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# Application of buccal fat pad-derived stem cells in combination with autogenous iliac bone graft in the treatment of maxillomandibular atrophy: a preliminary human study

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*Abstract.* Stem cell therapy for the treatment of bone defects is an alternative or adjunct to autologous bone grafting. This study assessed the efficacy of buccal fat pad-derived stem cells (BFPSCs) with iliac bone block grafting for the treatment of extensive human alveolar ridge defects. Eight patients with extensive jaw atrophy were selected for this study. The jaws were reconstructed with non-vascularized anterior iliac crest bone blocks. Gaps between the blocks were filled with freeze-dried bone granules and covered with a collagen membrane. In the test group ($n = 4$), these granules were seeded with BFPSCs. Cone beam computed tomography scans were used to assess the amount of new bone formed at six sites in each patient. Trephine biopsies of 2-mm were also taken from the graft site during implant placement for histomorphometric analysis. The mean bone width change at the graft site was greater in the test group than in the control group ($3.94 \pm 1.62$ mm vs. $3.01 \pm 0.89$ mm). New bone formation was 65.32% in the test group versus 49.21% in the control group. The application of BFPSCs in conjunction with iliac bone block grafts may increase the amount of new bone formation and decrease secondary bone resorption in extensively atrophic jaws.

Key words: mesenchymal stem cell; tissue engineering; regenerative medicine; bone defect; bone graft.

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The reconstruction of severely resorbed jaws is a challenging procedure, depending on the severity of bone atrophy. Bone defects in the maxillofacial region vary from small peri-implant defects to extensive bone atrophy (approximately equal to a span of six teeth).[1,2] For extensive defects, autogenous bone grafting from an extraoral donor site, such as the anterior iliac crest, remains the workhorse technique for jaw reconstruction.[3] Autografts concurrently provide osteogenic cells and a suitable scaffold for osteogenesis.[4] However, donor site morbidity, prolonged hospitalization, and high costs are all limitations of this technique.[5] Moreover, secondary bone resorption is a major shortcoming for dental implant success.[6,7] It has been reported that the entire grafted iliac crest bone may be resorbed within 6 years post-surgery.[7]

Tissue engineering is a new minimally invasive method to restore and reconstruct tissues or organs that involves the morphogenesis of new tissue using constructs formed from isolated cells, growth factors, and scaffolds.[8] Mesenchymal stem cells (MSCs) form part of the tissue engineering triangle and are a novel modality for bone autografts. These cells may be obtained from several sources including the bone marrow, adipose tissue, dental pulp, peripheral blood, or periosteum.[9] In the trial reported here, the buccal fat pad was used as a source of MSCs. Adipose stem cells (ASCs) – initially called processed lipoaspirate cells – were first discovered in 2001 by Zuk et al.[10] They have been evaluated extensively for tissue engineering, and in particular for bone formation. Many studies have shown the excellent efficacy of MSCs in bone regeneration,[11–14] but in larger and critical-sized defects, this stem cell treatment as the sole therapy has shown a lesser amount of new bone formation.[15,16] Furthermore, onlay block grafts have shown reduced osteogenic activity and slow revascularization when compared to particulate bone marrow.[17]

To increase the osteogenic activity of non-vascularized iliac bone block grafts, this study sought to assess the concomitant application of buccal fat pad (BFP)-derived MSCs (BFPSCs) and anterior iliac crest bone grafting in the reconstruction of extensive alveolar ridge defects.

## Materials and methods

### Patient selection

Eight patients presenting to the maxillofacial clinic of a university hospital in Tehran, Iran, were selected for this study. All of the patients enrolled in this study had extensive jaw atrophy (the need for augmentation to accommodate more than six teeth). The patients were aged between 25 and 60 years. They were non-smokers with no underlying systemic conditions. Of these eight patients, four were assigned to the control group. In these cases, reconstruction of the atrophic ridge was performed using onlay bone block grafting from the anterior iliac crest. Freeze-dried bone allograft (FDBA) granules (SureOss; Hans GBR Biomaterial, Seoul, Korea) were applied to fill the gaps, and collagen membranes (Jason membrane; Botiss Biomaterials GmbH, Zossen, Germany) were used to cover the defect. In the test group ($n = 4$), the FDBA granules were loaded with autogenous MSCs harvested from the BFP.

The institutional ethics committee approved all of the studies, and informed consent was obtained from all donors. The isolation and cultivation of the MSCs was performed without xenogenic supplements such as foetal bovine serum (FBS). All methods used to cultivate and extract MSCs have been described in previous projects using human patients.[13,14]

### Buccal fat pad-derived stem cells (BFPSCs)

The BFP was harvested from healthy donors through a vestibular incision distal to the maxillary second molar (Fig. 1). It was exposed by blunt dissection while preserving the thin covering membrane. Approximately 3–5 ml was excised and delivered to the laboratory in Dulbecco's modified Eagle's medium (DMEM). The dissected tissue was minced and incubated in 3 mg/ml type I collagenase and 4 mg/ml dispase in phosphate buffered saline (PBS)

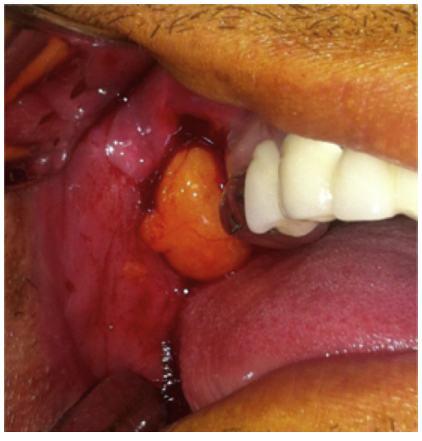

*Fig. 1.* Buccal fat pad (BFP) exposure through a 1-cm lateral vestibular incision. With blunt dissection, 3–4 ml of BFP can be harvested, and this is sufficient for the culture of adipose-derived stem cells.

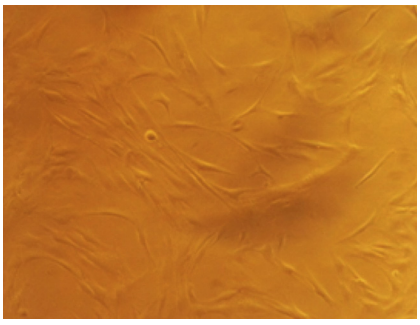

*Fig. 2.* Stellate cells form after the third passage of cell culture, as seen on light microscopy.

(Gibco Laboratories, Grand Island, NY, USA) at 37 °C for 30 min. The fragments were then immersed in minimum essential medium alpha (α-MEM) supplemented with 10% FBS and 1% antibiotics (Gibco), and placed in 25-cm$^2$ culture flasks, followed by incubation in a humidified atmosphere containing 5% $CO_2$ at 37 °C. The culture medium was changed twice weekly and after reaching the subconfluent stage, and cells were removed by enzymatic digestion (0.25% trypsin–ethylenediaminetetraacetic acid (EDTA)) and passaged. MSCs of the third to fourth passage were used in the experiments. Adherent cells were expanded as monolayer cultures in a 95/5 air/$CO_2$ (v/v) atmosphere at 37 °C, with medium changes every 3 days. Confluent cells were dissociated with trypsin and subcultured in new six-well culture dishes at a plating density of $6 \times 10^4$ cells/dish. The cells could be seen under light microscopy (Fig. 2).

### Preparation of human serum

Human autologous serum was used in this study. Twenty millilitres of whole blood was collected from each patient and drained into two 10-ml tubes without anticoagulants (BD, Plymouth, UK); this was allowed to clot for 4 h at 4–8 °C. Subsequently, the blood was centrifuged at 2800 × *g* at 4 °C for 15 min. Serum was collected and stored at −20 °C. FBS was omitted from the laboratory procedure for ethics committee considerations.[18]

### Evaluation of the mesenchymal stem cell nature of the isolated cells

*Flow cytometry analysis*

Fluorescence absorbance cell sorting (FACS) analysis was performed using standard protocols and quantification criteria to confirm the cell surface expression of CD44, CD90, CD73, CD105, CD45, and CD34. The gate used to distinguish positive