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Ankaferd blood stopper enhances healing after osseous grafting in patients with intrabony periodontal defects

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Background and Objective: The aim of this clinical study were to compare the clinical efficacy of ankaferd blood stopper (ABS) when used in combination with autogenous cortical bone graft (ACB) in the treatment of intrabony periodontal defects.

Material and Methods: The study was planned as a split-mouth design. Fifteen patients with chronic periodontitis at 30 sites (six men, nine women; 42 ± 7 years) were included. Treatment sites had probing pocket depths (PPD) of ≥ 6 mm and osseous defect depths of ≥ 4 mm as radiographically assessed. Following the initial periodontal therapy, patients were randomly assigned to two treatments in contralateral areas of the dentition: ACB + ABS or ACB alone. At baseline and 6 mo after surgery, clinical parameters of plaque index, gingival index, PPD, clinical attachment level and gingival recession (GR) were recorded. The primary outcome variable was the change in clinical attachment level between baseline and 24 wk after surgery. Gingival crevicular fluid samples were collected immediately before surgery and at 2, 4, 6, 12 and 24 wk after the surgery. Gingival crevicular fluid volume was calculated and vascular endothelial growth factor levels in gingival crevicular fluid were measured.

Results: PPD decreased, clinical attachment level improved and gingival index decreased significantly in response to both modes of treatment ($p < 0.05$). Both treatment modalities resulted in a significant gain in radiographic bone levels compared to baseline ($p < 0.05$). Intergroup comparisons showed that there was a significantly higher gain in clinical attachment level in the ABS/ACB group compared to ACB group ($p < 0.05$) with significantly less GR ($p < 0.05$). Similarly, vascular endothelial growth factor concentration in gingival crevicular fluid was significantly higher in the ABS/ACB group at postoperative weeks 2 and 4 compared to the ACB group ($p < 0.01$).

Conclusions: The findings suggest that ABS enhances the soft tissue healing during the periodontal defect fill by the ACB by stimulating angiogenesis and vascular endothelial cell function, prevents GR and thereby increases the clinical attachment gain.

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Periodontitis is an oral infectious disease, characterized by clinical attachment loss, alveolar bone resorption, periodontal pocketing and gingival inflammation (1,2). One of the main objectives of periodontal therapy is regeneration of the tooth's supporting periodontal tissues to their original levels (3). The healing after non-surgical and conventional surgical methods is histologically characterized by a long junctional epithelium along the root surfaces (4,5). Therefore, reconstructive techniques are required (6). The most widely used methods include autogenous bone and substitutes, barrier membranes for guided tissue regeneration or a combination of these techniques. All these approaches result in superior clinical outcomes in terms of probing pocket depth (PPD) reduction and clinical attachment gain compared to open flap debridement (7,8) with histological gain in clinical attachment and bone fill (9–13). On the other hand, accomplishing a complete and predictable reconstruction of periodontal tissues is still a challenge (7). One of the main challenges after the regenerative procedures is recession at the gingival margin (14–16). While hard tissues are being regenerated, the attachment gain is usually limited by the apical migration of the gingival margin (14–16). This outcome would be highly dependent on the microvascularization of the gingival tissues; therefore, understanding the biology of the clinical response to grafting procedures and how each tissue compartment responds to regenerative techniques for an ideal outcome of homeostasis is critical.

Soft tissue healing after the periodontal regenerative procedures is critical for success in increasing attachment gain by preventing recession (16–18). This process heavily depends on the vascular response and angiogenetic events during healing (16–18) and is mediated by various cytokines and growth factors. Angiogenesis is regulated by vascular endothelial growth factor (VEGF), which potently increases microvascular permeability, stimulates endothelial cell proliferation, induces proteolytic enzyme expression and the migration of endothelial cells, monocytes and osteoblasts, all of which are essential for angiogenesis (19–24). VEGF is also required for the development, remodeling, repair and regeneration of periodontal tissues (25–27). Studies about the role of VEGF suggested that it promotes the progression or healing of periodontal lesions (28–31). We have previously demonstrated that VEGF expression was related to the healing stage of periodontal disease than to the destruction stage of the lesion (31). While angiogenesis regulates a wide array of biological processes, including the inflammatory healing and restoration of tissue architecture, its involvement during regenerative periodontal treatment and tissue response has been investigated in only a limited number of studies (32,33).

Ankaferd blood stopper (Ankaferd Ilac Kozmetik AS, Istanbul, Turkey) has been recently introduced as a medicinal plant extract product and approved for the management of hemorrhage. ABS includes a standardized mixture of five plants: *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*. The hemostatic effect of ABS is based on the formation of an encapsulated protein network that provides focal points for vital erythrocyte aggregation (34–36). ABS provides tissue oxygenation while inducing a physiological hemostatic process without disturbing any individual clotting factor. ABS has been shown to affect endothelium, blood cells, angiogenesis, cellular proliferation, vascular dynamics and cell mediators as well as exerting antibacterial effects (34,37). In addition to the direct usage of ABS in the management of hemorrhagic disorders, off-label applications have been tested (34,38,39). Such applications demonstrated that ABS could enhance wound healing, infection control, early stage bone tissue healing and formation (34,38,39). These studies demonstrated that the pro-regenerative and pro-angiogenic properties of the ABS could be beneficial for a wide spectrum of clinical applications, including the restoration of hard and soft tissues. Limited data have suggested that ABS could be used as a pulp dressing in primary molars (40) and can decrease the occurrence of inflammation and necrosis, while increasing new bone formation during the early bone healing period (38).

In this clinical study, we have hypothesized that ABS will enhance soft and hard tissue healing for periodontal tissue reconstruction due to its impact on the vascularization of gingival tissues. The purpose was therefore to evaluate the clinical efficacy of ABS with autogenous cortical bone graft (ACB) in the treatment of intrabony periodontal defects and to evaluate the expression of VEGF during the healing period.

Material and methods

Study population

The study protocol was approved by the Local Ethics Committee of Istanbul University (Ethical Committee Approval Number: 2013/642), and written informed consent was obtained from all study participants in accordance with the Helsinki Declaration. The study was designed as split-mouth study. Fifty-nine patients were screened; 15 patients meeting the inclusion and exclusion criteria and with chronic periodontitis (six men, nine women; 42 ± 7 years) and with 30 sites exhibiting radiographic evidence of bone loss were included. For inclusion, the subjects had to have similar intrabony defects without furcation involvement in each of the contralateral quadrants, including the premolars and molars. The exclusion criteria were systemic diseases (i.e., diabetes mellitus, cancer, HIV, bone metabolic diseases or disorders that compromise wound healing), chronic high-dose steroid therapy, radiation or immunosuppressive therapy, allergy or sensitivity to any drug, pregnancy, lactation and smoking. The subjects had no history of drug therapy for at least 6 mo before recruitment to the study. There were no drop-outs during the study. Change in clinical attachment level was the primary outcome variable. Change in PPD, gingival recession (GR) and radiographic alveolar bone levels were used as secondary outcome variables.

Initial periodontal therapy

Initial periodontal therapy in all patients consisted of an oral hygiene instruction, full-mouth scaling and root planing, and occlusal adjustments if necessary. Periodontal re-evaluation was performed to determine the patient's response and the need for periodontal surgery was assessed 4–6 wk after completion of the initial phase. The following selection criteria had to be met: (i) PPD, ≥ 6 mm; (ii) radiographic and intrasurgical osseous defect depth, ≥ 4 mm; (iii) two or three osseous walls; and (iv) no previous prosthetic restoration or endodontic treatment on the related tooth.

Measurements and sample collection

PPD, clinical attachment level, plaque index (PI), gingival index and GR were measured. PPD, GR and clinical attachment level measurements were made by the Florida Probe (Florida Probe Corp., Gainesville, FL, USA) immediately before surgery and 6 mo postoperatively. PPD was measured as the distance from the gingival margin to the base of the periodontal pocket. Clinical attachment level was recorded by combining the distance from the cemento-enamel junction to the gingival margin with probing depth. Measurements were made in six areas per tooth: mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual and midlingual. All clinical measurements were performed by the same investigator, who was blinded with respect to treatment modality. Before the actual measurement, 10 subjects were randomly selected and used to calibrate the investigator. The investigator evaluated the subjects on two separate occasions, 48 h apart. Calibration of the investigator was accepted if measurements at baseline and 48 h were > 90% similar at the millimeter level.

Gingival crevicular fluid samples were collected immediately before surgery and at 2, 4, 6, 12 and 24 wk after surgery, and were taken at each experimental site. Before gingival crevicular fluid sampling, the sites were isolated with cotton rolls and saliva was removed. Gingival crevicular fluid was sampled with filter paper (Periopaper; ProFlow, Inc., Amityville, NY, USA).

Paper strips were placed in the crevice until mild resistance was felt, and left in position for 30 s. Strips with visible signs of saliva or blood contamination were discarded. The gingival crevicular fluid volume of each strip was determined by electronic impedance (Periotron 8000; ProFlow Inc.). Samples were placed in a sterile polypropylene tube and stored at −70°C until enzyme-linked immunosorbent assay analysis. VEGF concentrations in gingival crevicular fluid were analyzed at 450–550 nm using a commercially available assay (Hu VEGF 165; Biosource, Carlsbad, CA, USA).

Surgical procedure

The same surgical procedures were performed in all patients: the application of either ABS/ACB grafting or ACB grafting materials alone was the only difference between the groups. All surgical procedures were performed on an outpatient basis by two experienced periodontal clinicians, using aseptic conditions and under local anesthesia. The same clinician performed all surgical procedures and the other assisted during the procedures.

Following local anesthesia, buccal and lingual intrasulcular incisions were made, and full-thickness mucoperiosteal flaps were raised. After flap elevation, blood was obtained from the defect area and was put into a glass bowl for the preparation of membrane. All granulation tissues were removed from the defects, and the roots were thoroughly scaled and planed using hand and ultrasonic instruments. The surgical sites were then rinsed with sterile saline. Fifteen paired interproximal intrabony defects were randomly treated with either ABS/ACB grafting or ACB grafting, via a split-mouth design (41). Randomization was carried out in each case during surgical treatment and before allocation of the graft materials by a coin toss.

An adequate amount of particulate cortical bone was harvested from the buccal cortical plate adjacent to the intrabony defect using a bone scraper and implanted into the intrabony defect. First, ACB granules were mixed with ABS (Trend Teknoloji Ilaç AS, Istanbul, Turkey); then applied to the created space, and finally ABS application on blood to prepare a gel form was positioned as a membrane over the ACB graft. Finally, the flaps were repositioned and secured with 4-0 silk suture material by using the interrupted and vertical mattress suturing technique to achieve primary closure.

Postoperative care

The patients were prescribed amoxicillin plus clavulanic acid (2 g/d for 7 d), flurbiprofen (200 mg/d for 3 d) and a 0.2% chlorhexidine gluconate mouth rinse (twice a day for 2 wk). The silk sutures were removed 1 wk after surgery. Patients were instructed to start tooth brushing and flossing or interproximal brushing, at the end of the second postoperative week. Re-call appointments for supragingival professional tooth cleaning and oral hygiene reinforcement weekly up to 1 mo after the surgeries, and 2 and 3 mo for the rest of the study period.

Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS version 15.0; SPSS, Chicago, IL, USA). For the statistical analysis of clinical data, only recordings representing the deepest site in each defect were used. The Shapiro–Wilk test was used to investigate whether data were normally distributed or not. The Wilcoxon signed-ranks test was used for intragroup comparisons and Mann–

Whitney *U* non-parametric test for intergroup comparisons of the clinical and biochemical findings. Power analysis indicated that 15 defects for each treatment modality would be sufficient to demonstrate statistical significance at the $p < 0.05$ level with a power of (at least) $\geq 80\%$. The data are shown as mean ± standard deviation.

Results

In total, 9 two-wall and 6 three-wall defects were treated with ABS/ACB grafting; 8 two-wall and 7 three-wall defects were treated with ACB alone. All patients participating in this study tolerated the surgical procedures well. Healing was uneventful with no adverse reactions in both groups. Figure 1 demonstrates representative intraoperative photographs where defects were treated with either the ACB or the ABS/ACB. The ACB graft is prepared and applied to an infrabony defect (Fig. 1A–C). In the group where the ABS was tested, the ACB was prepared and applied to the infrabony defect and covered by the ABS (Fig. 1D–F). When mixed with the blood, the ABS gains a gel form allowing an easy application. Figure 2 demonstrates a representative case for each treatment group before and after 6 mo of healing. The hard tissue defect fill was accomplished in both groups. Values in PI are shown in Fig. 3A. No significant differences in PI were observed at 24 wk postoperatively, compared to the preoperative data ($p > 0.05$) or between the groups ($p > 0.05$). The change in gingival index showed a significant reduction in both groups compared to baseline values after 24 wk ($p < 0.001$) with no significant differences among treatment modalities (Fig. 3B). There was a statistically significant reduction in gingival crevicular fluid volume in the ABS/ACB group compared to the ACB group throughout the treatment period ($p = 0.012$) suggesting that the ABS could regulate the gingival inflammatory response and the inflammatory exudate at tissue level (Fig. 3C).

Both treatment modalities resulted in a significant decrease in PPDs compared to baseline values after 24 wk ($p < 0.001$; Fig. 4A). No significant differences were observed in the PPD between the values of groups ($p > 0.05$). Intragroup comparisons showed that clinical attachment level as the primary outcome variable improved significantly at 24 wk postoperatively, compared to the preoperative data in both groups ($p < 0.001$; Fig. 4B). Intergroup comparisons at 24 wk showed a significant gain in clinical attachment level in the ABS/ACB grafting group compared to the ACB grafting alone ($p < 0.01$). Table 1 demonstrates the frequency distribution for the PPD and clinical attachment level changes during treatment. There was a significant difference in both treatment groups compared to baseline ($p < 0.05$) while no statistically significant difference was observed between groups with regards to the frequency of pockets of at least 5 mm in depth and clinical attachment level of at least 5 mm after 24 wk. In parallel, while there was a statistically significant increase in GR in the ACB grafting-alone group compared to preoperative values ($p < 0.05$) there was no significant GR in the ABS/ACB grafting group compared to preoperative data ($p > 0.05$); the difference was statistically significant compared to the ACB-alone group (Fig. 4C). These data suggest that the ABS prevents the GR. Both treatment modalities resulted in a significant decrease in radiographic bone level compared to baseline values after 24 wk ($p < 0.001$). There was no significant difference in radiographic bone level between the values of groups ($p > 0.05$; Fig. 4D).

Intragroup analysis showed significant increases in the VEGF concentration for both groups at postoperative weeks 2, 4, 6, 12 and 24 compared to preoperative values ($p < 0.01$; Fig. 5A). Intergroup analysis demonstrated significantly higher

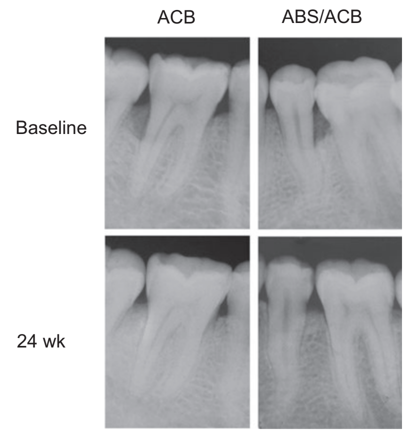

Fig. 2. Radiographic appearance at baseline and 24 wk postsurgery for site treated with ACB and ABS/ACB. ABS, ankaferd blood stopper; ACB, autogenous cortical bone graft.

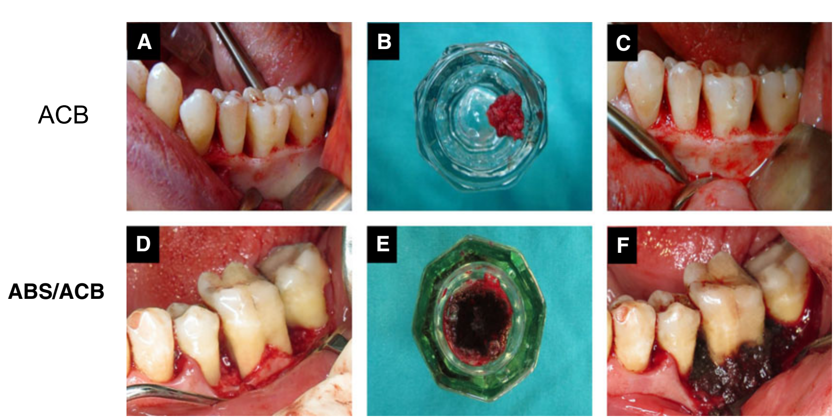

Fig. 1. Intraoperative photographs for representative cases treated with ACB and ABS/ACB. (A) Infrabony defect treated with ACB. (B) Preparation of the ACB. (C) Application of the ACB in the defect. (D) Infrabony defect with ABS/ACB. (E) Preparation of the ABS as a gel form and mixed with autogenous blood obtained from the defect site. (F) Application of the ABS and ACB in the defect. ABS, ankaferd blood stopper; ACB, autogenous cortical bone graft.

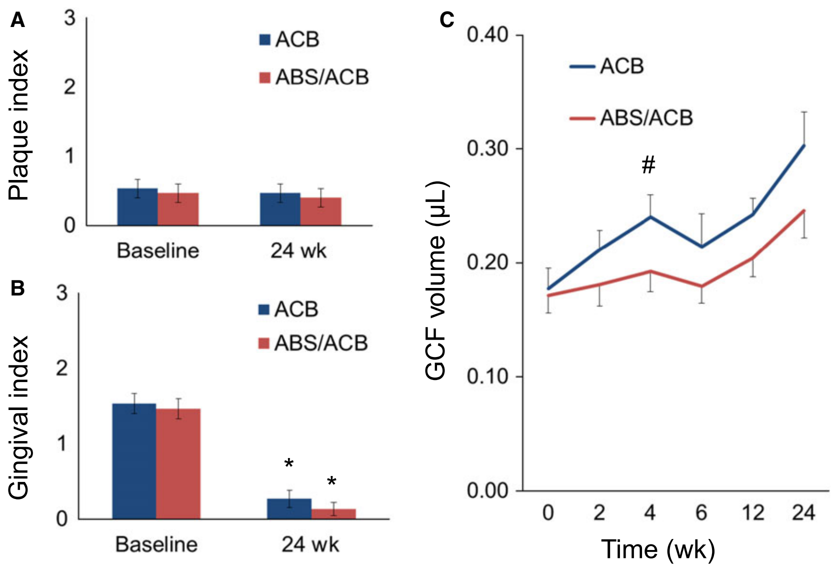

Fig. 3. (A) Changes in plaque index. (B) Changes in gingival index. (C) Changes in GCF volume. $*p < 0.001$ compared to baseline; $^{\#}p = 0.012$ compared to the ACB group. ABS, ankaferd blood stopper; ACB, autogenous cortical bone graft; GCF, gingival crevicular fluid.

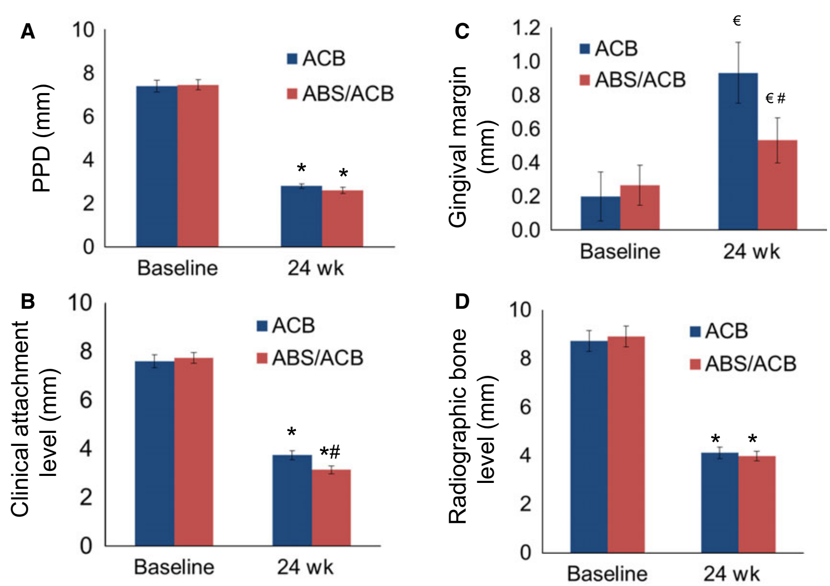

Fig. 4. (A) Changes in PPD. (B) Changes in clinical attachment level. (C) Changes in gingival margin. (D) Changes in radiographic bone level. $*p < 0.001$ compared to baseline; $^{\#}p < 0.01$ compared to the ACB grafting alone. $^{€}p < 0.05$ compared to baseline. ABS, ankaferd blood stopper; ACB, autogenous cortical bone graft; PPD, probing pocket depth.

VEGF levels in the ABS/ACB grafting group at postoperative weeks 2 and 4 ($p < 0.01$) compared to levels in the ACB grafting-alone group whereas no significant differences were found in VEGF levels between the groups at other time intervals ($p > 0.05$). There was no difference in total VEGF levels between the treatment groups ($p > 0.05$; Fig. 5B). No correlation between VEGF levels and clinical parameters was found ($p > 0.05$).

Discussion

The objective of this clinical study was to evaluate efficacy of the ABS on periodontal defect restoration when the defects were treated with the ACB graft. The findings demonstrated that ABS enhances the clinical attachment gain by reducing postoperative GR. This outcome could be at least in part due to a reduced gingival inflammation, inflammatory exudation and increased angiogenetic activity suggesting that the ABS-mediated vascular changes lead to an increased regenerative healing in periodontal infrabony defects treated with the autogenous cortical bone graft.

Periodontal regeneration of intrabony defects using bone grafts has been extensively studied (42,43). Different strategies such as guided tissue regeneration, growth factors and combination therapies with bone grafts were used (14,44–48). The autogenous cortical bone graft is considered as the gold standard (49,50) with excellent regenerative potential supported by histologic evidence (51). Autografts are bioabsorbable, non-allergic, easy to handle and not costly (49,52–54). Various researchers have reported on the clinically successful use of intraoral autogenous bone grafts in the treatment of intrabony defects (11,55–57). Bone grafting and periodontal regenerative surgery result in an increased GR while leading to a reduction in probing depth and a gain of clinical attachment (14,44,58). Various methods including guided tissue regeneration, applications of enamel matrix derivative, platelet concentrates as platelet-rich plasma, plasma rich in growth factor, platelet-rich fibrin and various polypeptide growth factors that modulate the wound healing have been used alone or in combination to minimize GR (44,45,59,60). Results of a controlled clinical study comparing hydroxyapatite and platelet-rich plasma combined with hydroxyapatite, showed that an increase in mean GR after 12 mo without any impact of the platelet-rich plasma (46) and these data were further confirmed when a combination of enamel matrix derivate with bioactive glass was compared to bioactive glass alone (61). In a case series comparing the treatment effects of autologous platelet concentrate or

Table 1. The frequency distribution for PPD and CAL changes

	PPD ≥ 5 mm (%)		CAL ≥ 5 mm (%)	
	Baseline	24 wk	Baseline	24 wk
ACB	74/90 (82)	12/90 (13)*	76/90 (84)	32/90 (36)*
ABS/ACB	68/90 (76)	11/90 (12)*	70/90 (78)	20/90 (22)*

ABS, ankaferd blood stopper; ACB, autogenous cortical bone graft; CAL, clinical attachment level; PPD, probing pocket depth.
*Significantly compared to baseline ($p < 0.05$).

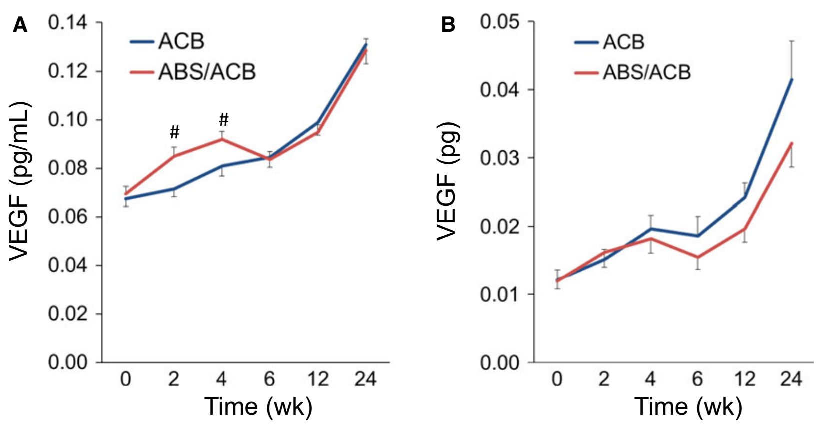

Fig. 5. (A) Changes in VEGF concentration. (B) Changes in total VEGF levels. $^{\#}p < 0.01$ compared to the ACB grafting alone. ABS, ankaferd blood stopper; ACB, autogenous cortical bone graft; VEGF, vascular endothelial growth factor.

bioabsorbable barrier membrane, similar results were obtained (44). Thus, attempts in reducing the GR after regenerative procedures with additional strategies have resulted in minimal or no gain so far. As an apical migration of the gingival attachment to the dental surfaces limits the regeneration of the hard tissues and attachment on root surfaces, GR continues to be a major issue in regenerative periodontal medicine (16–18). In addition, sensitivity of exposed root surfaces and esthetic issues present concerns due to GR. Our data demonstrate that ABS can prevent the GR when used for defect fill after the ACB grafting. This observation is statistically significant resulting in 30% less GR compared to grafting alone and suggests a novel mechanism through which soft tissue healing can be regulated during the regenerative periodontal treatment.

ABS can enhance tissue oxygenation, local hemostasis, and promote wound healing and clot stability into intrabony periodontal defects. Wound healing begins with hemostasis, which includes fibrin clot formation, platelet adhesion and aggregation (44). Our results suggested that the prevention on GR in the ABS/ACB grafting group might be related to healing modulating by the ABS. This function at least in part, depends on the VEGF concentration and regulation of tissue inflammation suggesting a role for angiogenesis in tissue homeostasis in periodontium. Consistent with our results, application of the ABS has been shown to decrease inflammation and necrosis, while increasing new bone formation during the early bone healing period (38). Likewise, in another experimental study, the findings suggested that ABS had an accelerator effect on short-term bone healing (39). ABS has also been shown to exert anti-infective and healing modulator properties that are also crucial periodontal interventions (62). Collectively, our data and the findings reported so far by other groups suggest that the ABS controls the inflammation and regulates the vascular response during healing,

There are several limitations in the use of the ABS. The ABS is denser and more resistant than other biological preparations, such as enamel matrix proteins or platelet-rich plasma; however, its space-maintaining ability in periodontal defects is not ideal due to its non-rigid feature. Therefore, its current delivery may require a stable bone graft or space provision for its biological efficacy. Likewise, its resorption is not known when delivered into the periodontal defects. Our data suggest that the ABS-enhanced healing occurs quickly and may not be long-lived. Thus, larger defects and other applications need to be tested for the ABS-mediated healing efficacy. Nevertheless, its biological impact seems to be profound, where a reduction in recession was accompanied by an increased attachment gain and pro-angiogenic properties mediated by the VEGF. The VEGF is a multifunctional angiogenic cytokine involved in inflammation and wound healing (29). It has been shown in periodontal tissues within plasma cells, macrophages, endothelial cells and in junctional, sulcular and gingival epithelia (28) with controversial findings during disease process (24,30,63). The VEGF is critical for the development, remodeling, repair and regeneration of periodontal tissues (25–27,31). Therefore, VEGF is inevitably associated with healing of wounds within the periodontal tissues and after periodontal surgical procedures. The present study showed that VEGF levels were significantly higher in the ABS/ACB grafting group compared to levels in the ACB grafting-alone group suggesting that increased VEGF might be a regulator of vascularization and faster recovery of the gingival tissues. This finding is consistent with a previous study from our group where we have demonstrated that the VEGF expression is possibly related to the healing phase of periodontal disease than to the destruction phase (31). To this end, in a clinical study evaluating local levels of the VEGF after reconstructive periodontal surgery with or without local platelet-derived growth factor-BB application in humans, increased VEGF levels were correlated with platelet-derived growth factor-BB-enhanced periodontal wound healing (27) suggesting a role for the VEGF.

Within the limits of the present clinical study, the results indicate that (i) both treatment modalities resulted in statistically significant clinical improvements compared with baseline, and (ii) ABS may improve the regenerative process and cause less GR and (iii) ABS may lead to an increase in levels of the VEGF in the healing stage of periodontal surgery.

References

1. Socransky SS, Haffajee AD. The nature of periodontal disease. *Ann Periodontol* 1997;**2**:3–10.
2. Flemmig TF. Periodontitis. *Ann Periodontol* 1999;**4**:32–38.
3. Polimeni G, Xiropaidis AV, Wikesjö UM. Biology and principles of periodontal wound healing/regeneration. *Periodontol 2000* 2006;**41**:30–47.
4. Caton JG, Greenstein G. Factors related to periodontal regeneration. *Periodontol 2000* 1993;**1**:9–15.
5. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: combinations of barrier membranes and grafting materials – biological foundation and preclinical evidence: a systematic review. *J Clin Periodontol* 2008;**35**(8 suppl):106–116.
6. Trombelli L, Farina R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *J Clin Periodontol* 2008;**35**(8 suppl):117–135.
7. Trombelli L. Which reconstructive procedures are effective for treating the periodontal intraosseous defect? *Periodontol 2000* 2005;**37**:88–105.
8. Jepsen S, Eberhard J, Herrera D, Needleman I. 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* 2002;**29**(suppl 3):103–116; discussion 160-2.
9. Dragoo MR, Sullivan HC. A clinical and histological evaluation of autogenous iliac bone grafts in humans. I. Wound healing 2 to 8 months. *J Periodontol* 1973;**44**:599–613.
10. Hiatt WH, Schallhorn RG, Aaronian AJ. The induction of new bone and cementum formation. IV. Microscopic examination of the periodontium following human bone and marrow allograft, autograft and nongraft periodontal regenerative procedures. *J Periodontol* 1978;**49**:495–512.
11. Stahl SS, Froum SJ, Kushner L. Healing responses of human intraosseous lesions following the use of debridement, grafting and citric acid root treatment. II. Clinical and histologic observations: one year postsurgery. *J Periodontol* 1983;**54**:325–338.
12. Harris RJ. Human histologic evaluation of a bone graft combined with GTR in the treatment of osseous dehiscence defects: a case report. *Int J Periodontics Restorative Dent* 2000;**20**:510–519.
13. Yukna RA, Mellonig JT. Histologic evaluation of periodontal healing in humans following regenerative therapy with enamel matrix derivative. A 10-case series. *J Periodontol* 2000;**71**:752–759.
14. Kotsovilis S, Markou N, Pepelassi E, Nikolidakis D. The adjunctive use of platelet-rich plasma in the therapy of periodontal intraosseous defects: a systematic review. *J Periodontal Res* 2010;**45**:428–443.
15. Martínez-Zapata MJ, Martí-Carvajal A, Solà I *et al*. Efficacy and safety of the use of autologous plasma rich in platelets for tissue regeneration: a systematic review. *Transfusion* 2009;**49**:44–56.
16. Del Fabbro M, Bortolin M, Taschieri S, Weinstein R. Is platelet concentrate advantageous for the surgical treatment of periodontal diseases? A systematic review and meta-analysis. *J Periodontol* 2011;**82**:1100–1111.
17. Marx RE. Bone graft physiology with use of platelet-rich plasma and hyperbaric oxygen. In: Jensen O, ed. *The Sinus Bone Graft*. Chicago: Quintessence, 1999:183.
18. Marx RE. Platelet concentrate: a strategy for accelerating and improving bone regeneration. In: Davies JE, ed. *Bone Engineering*. Toronto: Em Squared, 2001:447.
19. Connolly DT. Vascular permeability factor: a unique regulator of blood vessel function. *J Cell Biochem* 1991;**47**:219–223.
20. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995;**146**:1029–1039.
21. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 1992;**13**:18–32.
22. Nakagawa K, Chen YX, Ishibashi H *et al*. Angiogenesis and its regulation: roles of vascular endothelial cell growth factor. *Semin Thromb Hemost* 2000;**26**:61–66.
23. Sakuta T, Matsushita K, Yamaguchi N *et al*. Enhanced production of vascular endothelial growth factor by human monocytic cells stimulated with endotoxin through transcription factor SP-1. *J Med Microbiol* 2001;**50**:233–237.
24. Unlu F, Guneri PG, Hekimgil M, Yesilbek B, Boyacioglu H. Expression of vascular endothelial growth factor in human periodontal tissues: comparison of healthy and diabetic patients. *J Periodontol* 2003;**74**:181–187.
25. Suthin K, Matsushita K, Machigashira M *et al*. Enhanced expression of vascular endothelial growth factor by periodontal pathogens in gingival fibroblasts. *J Periodontal Res* 2003;**38**:90–96.
26. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 1999;**5**:623–628.
27. Cooke JW, Sarment DP, Whitesman LA *et al*. Effect of rhPDGF-BB delivery on mediators of periodontal wound repair. *Tissue Eng* 2006;**12**:1441–1450.
28. Booth V, Young S, Cruchley A, Taichman NS, Paleolog E. Vascular endothelial growth factor in human periodontal disease. *J Periodontal Res* 1998;**33**:491–499.
29. Prapulla DV, Sujatha PB, Pradeep AR. Gingival crevicular fluid VEGF levels in periodontal health and disease. *J Periodontol* 2007;**78**:1783–1787.
30. Johnson RB, Serio FG, Dai X. Vascular endothelial growth factors and progression of periodontal diseases. *J Periodontol* 1999;**70**:848–852.
31. Cetinkaya BO, Keles GC, Ayas B, Sakallioglu EE, Acikgoz G. The expression of vascular endothelial growth factor in a rat model at destruction and healing stages of periodontal disease. *J Periodontol* 2007;**78**:1129–1135.
32. Nokhbehsaim M, Deschner B, Bourauel C *et al*. Interactions of enamel matrix derivative and biomechanical loading in periodontal regenerative healing. *J Periodontol* 2011;**82**:1725–1734.
33. Bizenjima T, Seshima F, Ishizuka Y, Takeuchi T, Kinumatsu T, Saito A. Fibroblast growth factor-2 promotes healing of surgically created periodontal defects in rats with early, streptozotocin-induced diabetes via increasing cell proliferation and regulating angiogenesis. *J Clin Periodontol* 2015;**42**:62–71.

34. Tasdelen Fisgin N, Tanriverdi Cayci Y, Coban AY *et al*. Antimicrobial activity of plant extract Ankaferd Blood Stopper. *Fitoterapia* 2009;**80**:48–50.
35. Haznedaroglu BZ, Haznedaroglu IC, Walker SL *et al*. Ultrastructural and morphological analyses of the in vitro and in vivo hemostatic effects of Ankaferd Blood Stopper. *Clin Appl Thromb Hemost* 2010;**16**:446–453.
36. Baykul T, Alanoglu EG, Kocer G. Use of Ankaferd Blood Stopper as a hemostatic agent: a clinical experience. *J Contemp Dent Pract* 2010;**11**:E088–E094.
37. Goker H, Haznedaroglu IC, Ercetin S *et al*. Haemostatic actions of the folkloric medicinal plant extract Ankaferd Blood Stopper. *J Int Med Res* 2008;**36**:163–170.
38. Isler SC, Demircan S, Cakarer S *et al*. Effects of folk medicinal plant extract Ankaferd Blood Stopper on early bone healing. *J Appl Oral Sci* 2010;**18**:409–414.
39. Şimşek HO, Tüzüm MŞ, Baykul T, Gürer İE, Başsorgun Cİ. Experimental investigation of the effects of a blood stopper agent (Ankaferd Blood Stopper) on bone surfaces. *Turk J Haematol* 2013;**30**:177–183.
40. Yaman E, Görken F, Pinar Erdem A, Sepet E, Aytepe Z. Effects of folk medicinal plant extract Ankaferd Blood Stopper(®) in vital primary molar pulpotomy. *Eur Arch Paediatr Dent* 2012;**13**: 197–202.
41. Cetinkaya BO, Keles GC, Pamuk F, Balli U, Keles ZP. Long-term clinical results on the use of platelet concentrate in the treatment of intrabony periodontal defects. *Acta Odontol Scand* 2014;**72**:92–98.
42. Froum SJ, Ortiz M, Witkin RT, Thaler R, Scopp IW, Stahl SS. Osseous autografts. III. Comparison of osseous coagulum-bone blend implants with open curetage. *J Periodontol* 1976;**47**:287–294.
43. Cortellini P, Tonetti MS. Clinical performance of a regenerative strategy for intrabony defects: scientific evidence and clinical experience. *J Periodontol* 2005;**76**:341–350.
44. Papli R, Chen S. Surgical treatment of infrabony defects with autologous platelet concentrate or bioabsorbable barrier membrane: a prospective case series. *J Periodontol* 2007;**78**:185–193.
45. Keles GC, Cetinkaya BO, Baris S, Albayrak D, Simsek SB. Comparison of platelet pellet with or without guided tissue regeneration in the treatment of class II furcation defects in dogs. *Clin Oral Investig* 2009;**13**:393–400.
46. Okuda K, Tai H, Tanabe K *et al*. Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: a comparative controlled clinical study. *J Periodontol* 2005;**76**:890–898.
47. Lekovic V, Camargo PM, Weinlaender M, Nedic M, Aleksic Z, Kenney EB. A comparison between enamel matrix proteins used alone or in combination with bovine porous bone mineral in the treatment of intrabony periodontal defects in humans. *J Periodontol* 2000;**71**:1110–1116.
48. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Kenney EB. Comparison of platelet-rich plasma, bovine porous bone mineral and guided tissue regeneration against platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: a reentry study. *J Periodontol* 2002;**73**:198–205.
49. Kim CS, Choi SH, Cho KS, Chai JK, Wikesjö UM, Kim CK. Periodontal healing in one-wall intra-bony defects in dogs following implantation of autogenous bone or a coral-derived biomaterial. *J Clin Periodontol* 2005;**32**:583–589.
50. Deliberador TM, Nagata MJ, Furlaneto FA *et al*. 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* 2006;**77**:780–789.
51. Mellonig JT. Alveolar bone induction: autografts and allografts. *Dent Clin North Am* 1980;**24**:719.
52. MacNeill SR, Cobb CM, Rapley JW, Glaros AG, Spencer P. In vivo comparison of synthetic osseous graft materials. A preliminary study. *J Clin Periodontol* 1999;**26**:239–245.
53. Carranza FA, Takei HH, Cochran DL. Reconstructive periodontal surgery. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, eds. *Clinical Periodontology*, 10th edition. St. Louis: Saunders, 2006:968–990.
54. Guida L, Annunziata M, Belardo S, Farina R, Scabbia A, Trombelli L. Effect of autogenous cortical bone particulate in conjunction with enamel matrix derivative in the treatment of periodontal intraosseous defects. *J Periodontol* 2007;**78**:231–238.
55. Renvert S, Garrett S, Shallhorn RG, Egelberg J. Healing after treatment of periodontal intraosseous defects. III. Effect of osseous grafting and citric acid conditioning. *J Clin Periodontol* 1985;**12**:441–455.
56. Ellegaard B, Low H. New attachment of periodontal tissues after treatment of intrabony lesions. *J Periodontol* 1971;**42**:648–652.
57. Rosenberg MM. Free osseous tissue autografts as a predictable procedure. *J Periodontol* 1971;**42**:195–209.
58. Park JS, Suh JJ, Choi SH *et al*. Effects of pretreatment clinical parameters on bioactive glass implantation in intrabony periodontal defects. *J Periodontol* 2001;**72**:730–740.
59. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. *Periodontol 2000* 2000;**24**:253–269.
60. Kalpidis CD, Ruben MP. Treatment of intrabony periodontal defects with enamel matrix derivative: a literature review. *J Periodontol* 2002;**73**:1360–1376.
61. Sculean A, Barbé G, Chiantella GC, Arweiler NB, Berakdar M, Brecx M. Clinical evaluation of an enamel matrix protein derivative combined with a bioactive glass for the treatment of intrabony periodontal defects in humans. *J Periodontol* 2002;**73**:401–408.
62. Beyazit Y, Kart T, Kuscu A *et al*. Successful management of bleeding after dental procedures with application of blood stopper: a single center prospective trial. *J Contemp Dent Pract* 2011;**12**:379–384.
63. Pradeep AR, Prapulla DV, Sharma A, Sujatha PB. Gingival crevicular fluid and serum vascular endothelial growth factor: their relationship in periodontal health, disease and after treatment. *Cytokine* 2011;**54**:200–204.