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Efficacy of adipose-derived stem cells for regeneration of localized mandibular bone defects

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ABSTRACT

Introduction: Use of adipose-derived stem cells (ADSCs) is a suggested hypothesis to enhance the regeneration of bone defects. This technique is of particular interest since ADSCs are more easily accessible via a non-invasive harvesting technique compared to bone marrow stem cells (BMSCs). This study sought to assess the efficacy of ADSC implantation for regeneration of localized mandibular bone defects.

Materials and Methods: Eight patients (six females and two males) between 18 and 28 years with indication for extraction of all four third molars were enrolled in this split-mouth randomized clinical trial. A total of 16mandibular bone defectswere divided into two groups of experimental and control. Cell-containing and cell-free scaffolds were used in the experimental and control groups, respectively. Size of bone defects and percentage of the newly formed bone were assessedi mmediately after surgery and at two, four and six months post-operatively.

Results: No statistically significant difference was foundin bone regeneration between the two groups at the afore-mentioned time points. However, at all time points in both sides (with and without ADSCs), bone regeneration significantly increased over time (P<0.000).

Conclusion: The efficacy of ADSC-containing scaffold was similar to that of cell-free scaffold for enhancing the regeneration of bone defects. Further investigations are required to assess the efficacy of stem cells of different origins in combination with different scaffolds in this regard.

Key words: Stem cells, Bone defects, Mandible, Adipose-derived stem cells.

Introduction

Regeneration of bone defects is a challenge in reconstructive and reparative surgeries because not only it is imperative to preserve the anatomy and function of the area, but also facial defects leave psychological wounds, which further emphasizes the significance of their efficient management [1]. The HCUP reported that the cost of surgical procedures for facial traumas alone was 1.4 million dollars in 2004 [2]. Fresh autogenous bone [3], biomaterials such as hydroxyapatite (HA) and tricalcium phosphate (TCP) [4] and demineralized bone matrix (DBM) [5,6] are currently used to enhance the regeneration of bone defects; however, these materials have many shortcomings [7-9]. Fridentein in 1987 was the first to

and bone [10]. At present, osteogenic cells are seeded on three-dimensional scaffolds resembling bone in regenerative medicine [11,12]. These cells have been isolated from the bone marrow [13,14], muscles, hair follicles [15] and adipose tissue [16,17] and successfully differentiated into osteoblasts, chondroblasts, lipoblasts, tenoblasts and myoblasts in vitro and in vivo [18]. Adult mesenchymal stem cells are highly versatile and can differentiate to various types of tissues depending on the expressed genes and the environment in which they are stored. Currently, bone marrow serves as a common source of stem cell isolation [19]. However, this method is invasive and painful and causes scratches and abrasion on the bone surface. Moreover, aging decreases the number of these cells and their differentiation potential [18-20]. ADSCs have osteogenic potential and can be largely harvested painlessly without requiring a complex procedure [21-23]. Furthermore, synthetic materials and allografts allow the seeding and proliferation of osteoblasts and preosteoblasts in vitro [24].

Considering the significance of this topic, in this clinical trial, an allograft containing ADSCs was implanted at the third molar extraction site defect for the first time to assess the process of bone regeneration compared to the control group.

Methods and Materials

This randomized split mouth match sequential double blind clinical trial was conducted on eight patients with indication for extraction of all four third molars. The patients signed written informed consent forms.

Age, sex, smoking status, systemic diseases and DMF index of patients were recorded in data forms. ASA 1 and 2 patients between 18 and 28 years with no history of chronic or underlying disease were included. Subjects with a history of maxillofacial malignancies, electrolyte disorders, diabetes mellitus, cardiovascular diseases, head or facial trauma and those with less than four third molars for extraction were excluded. Dissimilar defect morphology at the two sides was also an exclusion criterion. In other words, patients with two fully impacted mandibular third molars with similar angulation and morphology were entered in the study.

First, the maxillary third molars were aseptically extracted under local anesthesia. Considering the close vicinity of the buccal fat pad (BFP) to the maxillary third molar area, a mucoperiosteal flap was elevated; a small incision (approximately 5mm) was easily made at the periosteum and about 2g of fat was extracted from the BFP and placed in a container for transfer to cell culture laboratory of the Imam Khomeini Hospital Cell Research Center (for isolation of stem cells and their subsequent induction to osteogenic cells). The mucosa was sutured watertight bilaterally using 4-0 Prolenesuture [18].

Expression of stem cell markers namely CD73, CD90 and CD105 on the surface of isolated cells was assessed using flow cytometry. Expression of osteogenic differentiation markers, indicative of successful induction of mesenchymal stem cells to osteogenic cells (expression of osteocalcin and collagen I genes) was evaluated using polymerase chain reaction (RT-PCR) [25]. Cell culture was performed using the standard technique. After 30 days, third passage cells expressing osteogenic markers were seeded on the scaffold [16,26,27].

Patients were recalled and one quadrant of themandible was randomly chosen as the experimental, and the other as the control group. Impacted mandibular third molars were extracted by a maxillofacial surgeon via an osteotomy in such a way that similar defects were obtained at both sides. In the experimental group, an implant of stem cells induced to osteogenic cells and seeded on an allograft scaffold was placed at the site of defect. In the control group (the other mandibular quadrant), same-size cell-free allograft scaffold was implanted. Mucosa was sutured watertight bilaterally using 3-0 Prolenesuture. Panoramic radiographs were immediately obtained and bone defects were evaluated by two observers [28].

The patients and the observers (radiographically assessing bone defects) were not aware of the material used at each side of the jaw. This ensured double blind design of the study.

Eventually, data related to each side including the changes in size of bone defects and the percentage of the newly formed bone at different time points were recorded and analyzed. Normally distributed changes at each time point were statistically analyzed using t-test. Data with non-normal distribution were analyzed by the Mann Whitney test. Significant differences were subjected to ANOVA and multiple comparisons.

Results

This study was conducted on eight patients. Since our study had a split mouth design, 16 mandibular defects were evaluated. There were six males and two females

with a mean age of 20±1.1 years (range 19-25 years). Patients were all ASA 1 and 2 and had no history of chronic or underlying diseases. Expression of CD90, CD105 and CD73 mesenchymal cell surface markers (Figure1) and no expression of CD45 and CD34 by the BFP cells in flow cytometry were indicative of stem cell characteristics (Figure2). Osteogenic differentiation of these cells after the third passage was approved by RT-PCR.

As seen, at two months, the percentage of new bone formation was 75±24% in the control and 74±18% in the experimental group; this difference was not statistically significant. At four and six months, no significant difference was noted in this regard either (P<0.9). At all time points in both sides (with and without ADSCs), bone regeneration significantly increased over time (P<0.000).

X-ray images of the patient before removing the tooth, repairing the cavity after 2 months of treatment and repair the cavity after 6 months of treatment is shown in Figure 3.

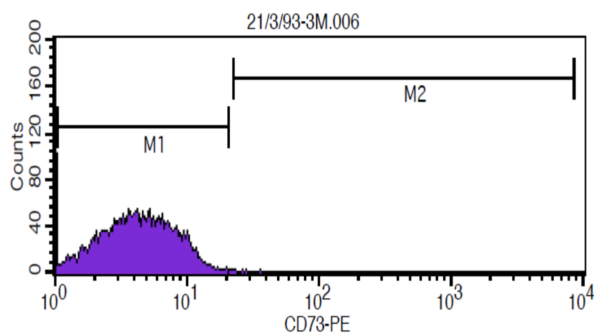


Figure 1: Expression of CD73 on mesenchymal stem cell surface.

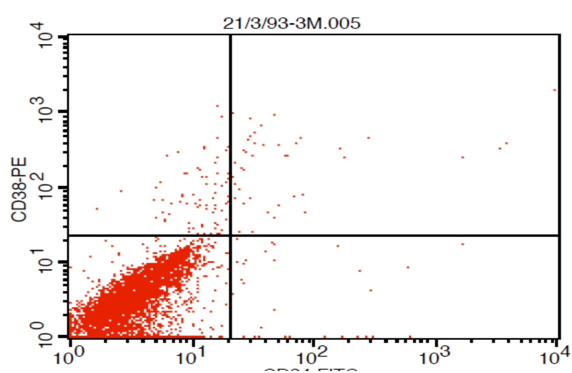
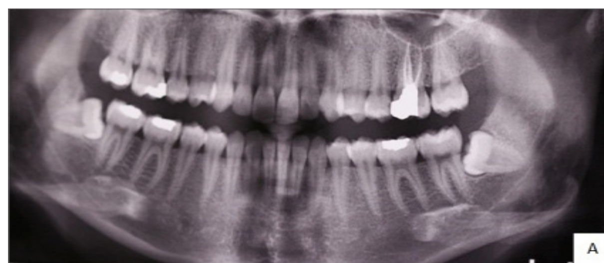
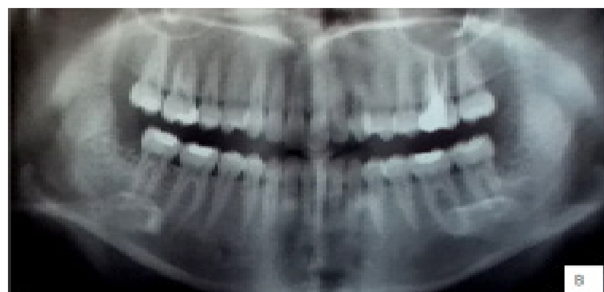


Figure 2: No expression of CD34 on mesenchymal stem cell surface





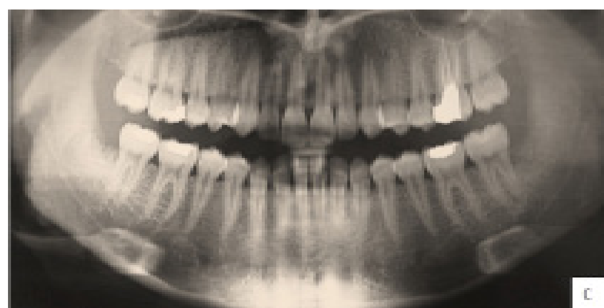


Figure 3: X-ray images of the patient before removing the tooth, repairing the cavity after 2 months of treatment and repair the cavity after 6 months of treatment.

Discussion

This study showed that cell-containing and cell-free scaffolds had equal efficacy for bone regeneration, and the difference in this regard between the control and experimental groups was insignificant. However, the results of previous studies in this respect are controversial. Many researchers believe that stem cells of different origins are not efficacious for this purpose while some others stated otherwise and showed optimal efficacy of different combinations of stem cells and scaffolds for bone regeneration. many shortcomings [7-9]. Fridentein in 1987 was the first to propose the use of mesenchymal stem cell.

This study showed that ADSCs present in the BFP had characteristics of mesenchymal stem cells and expressed CD90, CD105 and CD73 markers while they did not express CD34 or CD45 markers. Also, they had osteogenic potential to differentiate into osteoblasts based on the results of RT-PCR. Moreover, these cells were well seeded on HA-TCP scaffold (60% TCP-40% HA) with 300μ pore size and were observable by elec-

tron microcopy.

Search of the literature yielded no similar study directly assessing the use of BFP for regeneration of alveolar bone defects at the third molar sites in humans to compare our results with. Thus, our findings were compared with the results of studies on the efficacy of ADSCs and scaffolds for regeneration of bone defects in animal models as well as studiesassessing the differences in regeneration by the use of stem cells versus autogenous cells.

In 2006, Hbii et al. treated an alveolar cleft by tissue engineering and use of BMSCs and reported that 80% of the cleft was regenerated and filled by the implanted cells at nine months. However, they only evaluated one case in their study, which is not enough to confirm the success of this technique as an alternative modality. Moreover, they obtained several CT scans for assessment of bone regeneration, which decreases the accuracy of their study in comparison with histological and histomorphometric studies. No comparison was made with any other graft technique either.

In an experimental study on four dogs, Shah Naseri compared the amount of new bone formation following tissue engineering with the use of ADSCs with the amount of newly formed bone after autogenous bone grafting. Incisor teeth were bilaterally extracted in dogs and each of the above-mentioned techniques was used in one quadrant. Significant differences were noted in the amount of newly formed bone at 15 and 60 days. At the autograft site, percentage of newly formed bone was 45% at 15 and 95% at 60 days. These values were 50% and 70%, respectively on the other side (P=0.004 and P=0.001, respectively). Although the regeneration rate was lower at the site treated with ADSCs compared to autogenous graft, this rate was still significant [29]. Although their study was an animal study with a small sample size, their results confirmed the optimal efficacy of ADSCs for regeneration of bone defects in dogs, which are physiologically close to humans and thus, optimal results may be expected in humans as well.

In another study, Behnia et al. seeded BMSCs on a calcium sulfate (Osteoset®) scaffold and used it in cone junction with DBM for treatment of bilateral alveolar clefts in two children and reported successful results. However, since they used DBM along with stem cells, the share of stem cells in this outcome cannot be determined and the final result cannot be attributed to the use of stem cells alone since DBM can directly enhance regeneration and its effect on stem cells cannot be overlooked either [28]. Moreover, due to small size and differences in the process of regeneration in children and adults, the optimal efficacy of stem cells for bone regeneration in children cannot be generalized to other age groups.

Based on all the above, the efficacy of stem cells and their cost effectiveness are still mattersof controversy. Our study showed that application of stem cells was cost-effective in terms of esthetic and functional results and lack of significant short-term or long-term complications. However, further studies on larger sample sizes are required to assess the efficacy of different scaffolds and techniques. Also, future studies are needed to assess whether ADSCs have any advantage over other stem cells for bone regeneration.

The current study had some limitations. The sample size was small because finding qualified patients was difficult. Attempts were made to standardize the bone defect morphology. Also, different surgeons might have performed differently depending on the case. Moreover, considering the differences in age, gender and alveolar bone shape in different patients, defects could not be standardized completely. Standardization of bone defects does not mean that the experimental and control groups are 100% similar; because considering the bone tissue, this is not feasible. Another issue is the placement of ADSCs in the defect. If defects were not standardized completely, application of these cells would be different as well. For assessment of bone regeneration, radiographs were obtained and assessed. This process, when performed by different technicians, may also result in errors in data registry. Also, even if the same person measures the defect size, the errors related to the method of measurement cannot be overlooked. Last, but not least, patients were followed up for six months. Longer follow-ups could have resulted in different findings or possibly significant differences between the two groups.

We tried our best to avoid bias in this study. Also, this study was not financially supported by any company. Patients and technicians that performed measurements were blinded to the group allocation of specimens. Also, this study was performed on humans and thus, our results are more reliable than those of animal studies. Patients were evaluated at two, four and six months post-operatively to accurately assess bone regeneration and post-operative complications. Moreover, since defects were matched in terms of morphology and angulation, the effect of this confounder on the results was minimized.

Conclusion

Considering the undeniable role of bone regeneration following many medical and dental procedures, finding non-invasive methods to enhance bone regenerationwill eliminate the problems associated with the use of invasive techniques such as bone graftharvesting. Future studies with larger sample sizes are required to further assess the efficacy of ADSCs for bone regeneration.

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