Clin Oral Invest (2012) 16:251–258
DOI 10.1007/s00784-010-0486-7

ORIGINAL ARTICLE

Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs

S. Burcak Simsek · Gonca Cayir Keles · Sancar Barıs · Burcu Ozkan Cetinkaya

Received: 25 May 2010 / Accepted: 28 October 2010 / Published online: 18 November 2010

Abstract The purpose of this study was to compare the effectiveness of mesenchymal stem cells (MSCs) with platelet-rich plasma (PRP) as scaffold and autogenous cortical bone (ACB) graft with and without PRP in the regenerative treatment of class II furcation defects in dogs. The mandibular second, third, and fourth premolars (P2, P3, P4) and maxillary P3 and P4 of both sides in three dogs were selected for experimentation. Class II furcation defects (5 mm in height and 2 mm in depth) were surgically created. Five weeks after the first operation, scaling + root planning (group 1), PRP (group 2), ACB (group 3), combination of ACB/PRP (group 4), and combination of MSCs/PRP (group 5) treatments were performed during open flap debridement. The percentage of cementum and alveolar bone formation was evaluated by histomorphometric analysis after a healing period of 8 weeks. There was new cementum along with periodontal ligament and coronal growth of alveolar bone in all groups. Cementum formation was significantly higher in groups 3, 4, and 5 compared to the control group ($P<0.05$) with no significant difference between groups 2, 3, 4, and 5. Alveolar bone formation was similar in all groups ($P>0.05$). It can be concluded that periodontal regeneration with complete filling of class II furcation defects with cementum, alveolar bone, and periodontal ligament is obtained 8 weeks after ACB, ACB/PRP, and MSCs/PRP treatments; however, efficacy of none is higher than another.

Keywords Autogenous bone graft · Furcation defects · Mesenchymal stem cells · Periodontal regeneration · Platelet-rich plasma

S. B. Simsek
Medipol Hospital,
Istanbul, Turkey

G. C. Keles (✉) · B. O. Cetinkaya
Department of Periodontology, Faculty of Dentistry,
Ondokuzmayis University,
55139, Samsun, Turkey
e-mail: goncakeles@hotmail.com

S. Barıs
Department of Pathology, Faculty of Medicine,
Ondokuzmayis University,
Samsun, Turkey

Introduction

Periodontitis, an oral infectious disease, is characterized by clinical attachment loss, alveolar bone resorption, periodontal pocketing, and gingival inflammation [1]. The purpose of periodontal therapy is to control the infection and regenerate the tissues that have been lost due to destructive periodontal disease [2, 3]. Although regeneration of periodontium is the ultimate goal of periodontal therapy, complete regeneration is not a predictable healing outcome following traditional periodontal treatment [4]. A number of studies have demonstrated successful regeneration of periodontal tissues using conventional regenerative periodontal therapies such as guided tissue regeneration, applications of enamel matrix derivative, platelet-rich plasma (PRP), various polypeptide growth factors, and combinations of these therapies [5–10].

The new technology tissue engineering [11] involves the morphogenesis of new tissue by combination of isolated cells with compatible scaffolds and growth factors [12, 13]. There is evidence that bone marrow-derived mesenchymal stem cells (MSCs) are multipotent cells that can replicate as undifferentiated cells and have the potential to differentiate into chondrocytes, tenocytes,

adipocytes, muscle cells, and nerve cells in vitro and in vivo [14, 15], and also into cementoblasts, osteoblasts, and periodontal fibroblasts [12, 16]. Moreover, MSCs have received widespread attention due to their potential utility in tissue engineering applications [17, 18].

Furcation involvement, bone resorption and attachment loss in the interradicular space results from periodontal disease, has been reported to considerably increase the risk for tooth loss [19, 20]. Furcation defects represent a formidable problem in the treatment of periodontal disease due to the complex and irregular anatomy of furcations [20]. When all of these findings were taken into consideration, it is conceivable that MSCs might be used in the regenerative treatment of class II furcation defects. The use of MSCs for periodontal regeneration offers an interesting and clinically useful modality to the clinician in treating class II furcation defects as MSCs are autologous and eliminates concerns about disease transmission and immunogeneic reactions associated with allogeneic or xenogeneic preparations. In recent years, PRP generally combined with graft materials has been used for the purpose of periodontal regeneration [21, 22]. PRP is a volume of autologous plasma that has a higher platelet concentration than baseline [23]. In tissue engineering procedures, MSCs have been used with PRP scaffold [13, 18, 24]. Data also suggest that the PRP scaffold provides effective enhancement of MSCs adhesion, proliferation, and differentiation to elicit bone formation [24].

Bone grafting procedures with autogenous bone grafts, allografts, xenografts, and alloplasts are also used to promote periodontal regeneration [25–27]. Among the different available graft materials, autogenous bone remains the gold standard for osseous regeneration [27–29]. Autogenous bone has osteogenic potential as it contains cells that participate in osteogenesis [27, 30]. Moreover, autografts are bioabsorbable (they are eventually replaced by the patient's own bone) [28], nonallergenic (they cause minimal tissue reaction without an immunological reaction) [27, 28], easy to handle, and not costly [31]. Rapid revascularization occurs around autogenous bone graft particles, and the graft can release growth and differentiation factors [27, 32]. Although autogenous bone grafts present some disadvantages, such as the need for secondary surgical sites and resulting additional surgical morbidity [28, 33], they can be minimized by using intraoral harvested bone [33]. The use of the latter graft material is, however, limited by the restricted donor sites in the oral cavity for extensive grafting [27, 33].

To date, MSCs with scaffold material alone or combined with the xenograft have been used to regenerate cylindrical mandibular bone defects, class III furcation defects, and periodontal fenestration defects in experimental studies [12, 16, 18, 24, 34, 35]. The present study was undertaken to test the hypothesis that using MSCs with PRP is a more effective regenerative treatment modality compared to autogenous cortical bone (ACB) graft alone and ACB graft with PRP in the treatment of class II furcation defects in dogs. No data are available on comparing MSCs/PRP and ACB for the regenerative periodontal treatment. Therefore, the purpose of this study was to compare the histomorphometrical effectiveness of MSCs/PRP and ACB graft for class II furcation defects in dogs.

Materials and methods

The research protocol was approved by the Ethical Committee for Animal Research of the University with the assignment protocol 2005/067.

Animals

Three young adult mongrel dogs, weighing about 15 kg, with intact maxillary and mandibular teeth showing good systemic and periodontal health were selected for the study. The animals received complete oral prophylaxis, antiparasitic treatment, multivitamins, and complete vaccines. All dogs were fed a water-softened dog food to prevent traumas of mastication to the surgical areas.

Preparation of class II furcation defects

The animals were not fed the night before surgery. All surgical procedures were performed under general anesthesia with xylazine (Rompun, Bayer, Istanbul, Turkey), ketamine HCl (Pfizer Inc., Istanbul, Turkey) (5 mg/kg, intramuscularly), and local infiltrated anesthesia with 2% lidocaine hydrochloride and 1/80000 andrenaline (Jetokain ampule, Adeka Pharmaceutical Company, Samsun, Turkey). The mandibular second, third, fourth, and premolars (P2, P3, P4) and maxillary P3 and P4 of both sides in each dog were selected for experimentation.

Under aseptic conditions, following sulcular incisions, mucoperiosteal flaps were elevated. With a round bur, class II furcation lesions, 5 mm in the apicoocclusal direction and 2 mm in the buccolingual direction, were surgically created on the buccal surface of these premolars. The roots were scaled to remove all periodontal ligament fibers. The bilateral defects were filled with a rubber base impression material (Zetaplus putty, Zhermack, Italy) to induce an inflammatory response and prevent spontaneous repair. The flaps were repositioned and the wounds were closed with bioresorbable sutures [36].

The impression material remained in place for 3 weeks. After this period, the impression material was removed with curettes, and scaling and root planing were performed on

the teeth. Plaque control by daily topical application of 0.2% chlorhexidine and mechanical tooth cleaning was maintained for 2 weeks [36].

Bone marrow MSCs isolation and PRP preparation

From each dog, a 10-ml sample of bone marrow was aspirated from the posterior iliac crest. Following the isolation of mononuclear cells from bone marrow in vitro, the isolated mononuclear cells of bone marrow were cultivated into plastic flasks by adding fetal bovine serum to proliferate the MSCs (ATI Technology, Trabzon, Turkey). The day of periodontal surgery, 30 ml blood was drawn from the *arteria femoralis* on the hind leg and placed in the bag that contained 4.5 ml citrate solution. Citrated blood was centrifuged in a standard laboratory centrifuge (ALC PK 130, Cologno Monzese, Italy) for 15 min at 1,250 revolutions per minute (rpm) to obtain PRP without erythrocyte and leukocyte. A second centrifugation was performed for 10 min at 4,000 rpm. The PRP was taken whereas the platelet-poor plasma was removed [37]. Platelet counts in the PRP were done with an automatic hematology analyzer (Mindray BC 3000 Plus, Shenzhen, China).

Treatment of defects

Five weeks after the first operation, mucoperiosteal flaps were raised to expose the inflamed furcations, granulation tissues were removed, and the root surfaces were scaled and planed. A reference notch was made on the mesial and distal root surfaces at the bone crest level with a small 1/2 round bur as a guideline for histomorphometric analysis. The surgical sites were then rinsed with sterile saline.

The P2s on both sides of the mandible were used as controls [group 1] and received scaling and root planing during flap procedure. The furcation defects of P3 and P4 on the right side of maxilla, P3 and P4 on the left side of mandible, P3 and P4 on the left side of maxilla, and P3 and P4 on the right side of mandible were filled by PRP [group 2], ACB [group 3], ACB + PRP [group 4], and MSCs + PRP [group 5], respectively (Fig. 1). At the time of application, PRP ($1.8–2.4\times10^6$ platelets/μl) was coagulated by adding 10% calcium chloride at 1:10 ratio (*v*/*v*) [37]. Coagulated PRP and 1×10^7 MSCs/ml were mixed in a sterilized syringe, and the gel was injected into the furcation defect. An adequate amount of cortical bone particulate was harvested from the buccal cortical plate, adjacent to the furcation defect, using a bone scraper and implanted to the defect.

The flaps were repositioned and secured with 3-0 silk suture material by using the interrupted suturing technique. The sutures were removed 1 week after surgery. Chemical plaque control was performed once a day by topical application of 0.2% chlorhexidine at the end of the experimental period. After a healing period of 8 weeks, the animals were euthanized by excess anesthesia.

Histomorphometric analysis

All tissues of dogs were fixed by 10% formalin perfusion perfected from the aorta. The maxillas and mandibles were dissected and decalcified in Gooding and Steward's solution (10% formic acid and 5% formaldehyde). After decalcification, blocks containing the individual teeth were immersed in paraffin. Semiserial sections (5 μm) were cut in the mesial–distal plane throughout the buccal–lingual extension of the teeth and stained with hematoxylin and eosin (H&E). Three sections which are representative of the central area of the furcation of each tooth were observed. The histomorphometric analysis was performed using a light microscope (Zeiss Axiophot Microscope, Carl Zeiss Inc., Thornwood, NY, USA). Images were digitized with a camera (Insight Firewire 2 MegaSample Colour Mosaic Camera, Spot; SciTech Pty. Ltd., Preston, VIC, Australia) and analyzed with the IPS 32 Software (Samba Technologies, Grenoble, France) by an examiner with no prior knowledge of the experimental design. Analysis of new cementum and new bone formation was carried out. The percentage of new cementum was evaluated at the dentin surface facing the defect, determined by the apical limits of the mesial and distal notches. The mineralized matrix deposited on the root dentin was considered to be new cementum [38]. The percentage of new bone from the furcation roof to the bone crest was used to evaluate new bone formation [38].

Statistical analysis

A commercially available software program (SPSS 12.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The values for each parameter analyzed represented the arithmetic mean of the three measurements obtained from each of the three histologic sections. The Shapiro Wilk test was used to investigate whether the data were normally distributed or not. Statistical comparisons between the treatment modalities were made with Friedman nonparametric test. Comparison among groups was carried out with Wilcoxon test ($P<0.05$, $N=6$).

Results

Clinical observations

Six class II furcation defects were created and surgically treated in each study groups. The surgical procedures were well tolerated by all animals, and there were no postoper-

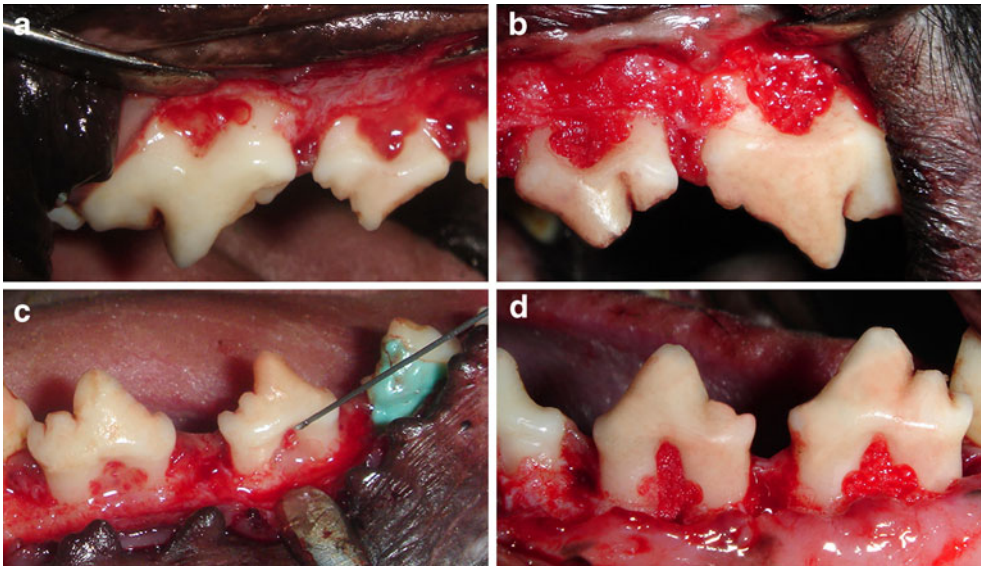

Fig. 1 Clinical aspects of class II furcation defects at surgery. **a** Treated with PRP (group 2), **b** treated with ACB + PRP (group 4), **c** treated with MSCs + PRP (group 5), **d** treated with ACB (group 3)

ative complications. No severe inflammation or swelling and dehiscence of the flaps were observed in any of the sites examined throughout the experimental period. There was no significant gingival recession in any of the study groups. The periodontal tissues were clinically healthy on the day of sacrifice.

Histomorphometric assessment

There was new cementum along with periodontal ligament and coronal growth of alveolar bone in all groups (Fig. 2a–e). These new tissues were observed in the areas above the reference notches. None of the specimens showed root resorption and ankylosis between new bone and the root surfaces.

The new cementum, deposited over dentin, was of variable thickness, composed of cells with intrinsic and extrinsic collagen fibers distributed randomly on the root surface. The new connective tissue between new bone and the root surface was cellular and composed of collagen fiber bundles. The newly formed bone was mainly woven bone.

Although the morphologic characteristics of the newly formed tissues were similar in all groups, statistically significant differences were found between test groups (groups 3, 4, and 5) and control group (group 1) in terms of the percentage of the values in cementum analyzed (Table 1). Cementum formation was significantly higher in groups 3, 4, and 5 compared to the control group ($P<0.05$) with no significant difference between groups 2, 3, 4, and 5. Alveolar bone formation was similar in all groups ($P>0.05$).

Discussion

To the authors' knowledge, this is the first report comparing healing activity after surgical therapies with PRP, ACB, ACB/PRP, MSCs/PRP, and flap surgery in class II furcation defects. The results of the present study indicate that the healing success in cementum formation was significantly higher after surgical therapies with ACB, ACB/PRP, and MSCs/PRP compared to controls with regard to the histomorphometric values. No significant difference in this parameter was found between these groups and the PRP group. Alveolar bone formation was higher in ACB, ACB/PRP, and MSCs/PRP treatments; however, this was not statistically significant compared to that of the control group and the PRP group. It is important to also consider that there may be spontaneous formation of alveolar bone in the control group. As the number of experimental animals and histological samples with the small statistical power was relatively small in the present study, furcation defects treated with MSCs showed a significant improvement in cementum and bone regeneration similar to autogenous bone graft which is consistent with previous experimental studies [24, 35].

Histologic analysis is necessary to evaluate the effectiveness of the regenerative techniques. The most widely used animal models are dogs and nonhuman primates in the periodontology literature [39]. Class II furcation defect is one of the main indications for the regenerative procedures [40]. A chronic class II furcation defect was chosen due to the possibility of spontaneous regeneration that reduces the sensitivity of the interpretation of regenerative techniques in acute defects [41]. However, acute class II furcation defects have been used in some experimental dog studies [41, 42]. Healing of class II furcation defects in dogs was analyzed histomorphometrically on a mesial–distal plane. Similar to our study, the mesial–distal section plane is the one most commonly reported for histologic analysis in the literature [36, 38–42].

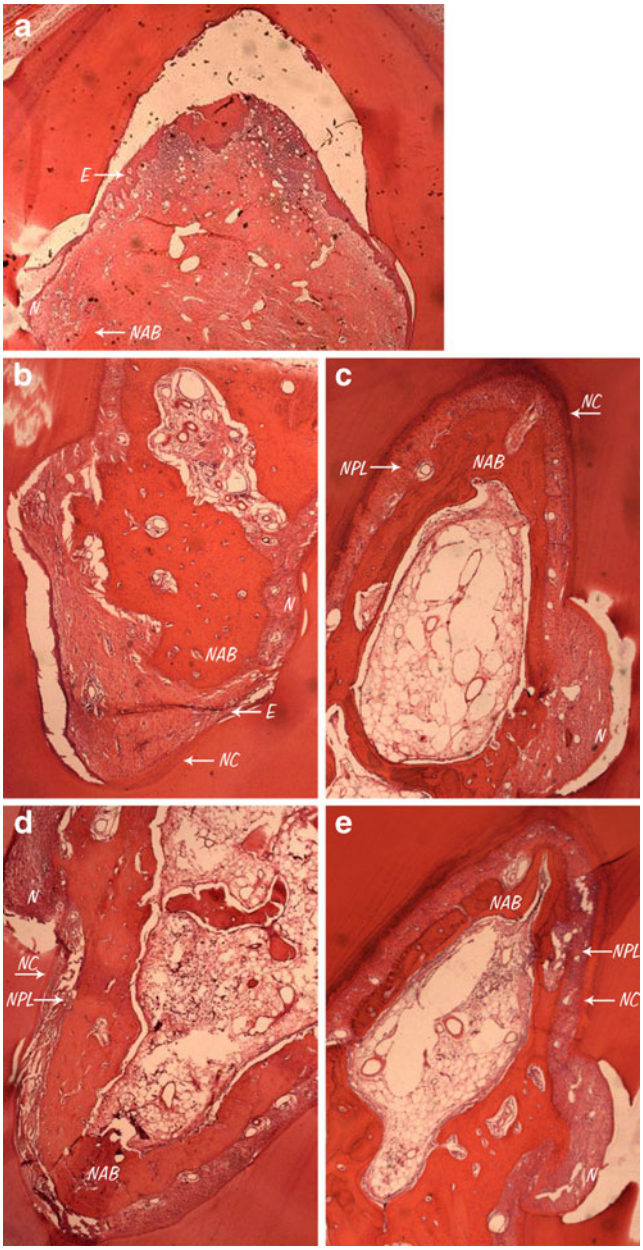

Fig. 2 Histologic overview of class II furcation defects in the mesial–distal plane at 8 weeks after surgical treatments (H&E, ×25). *N* the base of the defects is marked by notch, *NC* new cementum, *NAB* new alveolar bone, *NPL* new periodontal ligament, *E* epithelium. **a** Control (group 1), **b** treated with PRP (group 2), **c** treated with ACB (group 3), **d** treated with ACB + PRP (group 4), **e** treated with MSCs + PRP (group 5)

For the purpose of periodontal regeneration, an appropriate scaffold material, which is necessary for delivering cells and growth factors and maintaining temporary mechanical function, has been added to MSCs during the implantation [35, 43]. In the present study, PRP was used as scaffold material which is a source of polypeptide growth factors with jelly-like flexibility [24]. Data suggest that 10% PRP induce stem cell proliferation; cells expanded

Table 1 The percentage of new cementum and alveolar bone (n=6 class II furcation defects in each group)

	Cementum (%)	Alveolar bone* (%)
Group 1	0 (0–20)	35.95 (0–46.35)
(Control)	3.33 (±3.33)	31.98 (±6.67)
Group 2	9.81 (0–100)*	27.15 (0–92.00)
(PRP)	36.60 (±20.165)	33.95 (±15.39)
Group 3	100 (78.30–100)*·**	86.85 (62.60–95.00)
(ACB)	93.62 (±4.09)	84.60 (±4.85)
Group 4	57 (43–100)*·***	85.70 (23.40–97.00)
(ACB + PRP)	66.83 (±10.78)	68.80 (±14.20)
Group 5	69.90 (23.80–100)*·****	86.65 (40.00–95.20)
(MSCs + PRP)	70.47 (±11.75)	80.47 (±8.23)

Friedman and Wilcoxon tests. Data are expressed as the median (minimum–maximum) and as the mean ± SEM

*P>0.05, no significant difference was found between groups

**P=0.026, significantly different from the values of group 1

***P=0.027, significantly different from the values of group 1

****P=0.028, significantly different from the values of group 1

with 10% PRP can mineralize the extracellular matrix [44]. Since keeping MSCs in the defect region for regeneration is difficult, β-tricalcium phosphate, atelocollagen, and fluorohydroxyapatite have been used as a scaffold as well as PRP in the literature [12, 16, 18, 24, 34, 45, 46]. Ceramic materials were not selected due to the reasons that these materials have no interconnected pores for new bone to invade the defect and have slow resorption rate, and these materials have no osteoinductive properties [24, 45]. It is important to note that PRP works as a hemostatic and stabilizing agent and may aid blood clot formation because of its high fibrin content [22]. The blood clot formation and immobilization has been reported to be the essential events for successful regenerative procedures [38, 47] as replacement of the blood clot eventually leads to the new periodontal tissue formation [36]. Therefore, the individual role of PRP in the treatment of class II furcation defects was tested in this experimental study.

From a clinician's perspective, it is notable that cell density of MSCs is also an important factor for periodontal tissue regeneration because sufficient tissue fluids and blood supply may be necessary for MSCs to survive after transplantation and differentiation into periodontal cells [12]. The cell concentrations (2×10^6 to 2×10^7) have been used in various studies reporting effective tissue regeneration [12, 48–50]. In the current study, 1×10^7 cells/ml, which are among these cell concentrations, were used for periodontal regeneration in class II furcation defects in dogs.

Root resorption and ankylosis have been observed in animal and human studies after the use of autogenous grafts

[51–53]. However, via an experimental study, it was suggested that autogenous bone grafts from intraoral sources do not induce ankylosis [27]. In the present study, there was no evidence of ACB graft-induced root resorption and ankylosis, which appears to occur at sites where bone formation takes place without regeneration of periodontal ligament [54]. Despite the increase of clinical and experimental studies using ACB graft in periodontal regenerative therapy in recent years [29, 31, 55], it has been reported that ACB graft is not osteogenic because only a few cells survive in this type of graft, but has osteoconductive capacity [29, 56]. Periodontal healing has been similar whether using surgical debridement alone, ACB graft, or ACB graft with a calcium sulfate barrier in the treatment of surgically created class II furcation defects in an experimental study in dogs [29]. Moreover, there is evidence that the combination of ACB graft and enamel matrix derivative in the treatment of deep periodontal intraosseous defects has led to a significant improvement in clinical parameters [31, 55]. Class II furcation defects were almost regenerated with periodontal tissues after the treatments of ACB alone and ACB/PRP in the current study.

The animals were sacrificed 8 weeks after periodontal surgery in this study. The observation period of experimental studies in animals has varied from a couple of weeks up to 3 or 6 months [57]. It has been reported that regenerated tissues are in the process of formation and/or remodeling after 3 months of wound healing [36]. Data also suggest that cementum formation was completed within the 8-week healing interval in intrabony defects in dogs [58], contrary to these findings. Newly formed bone in the present study showed characteristics of immature bone. Histomorphometric analysis of new cementum formation demonstrated a high percentage of new cementum in the ACB, ACB/PRP, and MSCs/PRP-filled sites (93.62%, 66.83%, and 70.47%, respectively), and there was no significant difference among groups. The control group had the lowest values (3.33%). There was no significant difference between PRP-filled sites (36.60%) and control sites. It is important to consider that the major goals of regenerative periodontal therapy are the new cementum formation and restoration of soft tissue attachment to the cementum [12, 59] since epithelial cells may not be able to migrate on the root surface, which is covered by new cementum with extrinsic fibers [12]. There is evidence that MSCs on the root surface differentiate into cementoblasts in the early stage of the healing process and cementoblasts release various kinds of cytokines, leading to a subsequent process of periodontal regeneration [16, 60]. Therefore, transplantation of MSCs may be a useful treatment modality for periodontal tissue regeneration.

The use of MSCs in periodontal regeneration has recently attracted the attention of periodontal researchers. Data from an in vitro study suggest that MSCs have potency to develop periodontal ligament characteristics and the cells may have the potential to form other periodontal tissues [61]. This is consistent with the reports that class II furcation defects in dogs have almost been regenerated with cementum, alveolar bone, and periodontal ligament 4 weeks after the transplantation of MSCs with regard to histomorphometric and immunohistochemical analysis [12, 16]. When the results of the experimental study [24] on MSCs' efficiency in mandibular bone defects were taken into consideration, no significant difference in newly formed bone has been observed between MSCs/PRP and autogenous particulate cancellous bone and marrow treatments at 2, 4, and 8 weeks, consistent to our findings.

When interpreting the findings of the present study, it also needs to be pointed out that PRP alone and with MSCs show positive influence on cementum and alveolar bone formation. However, PRP-filled defects did not regenerate exactly and showed fewer amounts of cementum and alveolar bone formation compared to ACB, ACB/PRP, and MSCs/PRP treatment of furcation defects. This finding is in accordance with a previous experimental dog study reporting that filling a mandibular bone defect with PRP alone has not allowed osteogenesis to occur in the affected areas [24]. MSCs combined with PRP showed a significant improvement in periodontal regeneration similar to autogenous bone in this study.

In the light of current knowledge, findings from studies on PRP have conflicting results [62–65]. In an experimental study, it has been suggested that PRP has a limited potential to promote local bone formation histologically in the rat calvaria defects [65]. As has been reported [62], PRP may also be effective in small (periodontal) and larger bone defects if the defects are treated with both autologous graft and PRP. The authors have also considered that PRP needs vital bone cells for stimulation [62]. However, an in vitro study [63] has suggested that PRP has the ability to increase periodontal ligament cell numbers and simultaneously upregulate extracellular matrix production. PRP has also been suggested to stimulate cell proliferation and increase alkaline phosphatase activity in periodontal ligament cells in a recent study [64]. When the results of the present study were taken into consideration, it is relevant to assume that enhanced periodontal regeneration may be obtained if the combinations of ACB/PRP and MSCs/PRP are applied instead of using PRP alone.

Based on the results of the present study, it can be concluded that periodontal regeneration with complete filling of class II furcation defects with cementum, alveolar bone, and periodontal ligament is obtained 8 weeks after ACB, ACB/PRP, and MSCS/PRP treatments; however, efficacy of none is higher than another.

Acknowledgments This study was supported by Ondokuzmayis University Research Fund (project DHF 052). We are especially grateful to Prof. Dr. Yuksel BEK (Chief of the Department of Biostatistics, Faculty of Medicine, University of Ondokuzmayis, Samsun, Turkey) for his precious contribution to the statistical analyses of our study.

Conflict of ınterest The authors declare that they have no financial relationships related to any products involved in this study.

References

1. Flemmig TF (1999) Periodontitis. Ann Periodontol 4:32–38
2. Park JS, Suh JJ, Choi SH, Moon IS, Cho KS, Kim CK, Chai JK (2001) Effects of pretreatment clinical parameters on bioactive glass implantation in intrabony periodontal defects. J Periodontol 72:730–740
3. Sculean A, Barbe G, Chiantella GC, Arweiler NB, Berakdar M, Brecx M (2002) Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. J Periodontol 73:401–408
4. Trombelli L (2005) Which reconstructive procedures are effective for treating the periodontal intraosseous defect? Periodontol 2000 37:88–105
5. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S (2000) Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontol 2000 24:253–269
6. Cortellini P, Tonetti MS (2000) Focus on intrabony defects: guided tissue regeneration. Periodontol 2000 22:104–132
7. Froum SJ, Gomez C, Breault MR (2002) Current concepts of periodontal regeneration. A review of the literature. N Y State Dent J 68:14–22
8. Kalpidis CD, Ruben MP (2002) Treatment of intrabony periodontal defects with enamel matrix derivative: a literature review. J Periodontol 73:1360–1376
9. Papli R, Chen S (2007) Surgical treatment of infrabony defects with autologous platelet concentrate or bioabsorbable barrier membrane: a prospective case series. J Periodontol 78:185–193
10. Keles GC, Cetinkaya BO, Baris S, Albayrak D, Simsek SB (2009) Comparison of platelet pellet with or without guided tissue regeneration in the treatment of class II furcation defects in dogs. Clin Oral Investig 13:393–400
11. Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920–926
12. Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, Takata T, Kato Y, Kurihara H (2004) Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 75:1281–1287
13. Yamada Y, Ueda M, Hibi H, Baba S (2006) A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: a clinical case report. Int J Periodontics Restorative Dent 26:363–369
14. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
15. Deans RJ, Moseley AB (2000) Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 28:875–884
16. Hasegawa N, Kawaguchi H, Hirachi A, Takeda K, Mizuno N, Nishimura M, Koike C, Tsuji K, Iba H, Kato Y, Kurihara H (2006) Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. J Periodontol 77:1003–1007
17. Bianco P, Robey PG (2001) Stem cells in tissue engineering. Nature 414:118–121
18. Yamada Y, Ueda M, Naiki T, Nagasaka T (2004) Tissue-engineered injectable bone regeneration for osseointegrated dental implants. Clin Oral Implants Res 15:589–597
19. Wang HL, Burgett FG, Shyr Y, Ramfjord S (1994) The influence of molar furcation involvement and mobility on future clinical periodontal attachment loss. J Periodontol 65:25–29
20. Cattabriga M, Pedrazzoli V, Wilson TG Jr (2000) The conservative approach in the treatment of furcation lesions. Periodontol 2000 22:133–153
21. Hanna R, Trejo PM, Weltman RL (2004) Treatment of intrabony defects with bovine-derived xenograft alone and in combination with platelet-rich plasma: a randomized clinical trial. J Periodontol 75:1668–1677
22. Demir B, Sengun D, Berberoglu A (2007) Clinical evaluation of platelet-rich plasma and bioactive glass in the treatment of intrabony defects. J Clin Periodontol 34:709–715
23. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR (1998) Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 85:638–646
24. Yamada Y, Ueda M, Naiki T, Takahashi M, Hata K, Nagasaka T (2004) Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: tissue-engineered bone regeneration. Tissue Eng 10:955–964
25. Low SB, King CJ, Krieger J (1997) An evaluation of bioactive ceramic in the treatment of periodontal osseous defects. Int J Periodontics Restorative Dent 17:358–367
26. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB (2002) Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. J Periodontal Res 37:300–306
27. Kim CS, Choi SH, Cho KS, Chai JK, Wikesjö UM, Kim CK (2005) Periodontal healing in one-wall intra-bony defects in dogs following implantation of autogenous bone or a coral-derived biomaterial. J Clin Periodontol 32:583–589
28. MacNeill SR, Cobb CM, Rapley JW, Glaros AG, Spencer P (1999) In vivo comparison of synthetic osseous graft materials. A preliminary study. J Clin Periodontol 26:239–245
29. Deliberador TM, Nagata MJ, Furlaneto FA, Melo LG, Okamoto T, Sundefeld ML, Fucini SE (2006) Autogenous bone graft with or without a calcium sulfate barrier in the treatment of class II furcation defects: a histologic and histometric study in dogs. J Periodontol 77:780–789
30. Carranza FA, Takei HH, Cochran DL (2006) Reconstructive periodontal surgery. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA (eds) Clinical periodontology, 10th edn. Saunders Elsevier, St. Louis, pp 968–990
31. Guida L, Annunziata M, Belardo S, Farina R, Scabbia A, Trombelli L (2007) Effect of autogenous cortical bone particulate in conjunction with enamel matrix derivative in the treatment of periodontal intraosseous defects. J Periodontol 78:231–238
32. Marx RE (1994) Clinical application of bone biology to mandibular and maxillary reconstruction. Clin Plast Surg 21:377–392
33. Chan C, Thompson I, Robinson P, Wilson J, Hench L (2002) Evaluation of Bioglass/dextran composite as a bone graft substitute. Int J Oral Maxillofac Surg 31:73–77
34. Li H, Yan F, Lei L, Li Y, Xiao Y (2009) Application of autologous cryopreserved bone marrow mesenchymal stem cells for periodontal regeneration in dogs. Cells Tissues Organs 190:94–101
35. Pieri F, Lucarelli E, Corinaldesi G, Fini M, Aldini NN, Giardino R, Donati D, Marchetti C (2009) Effect of mesenchymal stem

cells and platelet-rich plasma on the healing of standardized bone defects in the alveolar ridge: a comparative histomorphometric study in minipigs. J Oral Maxillofac Surg 67:265–272
36. Macedo GO, Souza SL, Novaes AB Jr, Grisi MF, Taba M Jr, Palioto DB (2006) Effect of early membrane removal on regeneration of class II furcation defects in dogs. J Periodontol 77:46–53
37. Keles GC, Cetinkaya BO, Albayrak D, Koprulu H, Acikgoz G (2006) Comparison of platelet pellet and bioactive glass in periodontal regenerative therapy. Acta Odontol Scand 64:327–333
38. Regazzini PF, Novaes AB, de Oliveira PT, Palioto DB, Taba M, de Souza SL, Grisi MF (2004) Comparative study of enamel matrix derivative with or without GTR in the treatment of class II furcation lesions in dogs. Int J Periodontics Restorative Dent 24:476–487
39. Bogle G, Garrett S, Stoller NH, Swanbom DD, Fulfs JC, Rodgers PW, Whitman S, Dunn RL, Southard GL, Polson AM (1997) Periodontal regeneration in naturally occurring class II furcation defects in beagle dogs after guided tissue regeneration with bioabsorbable barriers. J Periodontol 68:536–544
40. Jepsen S, Eberhard J, Herrera D, Needleman I (2002) A systematic review of guided tissue regeneration for periodontal furcation defects. What is the effect of guided tissue regeneration compared with surgical debridement in the treatment of furcation defects? J Clin Periodontol 29:103–116, discussion 160–162
41. Soares FP, Hayashi F, Yorioka CW, Pannuti CM, Gioso MA, de Lima LA, Romito GA, Pustiglioni FE (2005) Repair of class II furcation defects after a reparative tissue graft obtained from extraction sockets treated with growth factors: a histologic and histometric study in dogs. J Periodontol 76:1681–1689
42. Cetiner D, Unsal B, Parlar A, Gültekin E, Kurtis B (2004) Evaluation of periodontal healing in class II furcation defects following guided tissue regeneration with two different types of polylactic acid membranes. Chin Med J (Engl) 117:270–274
43. Hollister SJ, Lin CY, Saito E, Lin CY, Schek RD, Taboas JM, Williams JM, Partee B, Flanagan CL, Diggs A, Wilke EN, Van Lenthe GH, Müller R, Wirtz T, Das S, Feinberg SE, Krebsbach PH (2005) Engineering craniofacial scaffolds. Orthod Craniofac Res 8:162–173
44. Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, Meotti C, Bertoja AZ, Giardino R, Fornasari PM, Mercuri M, Picci P (2003) Platelet-derived growth factors enhance proliferation of human stromal stem cells. Biomaterials 24:3095–3100
45. Boo JS, Yamada Y, Okazaki Y, Hibino Y, Okada K, Hata K, Yoshikawa T, Sugiura Y, Ueda M (2002) Tissue-engineered bone using mesenchymal stem cells and a biodegradable scaffold. J Craniofac Surg 13:231–239, discussion 240–243
46. Yamada Y, Boo JS, Ozawa R, Nagasaka T, Okazaki Y, Hata K, Ueda M (2003) Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. J Craniomaxillofac Surg 31:27–33
47. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB (2005) A reentry study on the use of bovine porous bone mineral, GTR, and platelet-rich plasma in the regenerative treatment of intrabony defects in humans. Int J Periodontics Restorative Dent 25:49–59
48. Awad HA, Butler DL, Harris MT, Ibrahim RE, Wu Y, Young RG, Kadiyala S, Boivin GP (2000) In vitro characterization of mesenchymal stem cell-seeded collagen scaffolds for tendon repair: effects of initial seeding density on contraction kinetics. J Biomed Mater Res 51:233–240
49. Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J (2002) Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. J Bone Joint Surg Br 84:571–578
50. Iwasa J, Ochi M, Uchio Y, Katsube K, Adachi N, Kawasaki K (2003) Effects of cell density on proliferation and matrix synthesis of chondrocytes embedded in atelocollagen gel. Artif Organs 27:249–255
51. Schallhorn RG, Hiatt WH (1972) Human allografts of iliac cancellous bone and marrow in periodontal osseous defects. II. Clinical observations. J Periodontol 43:67–81
52. Dragoo MR, Sullivan HC (1973) A clinical and histological evaluation of autogenous iliac bone grafts in humans. II. External root resorption. J Periodontol 44:614–625
53. Levin MP, Getter L, Cutright DE (1975) A comparison of iliac marrow and biodegradable ceramic in periodontal defects. J Biomed Mater Res 9:183–195
54. Wikesjö UM, Lim WH, Thomson RC, Cook AD, Wozney JM, Hardwick WR (2003) Periodontal repair in dogs: evaluation of a bioabsorbable space-providing macroporous membrane with recombinant human bone morphogenetic protein-2. J Periodontol 74:635–647
55. Trombelli L, Annunziata M, Belardo S, Farina R, Scabbia A, Guida L (2006) Autogenous bone graft in conjunction with enamel matrix derivative in the treatment of deep periodontal intra-osseous defects: a report of 13 consecutively treated patients. J Clin Periodontol 33:69–75
56. Bauer TW, Muschler GF (2000) Bone graft materials. An overview of the basic science. Clin Orthop Relat Res 371:10–27
57. Selvig KA (1994) Discussion: animal models in reconstructive therapy. J Periodontol 65:1169–1172
58. Choi SH, Kim CK, Cho KS, Huh JS, Sorensen RG, Wozney JM, Wikesjö UM (2002) Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (rhBMP-2/ACS) on healing in 3-wall intrabony defects in dogs. J Periodontol 73:63–72
59. Karring T, Nyman S, Gottlow J, Laurell L (1993) Development of the biological concept of guided tissue regeneration—animal and human studies. Periodontol 2000 1:26–35
60. Grzesik WJ, Narayanan AS (2002) Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med 13:474–484
61. Kramer PR, Nares S, Kramer SF, Grogan D, Kaiser M (2004) Mesenchymal stem cells acquire characteristics of cells in the periodontal ligament in vitro. J Dent Res 83:27–34
62. Froum SJ, Wallace SS, Tarnow DP, Cho SC (2002) Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. Int J Periodontics Restorative Dent 22:45–53
63. Kawase T, Okuda K, Wolff LF, Yoshie H (2003) Platelet-rich plasma-derived fibrin clot formation stimulates collagen synthesis in periodontal ligament and osteoblastic cells in vitro. J Periodontol 74:858–864
64. Kawase T, Okuda K, Saito Y, Yoshie H (2005) In vitro evidence that the biological effects of platelet-rich plasma on periodontal ligament cells is not mediated solely by constituent transforming-growth factor-beta or platelet-derived growth factor. J Periodontol 76:760–767
65. Pryor ME, Polimeni G, Koo KT, Hartman MJ, Gross H, April M, Safadi FF, Wikesjo UM (2005) Analysis of rat calvaria defects implanted with a platelet-rich plasma preparation: histologic and histometric observations. J Clin Periodontol 32:966–972