Treatment of Peri-Implant Defects in the Rabbit's Tibia with Adipose or Bone Marrow-Derived Mesenchymal Stems Cells

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ABSTRACT

Background: Mesenchymal stem cell (MSC) treatment in conjunction with bone graft materials or space filler can be an alternative to autogenous bone grafts in the treatment of peri-implant bone defects.

Purpose: To evaluate the success of bone regeneration capacity of adipose-derived and bone marrow-derived MSCs for the treatment of peri-implant bone defects when applied with a beta-tricalcium phosphate/collagen-based scaffold.

Material and Methods: Forty implants were placed into the tibiae of 10 rabbits bilaterally. Surgical defects created around the implants were treated with one the following treatment modalities: 1) adipose-derived MSC transplanted scaffold + collagen membrane; 2) bone marrow-derived MSC transplanted scaffold + collagen membrane; 3) autogenous bone + collagen membrane; and 4) collagen membrane only. The bone regeneration capacity of each technique was determined by histomorphometry, micro-CT, and measuring the implant stability by resonance frequency analysis.

Results: One limb of one rabbit was excluded because of fracture, and another limb was excluded because of infection. All parameters on 36 implants revealed that both sources of MSC can form equivalently new bone that is comparable with autogenous bone. The defects treated with membrane only had significantly less bone formation compared with other groups.

Conclusion: Both adipose-derived and bone marrow-derived MSC treatments are feasible alternatives to autogenous bone grafts in the treatment of peri-implant osseos defects.

KEY WORDS: dental implants, guided bone regeneration, mesenchymal stem cells, rabbit

INTRODUCTION

Augmentation of the defects around dental implants is one of the most applied surgical procedures associated with dental implant surgery.[1] Several augmentation techniques and materials have been described in the literature. To date, autogenous bone grafts are considered the best graft materials due to their osteogenic capacity.[2] However, they require donor sites, and the patients' tolerance is less compared with the other techniques, in which no additional surgical site is created. Utilization of mesenchymal stem cells (MSCs) in conjunction with bone graft materials or space filler may add osteogenic features; thus, they can be alternatives to the autogenous bone grafts in the treatment of peri-implant bone defects.

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Stem cells are progenitor cells of self-renewing tissues that have a capacity of prolonged or unlimited self-renewal under controlled conditions and a potential to differentiate into variety of specialized cell types. MSCs are adult stem cells that are progenitors of mesodermal tissues. Because of their osteogenic differentiation capacity, MSCs have been used for bone regeneration purposes in oral and maxillofacial surgery since more than two decades.[3] MSCs can be delivered to the recipient site by means of two methods: with direct application of the collected MSCs or after ex vivo expansion in tissue cultures. Currently, the most popular sources of MSCs when used for bone regeneration procedures are bone marrow and adipose tissue. Bone marrow is obviously the best source when direct application method is used.[4,5] But it is associated with increased donor site morbidity and difficulty in the management and processing of the aspirate. As a source of MSC, adipose tissue has several additional advantages over bone marrow including availability of larger quantities of autogenous tissue supply and relatively higher osteoblastic cell yield.[4,5] But the literature indicates requirement of more studies about the bone regeneration capacity of adipose-derived MSCs.[6,7]

Unfavorable local conditions of the alveolar ridge are present in many of the patients, who need dental implant therapy. Dental implant placement may result in a dehiscence or a fenestration-type defect around the implant body, if the ridge lacks sufficient amount of bone volume (BV). Guided bone regeneration (GBR) procedure, in which osteogenesis occurs within the alveolar defect that is mechanically prevented from undesirable tissues, has been successfully used in the treatment of such defects since more than two decades.[1,8] As the single-stage GBR technique (concomitant with implant placement) is one of the most commonly applied surgical procedures in implant dentistry, the effectiveness of variety of graft and membrane materials in the GBR technique have been studied extensively in previous animal and clinical studies. To replicate a similar peri-implant bone defect that occurs after implant placement clinically, different types of animal models were developed previously. These include surgically created bone defects around the implants in the rabbit tibia or femur, the dog mandible, and the pig calvarium.[8–11]

In this study, the success of MSCs delivered with beta-tricalcium phosphate (β-TCP)/collagen scaffold was compared with autogenous bone graft in the restoration of peri-implant defect in the tibia of rabbits. MSCs were derived either from the adipose tissue or bone marrow of the same rabbit. The main purpose of this study was to find out whether synthetic scaffolds that are loaded with adipose-derived MSCs or bone marrow-derived MSCs can be alternative treatments to autogenous bone grafts in the restoration of peri-implant bone defects.

MATERIALS AND METHODS

Study Design

The study was reviewed and approved by the Chulalongkorn University Institutional Ethics Committee for the Local Use of Animals in Experiments. Ten skeletally mature male New Zealand white rabbits weighing between 3.1 and 3.8 kg (mean: 3.51 ± 0.46, at the time of the implant placement surgery) were used. Preoperatively, all rabbits were monitored daily with regard to their general health and food intake for 2 weeks. All rabbits survived this phase without any significant systemic or local pathology or impairment in their general health. Each rabbit underwent the same treatment procedure. Initially, adipose tissue and the bone marrow were collected from each rabbit. After isolation and expansion of the MSCs in tissue cultures, implant placements and augmentations of the peri-implant defects were carried out. Two implants were placed to each tibia of the rabbits. The defects around the implants were treated with one of the following modalities: 1) GBR with adipose-derived MSC transplanted scaffold + collagen membrane (group 1); 2) GBR with bone marrow-derived MSC transplanted scaffold + collagen membrane (group 2); 3) GBR with autogenous bone graft particles + collagen membrane (group 3); and 4) GBR with collagen membrane only (group 4). After 8 weeks of healing, the animals were killed, and the characteristics of the newly formed bone were evaluated.

Collection of Adipose Tissue and Bone Marrow

The rabbits were anesthetized with 35 mg/kg ketamine and 5 mg/kg xylazine via intramuscular route. The peritoneum and iliac crest regions were shaved and prepared with 10% povidone-iodine solution. About 5-cm long adipose tissue was collected from the peritoneum after the exposure the subcutaneous tissue. Subsequently, about 4 ml of bone marrow was aspirated from the posterior iliac crest using 16-gauge bone marrow aspiration needle that was coated with 5 IU heparin/ml.

Isolation and Culture of the MSCs from Bone Marrow and Adipose Tissue

Mononuclear cell populations from rabbit bone marrow were isolated using IsoPrep® (Robbins Scientific Corporation, Mountain View, CA, USA) density gradient centrifugation. The aspirates were washed twice with phosphate buffer saline (GIBCO™, Invitrogen Corporation, Grand Island, NY, USA) and re-suspended in complete medium, Alpha-minimum containing medium (GIBCO) supplemented with 10% (v/v) fetal bovine serum (Lonza, Allendale, NJ, USA), 1% Glutamax (GIBCO), 100 U/ml penicillin (General Drug House CO., Ltd, Bangkok, Thailand), and 100 µg/ml streptomycin (General Drug House CO., Ltd, Bangkok, Thailand). Cell suspensions were plated in 25 cm^2 cell culture flask (Corning Inc., Corning, NY, USA) at a density of 2×10^5 cells/cm^2. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and medium was replaced every 3 days during the entire culture period. For isolation of MSCs from the adipose tissues, small pieces of adipose tissue (approximately 2 cm^2 each) were washed with PBS, digested by incubating with 0.1% collagenase type II (Sigma-Aldrich, St. Louis, MO, USA) in Ham's F12 medium for 2 hours at 37°C. The digested tissues were then washed twice with PBS, re-suspended in complete medium, and plated in 25 cm^2 culture flask (Corning Inc., Corning, NY, USA). Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2, and medium was replaced every 3 days during the entire culture period.

Immunophenotyping of the MSCs by Flow Cytometry

At the third passage level, MSCs were characterized for their surface marker expression profiles with the following mouse or anti-human antibodies: anti-CD45-FITC (BD Pharmingen, San Diego, CA, USA), anti-CD34-PE (Biolegend, San Diego, CA, USA), anti-CD90-FITC (AbDSerotec, USA), anti-CD73-PE (BD Pharmingen, San Diego, CA, USA), and anti-CD105-PE (Miltenyi Biotec, Bergisch-Gladbach, Germany) for 30 minutes at 4°C in the dark. After incubation, cell pellets were washed twice with PBS and fixed with 1% (w/v) paraformaldehyde in PBS. Flow cytometry was performed by FACS Calibur™ flow cytometer (Becton Dickinson, Mountain View, CA, USA) using CellQuest™ software (Becton Dickinson).

Osteogenic Differentiation of the Cultured MSCs

After the third passage, 5×10^4 MSCs were cultured in osteogenic differentiation-inducing medium, which is complete medium supplemented with 0.1 µM dexamethasone and 50 µg/ml ascorbic acid. The medium was replaced every 3 days throughout an entire culture period. On culture day 7 onward, 10 mM β-glycerophosphate was added to the medium. After culture for 3 weeks, the cells were washed with PBS, and their alkaline phosphatase activity was determined by incubating with BCIP®/NBT Liquid substrate (Sigma-Aldrich, St. Louis, MO, USA) for 30 minutes at room temperature. After incubation, the cells were washed twice with distilled water and observed under inverted microscope (Nikon TS100, Nikon, Chiyoda, Tokyo, Japan).

Scaffold

The scaffold that was used in this study was a highly porous strip for defect filling, and it was prepared from a collagen slurry and β-TCP mixture (SupraFlex®, BMT Calsis Co., Ankara, Turkey). The production of the material was conducted in two consecutive steps following the preparation of this mixture, namely freeze-drying and dehydrothermal (DHT) processing. Freeze-drying was performed at –80°C for 24 hours, while DHT processing was conducted for 3 days (Monograph, BMT Calsis Co.). The graft material was sterilized via gamma-irradiation (25 kGy). Microcomputerized tomography revealed a porosity of around 65.63% (with an open porosity percentage of 65.54%). The average pore size of the scaffolds calculated from SEM analysis was around 35 µm. The manufacturer designed blocks of $3 \times 25 \times 100$ mm for this study.

Surgery and Experimental Procedure

The operations were carried out under sterile conditions. The rabbits were anesthetized with 35 mg/kg ketamine and 5 mg/kg xylazine via intramuscular route. After induction, the anesthesia level was maintained by 2% isoflurane in oxygen. Both tibiae of the rabbits were used for implant placement and augmentation of the peri-implant defects. After securing the animal in a supine position, both tibia regions were prepared and draped under aseptic conditions. Vital signs of the rabbits were monitored continuously during the operation. The surface of the tibia bone was approached via a

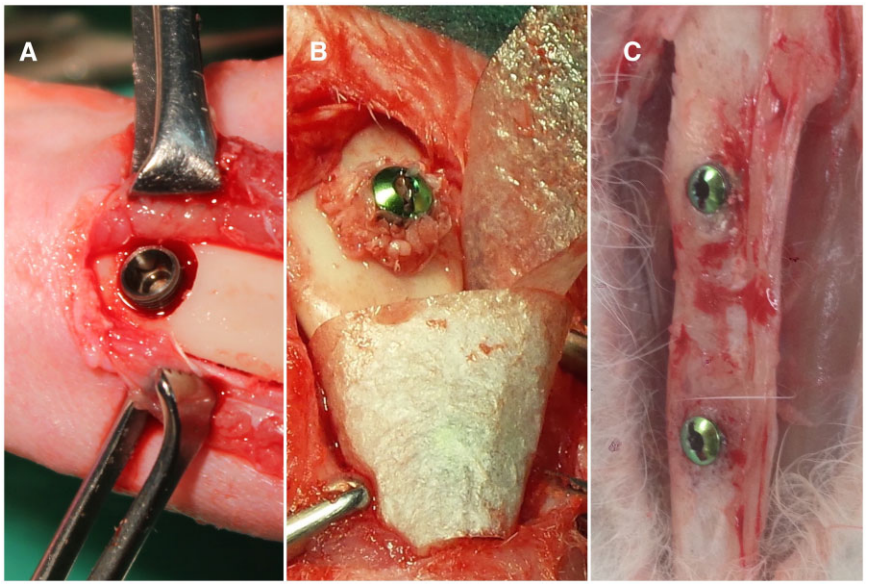

Figure 1 Intraoperative photographs displaying surgical steps. *A*, Situation after the implant placement. Note the defect that was surgically created around the implant. *B*, Situation after the bone augmentation. *C*, Macroscopic appearance after the healing period; note the bone healing status around the implants.

linear incision. Two implants with SLA surface (length = 8 mm, diameter = 3.4 mm; Implantium, Dentium, Seoul, Korea) were placed in each tibia. Implant osteotomies were prepared in accordance with the manufacturer drilling sequence under copious sterile saline irrigation. The distance between the centers of two implants was 30 mm. Before the implant installation, standardized surgical defects were prepared on the medial cortex that engages the coronal aspect of the implant with a 5-mm wide cylinder-shaped bur. Eventually, the implants were placed so that the coronal segments of the implant involving the six micro-threads closest to the implant neck protruded into the bone defects (Figure 1A). The defects around the implant neck was treated with one the four modalities, which were described above (Figure 1B). The scaffolds were cut out and shaped into the same dimensions for each implant using a trephine bur. For each scaffold, the MSCs were detached and suspended in 0.2 ml of saline solution. The number of MSCs transplanted to each scaffold was 2×10^6. In one of the experimental group, the defect around the implant neck was augmented with autogenous bone particles. The particles were collected from the same surgical site by using a commercially available bone scraper (Ebner502 Grafter, Maxilon Labs., Hollis, NH, USA). We standardized the amount of the particles for each defect by adjusting the volume to 1 ml in a sterile plastic syringe. All defects were covered with a rectangular-shaped (15×20 mm) collagen membrane (Genoss, Dentium). The type of the treatment for each implant was randomized by drawing pieces of paper from the bag. The flap was sutured in layers with resorbable sutures.

The rabbits were housed in separate cages postoperatively and were fed ad libitum. Analgesics (Tramadol 1 mg/kg) and antibiotics (Cefazolin 25 mg/kg) were administered intramuscularly preoperatively and twice per day over four postoperative days. Food and water intake and weights of the subjects were monitored and recorded daily.

Data Interpretation

At the end of the 8-week healing period, the animals were euthanized with an overdose of intravenous %5 sodium thiopental. The characteristics of the newly formed bone and bone-implant contact was evaluated by implant stability measurements, microcomputed tomographic (micro-CT) analysis, and histomorphometric analysis.

Implant stability was measured with resonance frequency analysis (RFA) technique. For this study, the wireless device was used (Ostell Mentor®, Integration diagnostics AB, Sävedalen, Sweden). The measurements were conducted at the baseline right after the placement and at the end of the healing period. The analyses were performed in two perpendicular directions, and a mean

value of implant stability quotient (ISQ) was calculated for each implant. After RFA analyses, the tibiae were harvested, the adhering soft tissues were stripped off, and the specimens underwent micro-CT and histomorphometric analyses.

Micro-CT images of the specimens, containing the implant with 0.5-cm surrounding bone, were obtained using Sky Scan micro-CT system (SkyScan 1172, Bruker, Kontich, Belgium). Initially, the specimens were fixed in 10% buffered formalin, dehydrated in increasing concentrations of ethanol, from 70% to 99% during 12 days, and embedded in methylmethacrylate (Technovit 7200, Haerus Kulzer GmbH, Wehrheiam/Ts, Germany). The specimens were placed vertically with the long axis of the implant perpendicular to the exposure beam. The scan was recorded at 30-μm voxel resolution. Using a CT analyzer software (CT-An, Bruker, Kontich, Belgium), three-dimensional images were reconstructed. The region of interest selected for the analysis was the newly formed tissue around coronal part involving six-microthread segment of the implant. After subtracting the implant material and calibrating the bone mineral density, tissue volume (TV) (mm^3) and BV (BV/TV) (%) parameters were calculated for each subject. The nomenclature and calculations for bone histomorphometry were applied in accordance with the report of the American Society for Bone and Mineral Research.[12]

Upon completion the micro-CT phase, 50-μm-thick undecalcified histologic sections that were sliced along the long axis of the implant were prepared using an electric diamond saw and grinding system (Exakt, Exakt Vertriebs, Norderstedt, Germany). The final sections were stained with Masson's trichrome. Digital images of the sections were obtained by a digital camera (Zeiss Axiocam Mrc, Zeiss, Oberkochen, Germany) attached to a microscope (Zeiss) at a magnification rate of ×10. The bone-to-implant contact ratio along the six micro threads closest to the implant shoulder was calculated using Image J software (ImageJ 1.33u; National Institutes of Health, Bethesda, MD, USA) on both sides of each implant. The mean values of both sites of the implant were considered for comparisons.

Statistical Analysis

The data were analyzed with spss 17.0 software for Windows (SPSS, Chicago, IL, USA) statistically. The variations in ISQ values, BV and BV/TV values obtained from micro-CT calculations, and bone-to-implant contact ratios among the groups were used for statistical comparisons. Data distribution was assessed using Kolmogorov–Smirnov test. There was normal distribution for all continuous variables. One-way analysis of variance (anova) was used for the comparisons. The Tukey's post hoc analysis was used to evaluate differences between each group. The level of significance was set at $p = .05$.

RESULTS

Both bone marrow and adipose tissue samples were collected in sufficient amount from all rabbits, and all animals recovered well from the tissue collection phase. All rabbits underwent implant placement surgery as planned. Despite all efforts, two limbs of two different rabbits had to be excluded from the study due to infection and bone fracture. Eventually, we had 36 specimens (nine in each group) for evaluation. Macroscopic evaluation at sacrifice of the specimens revealed that all implants were *in situ*. There was no evidence of collagen membrane remnants in any of the specimens (Figure 1C).

Cell Collection, Isolation, and Expansion

There were no complications in the cell collection phase. Adipose tissue and bone marrow samples were collected, and the MSCs were successfully isolated and expanded from all 10 animals. The freshly isolated bone marrow mononuclear cells (BM-MNCs) and adipose-derived cells consisted mostly of small circular-shaped cells. After cultured for 2 days, some BM-MNCs and adipose tissue-derived cells attached to the plastic culture flasks and formed small clusters of adherent cells consisting of spindle-shaped cells. At the end of 2-week culture, the adherent cells derived from the bone marrow and adipose exhibited fibroblast-like morphology rapidly proliferated and reached 80% confluence (Figure 2).

Immunophenotypes of MSCs from Bone Marrow and Adipose Tissue

The results showed that the MSCs derived from bone marrow expressed typical MSC marker CD73 but did not express CD90, while MSCs derived from adipose tissues did not express CD73 and CD90. Similar to human MSCs, MSCs derived from bone marrow and adipose tissues did not express hematopoietic markers CD34, CD45 (Figure 3). As we used antibodies, which are not specific to rabbit antigens, the negative results

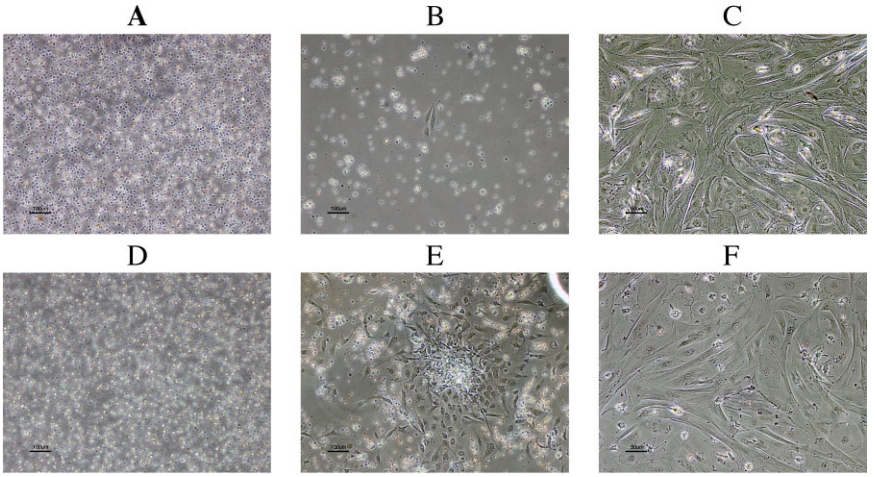

Figure 2 Morphology of the MSCs derived from bone marrow and adipose tissue. *A*, Freshly isolated bone marrow-derived cells. *B*, Bone marrow-derived cells after culture for 2 days. *C*, Bone marrow-derived cells after culture for 2 weeks. *D*, Freshly isolated adipose tissue-derived cells. *E*, Adipose-derived cells after culture for 2 days. *F*, Adipose-derived cells after culture for 2 weeks. Scale bar in *A*, *B*, *D*, and *E* = 100 μm. Scale bar in *C* and *F* = 50 μm.

might arise from the fact that these antibodies could not cross-react with rabbit antigens.

Osteogenic Differentiation Potential of the MSCs

Alkaline phosphatase activity assay of the MSCs derived from the adipose tissue and bone marrow after cultured for 3 weeks in osteogenic differentiation-inducing medium exhibited low level of alkaline phosphatase activity (Figure 4).

Implant Stability Scores

The mean values of the RFA measurements at implant placement were 63.7 ± 3.8, 64.7 ± 4.9, 62.8 ± 2.9, and

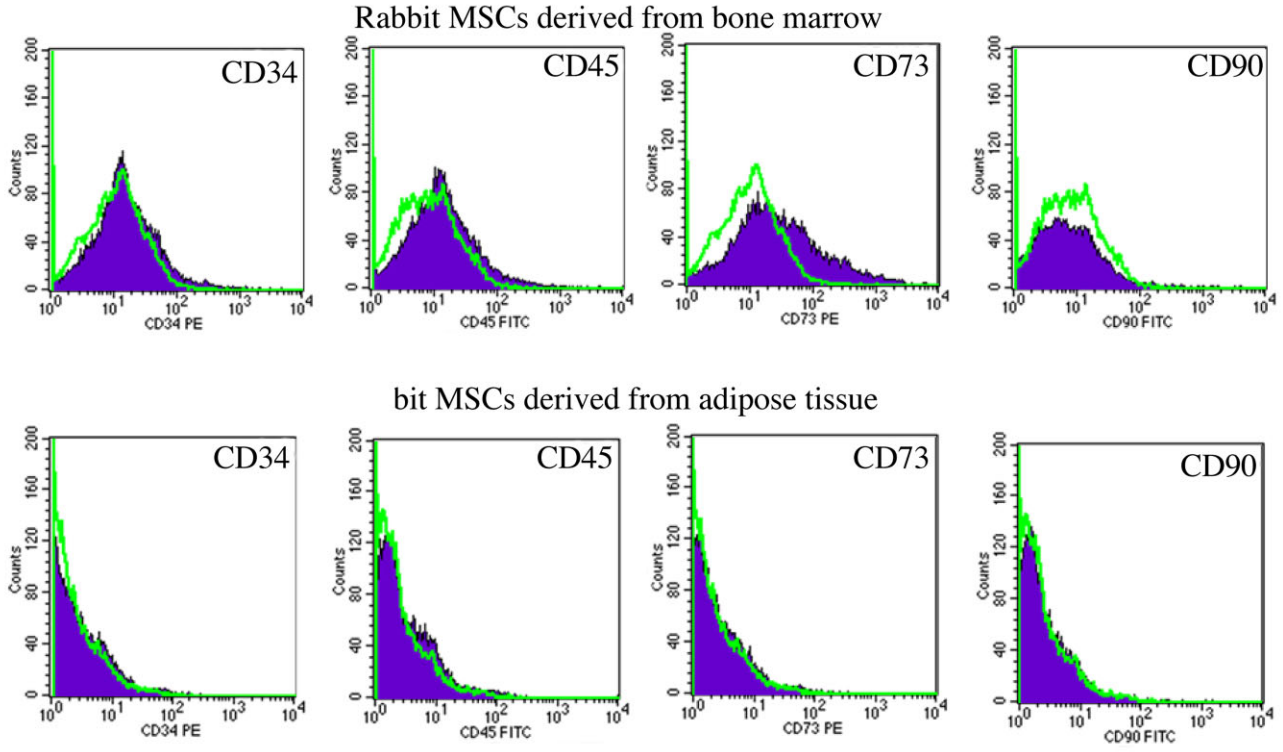

Figure 3 Immunophenotypic characteristics of the MSCs derived from bone marrow and adipose tissue.

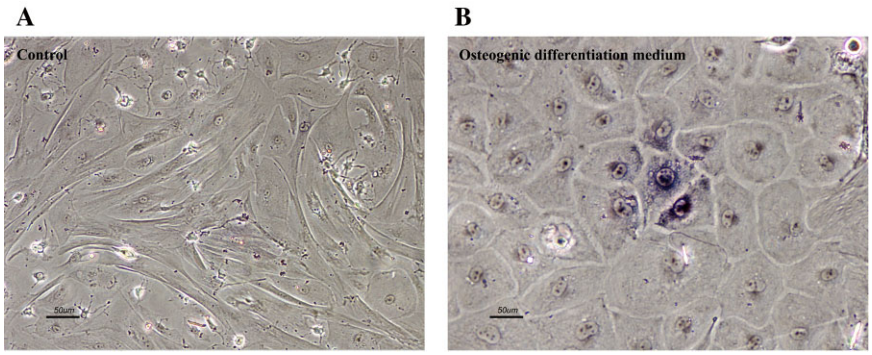

Figure 4 Osteogenic differentiation of MSCs derived from adipose tissue. *A*, Morphology of nondifferentiated MSCs after culture in complete medium for 3 weeks (control). *B*, Morphology of differentiated MSCs, which exhibited alkaline phosphatase activity (purple color) after culture in osteogenic differentiation-inducing medium for 3 weeks. Scale bar = 50 μm.

65.2 ± 2.9 ISQ for the groups 1, 2, 3, and 4, respectively. Posthealing ISQ values were 78.4 ± 6.2 for group 1, 77.8 ± 6.6 for group 2, 77.0 ± 3.1 for group 3, and 70.2 ± 4.1 for group 4 (Table 1). The baseline ISQ levels did not significantly differ among the groups ($p = .591$). There was a statistical difference among the groups with regard to final ISQ scores ($p = .007$). The ISQ scores in group 4 was significantly lower than any other group ($p = .011$ between groups 1 and 4, $p = .020$ between groups 2 and 4, and $p = .046$ between groups 3 and 4). There were no significant differences among the groups 1, 2, and 3 ($p = .996$ between groups 1 and 2, $p = .936$ between groups 1 and 3, and $p = .984$ between groups 2 and 3).

Micro-CT Calculations

Micro-CT observations revealed formation of new calcified tissue around the implants in all samples (Figure 5, A–H). The mean values of the TV and BV/TV calculations for each group are shown in Table 2. There were strong significant differences among the groups with regard to both parameters ($p = .000$). Both parameters were found significantly lower in group 4 compared with the other groups ($p = .000$ for all between-group comparisons). There was no significant difference for both parameters between the group 1, 2, and 3. The p values of the between-group comparisons for the TV parameter were as $p = .936$ between groups 1 and 2, $p = .619$ between groups 1 and 3, and $p = .920$ between groups 2 and 3. The p values of the between-group comparisons for the BV/TV parameter were as $p = .930$ between groups 1 and 2, $p = 0.745$ between groups 1 and 3, and $p = .977$ between groups 2 and 3.

TABLE 1 Comparisons of the Resonance Frequency Analysis (RFA) Measurements at the Baseline and End of the Healing Period

Group	*n*	Baseline ISQ	Final ISQ
1	9	63.7 ± 3.8	78.4 ± 6.2
2	9	64.7 ± 4.9	77.8 ± 6.1
3	9	62.8 ± 3.4	77.0 ± 4.1
4	9	65.2 ± 2.9	70.2 ± 5.9*

*$p = .007$ (Significantly lower ISQ value was encountered in the group 4 compared with any of the other group at the end of healing period. There was no significant difference among the groups at the baseline).

Bone-to-Implant Contact Ratios

Figure 6, A–D, displays examples from each group that were used for histologic measurements. The mean values of the BIC ratios were 64.3 ± 4.5 for group 1, 62.6 ± 6.1 for group 2, 60.6 ± 5.3 for group 3, and 43.5 ± 4.5 for group 4 (Table 2). Multigroup comparison by one-way ANOVA analysis showed a strong significant difference among the groups ($p = .000$). Post hoc analysis confirmed that there was no significant difference between group 1, 2, and 3, while the values were significantly lower in group 4 when compared with group 1, 2, and 3 separately ($p = .838$ between groups 1 and 2, $p = .277$ between groups 1 and 3, $p = .000$ between groups 1 and 4, $p = .751$ between groups 2 and 3, $p = .000$ between groups 2 and 4, and $p = .000$ between groups 3 and 4).

DISCUSSION

The present study demonstrated that MSCs from both adipose tissue and bone marrow that are expanded ex vivo were equally successful at bone regeneration

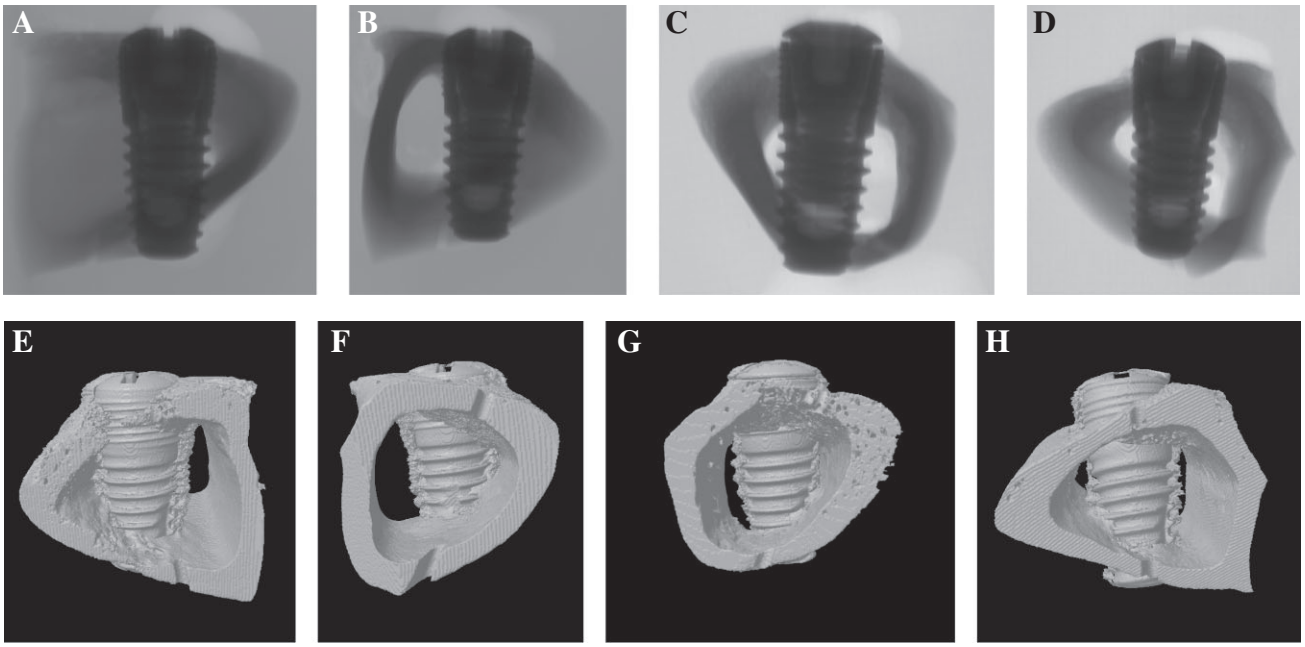

Figure 5 Samples of micro-CT images: two-dimensional micro-CT images of specimen from (A) group 1, (B) group 2, (C) group 3, and (D) group 4 (Figure 6D); three-dimensional reconstructed images of specimen from (E) group 1 (F) group 2, (G) group 3, and (H) group 4.

around dental implants. Contemporary literature suggests that MSCs can be successfully used for bone regeneration purposes without significant donor site morbidity and any autoimmune response, which are negative features of autogenous bone grafts and *rh*-BMP treatments respectively.[2,4,13] Therefore, MSC treatment has the potential for becoming a routine clinical application in the near future.[13] The results of the present study suggest that MSCs derived from bone marrow or adipose tissue are feasible alternatives of autogenous bone grafts in GBR concomitant with implant placement.

TABLE 2 Comparisons of the Mean and SDs of Tissue Volume (TV) and Bone Volume (BV/TV) Values Gathered from the Micro-CT Calculations and Bone-to-Implant Contact (BIC) Ratios Gathered from Histomorphometric Examinations

Group	*n*	TV (mm^3)	BV/TV (%)	BIC (%)
1	9	18.60 ± 1.90	74.4 ± 7.8	64.3 ± 7.9
2	9	17.39 ± 2.09	75.5 ± 6.2	62.6 ± 6.1
3	9	16.96 ± 1.61	76.2 ± 6.9	60.6 ± 5.3
4	9	9.30 ± 1.87*	65.7 ± 8.9*	43.7 ± 9.3*

*p = .000 (All parameters were significantly lower in group 4 than any of the other groups. There was no significant difference among groups 1, 2, and 3 for any of the parameters).

One of the earliest studies evaluating the effectiveness of MSCs in new bone formation around peri-implant defect was the study by Yamada and colleagues in 2004.[14] The authors created peri-implant defects in the mandibles of dogs. The defects were treated with three different materials. The group, in which a combination of MSC and platelet rich plasma was used, had the best results with regard to bone formation. The authors isolated MSCs from the bone marrow of the same dog. A following study by the same group showed similar results when MSCs were applied concomitant with implant placement.[15] A later study by Kim and colleagues used stem cells isolated either from periodontal ligament or bone marrow in the treatment of peri-implant bone defects in the mandibles of beagle dogs.[16] They reported increased bone regeneration in both groups compared with the control group. There was no difference with regard to stem cell origin. A similar study by Ribeiro and colleagues compared the efficacy of MSCs isolated either from periosteum or bone marrow in the treatment of the peri-implant bone defect in beagle dogs.[10] Both groups showed good results without significant difference between the groups. Another study by the same group evaluated the success of MSCs isolated and cultured from bone marrow using the same dog peri-implant defect model.[17] The authors concluded

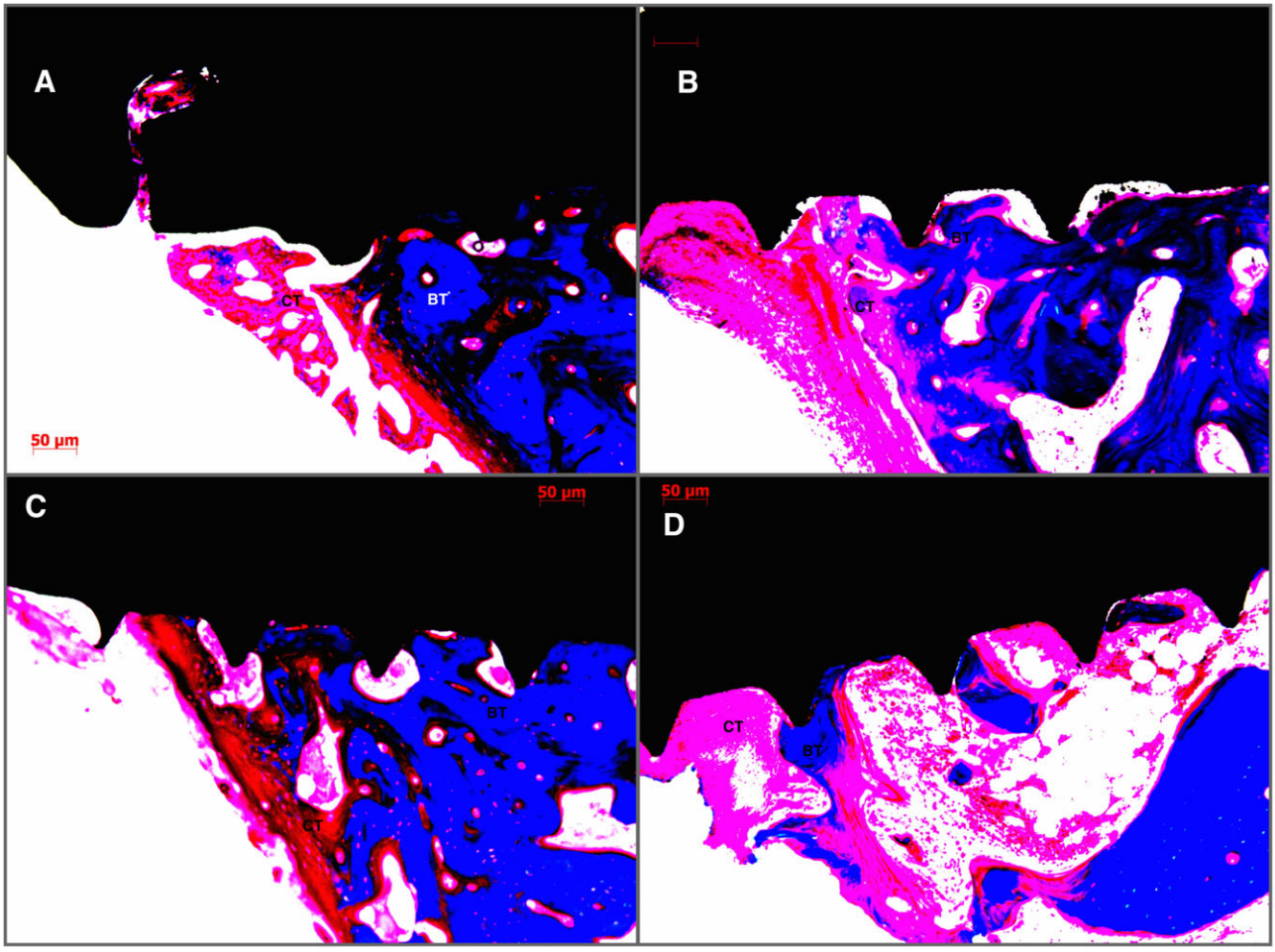

Figure 6 Samples of undecalcified histologic sections from each group. *A*, Group 1 (GBR with adipose-derived MSCs). *B*, Group 2 (GBR with bone marrow-derived MSCs). *C*, Group 3 (GBR with autogenous bone particles). *D*, Group 4 (control group – collagen membrane only). Stain: Masson's trichrome, Magnification: ×10. BT = bone issue; CT = connective tissue.

that the defects treated with MSC either covered with membrane or uncovered had better bone healing than nontreated defects. A recent study by Han and colleagues showed that MSCs derived from the peripheral blood induce new bone formation within the peri-implant defects around the immediately placed implants in the mandibles of Mongrel dogs.[18] A later study by Hao and colleagues, in which the same animal model was used, concluded that umbilical cord-derived MSCs successfully accelerate bone healing within the peri-implant bone defects.[19]

The number of prospective clinical studies that evaluate the effectiveness of MSCs in the treatment of bone defects of the maxillofacial region is relatively limited. Positive outcomes of the MSC treatment have been shown for different types procedures including rehabilitation of the alveolar cleft, sinus floor augmentation, onlay horizontal or vertical alveolar ridge augmentation, and two-stage GBR.[20–23] There is so far no prospective clinical study available, which evaluated the success of MSC application in the treatment of peri-implant bone defects. However, some case series indicate that MSC therapy may successfully provide new bone formation around dental implants.[24,25]

The bone marrow has been used in the majority of previous animal and clinical studies as the source of MSC. However, the interest in using adipose tissue is rapidly increasing because of the advantages of adipose-derived MSCs when compared with bone marrow-derived stem cells. Most notably that a large number of cells can be easily and quickly isolated from the adipose tissue. In addition, although bone marrow aspiration is considered a relatively safe and reliable procedure, it is often associated with pain and may cause serious complications such as postoperative infection and bleeding. Previous animal studies suggest that bone regeneration

with adipose-derived MSCs can be successfully performed; thus, we believe that adipose tissue will be the one of the most notable alternative MSCs sources in the bony rehabilitation of maxillofacial structures.[26,27]

The scaffold used to host the cells in the recipient site has a great importance in the success of the bone regeneration procedure with the aid of MSCs. Different types of scaffolds have been described previously.[7,28] The scaffold used in the present study was a semiflexible, biodegradable composite strip made by a mixture of collagen slurry and beta-tricalcium phosphate. Highly porous and flexible structure of the scaffold enabled a convenient condition for the cells to be seeded, easier adaptation to the recipient bed, and enhanced ingrowth of the vessels to the newly formed bone. These features are consistent with the characteristics of the ideal scaffold.[28] New bone formation around the dental implant detected by micro-CT images and histologic sections revealed that the scaffold showed good biocompatibility, proved to be a feasible carrier of the cells, and demonstrated osteoconductivity allowing new bone formation.

The study model used in the present study is a GBR technique concomitant with implant placement. In usual clinical condition and in the previous study models, more than one wall exist prior the bone augmentation. Increased number of walls around the bone defect facilitates relatively better vascular and cellular source for the regenerating bone and increases the chance of successful results when compared with one-wall defect conditions. Our study model constituted a relatively more challenging situation for the new bone to form. This allowed us to differentiate the results of the augmentation methods better since the effects of the pristine bone have been minimized.

We evaluated quantity of the newly formed bone around the implants and its osseointegration capacity by means of RFA, micro-CT, and histomorphometric analyses. Determination of the implant stability, which is related to histological osseointegration of the implant, with RFA measurement is considered a reliable method.[29] At the end of the healing period, there was substantial increase in the RFA values in all groups when compared with the baseline values. However, the RFA values in the control group were significantly lower than the study groups, in which MSCs from adipose tissue and bone marrow or autogenous bone particles were used. Three-dimensional reconstructed micro-CT images allowed us to evaluate the tissue around the implant, which consists of fibrous tissue and bone tissue. We determined the amount of the whole tissue as well as the percentage of the bone (mineralized) tissue within the whole tissue. The measurements indicate that the amount of the tissue present around the implants was equal in three study groups and significantly higher than the control group. In accordance with the TV parameter, the BV/TV ratio was also significantly higher in the experimental groups compared with the control group. Bone-to-implant contact, which is one of the most useful parameters of osseointegration,[30] showed that there was no difference among the experimental groups, whereas any of all groups showed higher BIC rates than the control group. In short, all parameters including mechanical, radiographic, and histologic evaluations were consistent with each other and revealed that adipose-derived MSCs and bone marrow-derived MSCs have similar osteogenic capability, which is comparable with the autogenous bone grafts, in the treatment of peri-implant osseos defects.

Comparisons of the adipose-derived and bone marrow-derived MSCs have been conducted in the treatment cranial osseos defects in two previous studies. Stockman and colleagues compared osteogenic potential of MSCs derived from different sources in a pig cranium defect model.[9] Their results indicate that the efficiency of adipose-derived and bone marrow-derived MSC transplantation following ex vivo cell expansion is not significantly different for the guided regeneration of bone defects. Han and colleagues created 10×10-mm cranial defects in 16 New Zealand rabbits.[31] Either bone marrow or adipose-derived MSCs were transplanted to the defects. The ossifications at the defects were compared with micro-CT and histology, and the results showed that the total amount of regenerated bone was almost the same in both groups. The results of our study are consistent with these studies indicating similar osteogenic capacity of MSCs from both sources.

One limitation of the present study was that alkaline phosphatase activity assay, which determines the osteogenic differentiation capacity of the MSCs, showed that both adipose-derived and bone marrow-derived MSCs exhibited low level activity. Due to the fact that the optimized standard culture condition for efficiently inducing osteogenic differentiation of rabbit MSCs has yet to be developed, we decided to use culture condition optimized for the induction of osteogenic differentiation of human MSCs in this study. The low level of alkaline

phosphatase activity could arise from the possibility that our osteogenic-inducing medium, which works well with human MSCs, might not be compatible with rabbit MSCs. However, our results demonstrated that when the rabbit-derived MSCs were transplanted into the suitable microenvironment of bone, they further differentiated and contributed in formation of new bone. According to this, we believe that the rabbit MSCs used in this study did possess an osteogenic differentiation capacity but could not be properly demonstrated in vitro due to the lack of suitable culture condition.

In conclusion, the results of this study showed that both bone marrow-derived MSCs and adipose-derived MSCs delivered with a collagen and β-TCP-based scaffold can provide equal amount of new bone formation with autogenous bone graft through a GBR technique in the rabbit tibia. The amount of the ossified tissue and bone-to-implant ratio were similar in the experimental groups and significantly higher than the control group.

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