbidity. Nowadays, BMMSCs are the most common source of osteoprogenitor cells in bone tissue engineering [3,16] that isolated from bone marrow as-

pirate. However, this invasive and painful process have only 0.001-0.1 percentage of BMMSCs in the obtained cells compared to lipoaspirates in which contain 1-5 percentage of AdCSs [17]. Moreover, the comparative investigations revealed similar osteogenic differentiation of AdSCs and BMMSCs [18-20]. In spite of the intensifying number of evidences on AdSCs and their excellent potential for bone regeneration, most of in vitro investigations and only limited clinical trials evaluated the safety and efficacy of AdSCs utilization in humans [21-23]. In accordance to our results, AdSCs showed beneficial effects on bone healing in anterior mandibular [21], maxillary [22], and cranial [23] bony defects. This study evaluated the efficacy of Adipose derived mesenchymal stem cells for three-dimensional bone regeneration in the alveolar defects. The finding of these cases represents that in large alveolar bone defects, application of AdSCs with GBR technique maintain appropriate bone regeneration for the following dental implant placement. One of our study's limitation is our evaluating methods. Although Radiographic images cannot separate areas of new bone formation from bone substitutes, histological assessments indicated lamellar trabecular pattern containing osteocyte that representing a new bone formation area. Another limitation is that due to ethical considerations, we did not have any control to compare our findings.

Conclusion

With the limitation of our study, this efficacious clinical application of AdSCs for regeneration of alveolar bone open a window in this prosperous new field of research that requires further efforts especially randomized controlled clinical trials and histomorphometric assessments to lead an evidence-based decision making.

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Conflict of Interest

There is no conflict of interest to declare.

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