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A comparison of different bone graft materials in peri-implant guided bone regeneration

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Abstract: The aim of this study was to compare the effects of hydroxyapatite (HA), deproteinized bovine bone (DPB), human-derived allogenic bone (HALG), and calcium sulfate (CAP) graft biomaterials used with titanium barriers for bone augmentation to treat peri-implant defects in rat calvarium treated by guided bone regeneration (GBR). Thirty-two female Sprague-Dawley rats were divided into four groups: DPB, HALG, HA, and CAP. One titanium barrier was fixed to each rat's calvarium after the titanium implants had been fixed. In total, 32 titanium implants and barriers were used. Ninety days after the surgical procedure, all the barriers were removed. After decalcification of bone tissue, the titanium implants were removed gently, and new bone regeneration in the peri-implant area was analyzed histologically. Immunohistochemical staining of vascular endothelial growth factor (VEGF) was also performed. There were no statistically significant between-group differences in new bone regeneration or VEGF expression after 3 months. According to the results of the histological and immunohistochemical analyses, none of the grafts used in this study showed superiority with respect to new bone formation.

Keywords: Bone Regeneration; Durapatite; Hydroxyapatites; Calcium Sulfate.

Introduction

The guided bone regeneration (GBR) method is used for the treatment of peri-implant bone tissue defects in oral-dental implantology.[1] In GBR, a barrier membrane is used to preserve blood formation and create a closed area around the bone tissue defect.[2,3,4] The GBR method encourages the proliferation of bone-forming cells called osteoblasts.[2,3,4] In GBR, the barrier membrane must be permeable to enable the diffusion of nutrients required for bone regeneration.[5] Previous experimental studies reported more successful bone tissue regeneration using the GBR procedure and a hermetically-closed, stiff occlusive titanium barrier as compared with permeable membranes in a rabbit calvarium model.[6,7,8]

Autogenous bone grafts contain growth factors and promote the recruitment of stem cells.[9,10] Due to their osteoinductive and osteoconductive properties, autogenous bone grafts are the current gold standard for bone augmentation procedures. However, autogenous bone grafts of extraoral origin also have a number of disadvantages. These include the need for

a second surgical procedure; a limited supply of bone grafts; and postsurgery morbidity, pain, and neural damage in the donor bone area, as well as patient discomfort.[11,12] Thus, various alternative bone graft materials, such as deproteinized bovine bone (DPB), human-derived allogenic bone (HALG), hydroxyapatite (HA), and calcium sulfate (CAP) bioceramic biomaterials, have been developed as alternative graft materials to autologous grafts.[9,10] Allografts, such as deproteinized human bone grafts, are one of the most commonly-used alternatives to autografts in the treatment of bone tissue defects. However, allografts have various disadvantages, including an increased risk of infections (hepatitis and HIV). Controversy also surrounds their osteoinductive potential. In experimental animal models, researchers reported increased bone regeneration using calcium phosphate ceramic-derived bone graft biomaterials (HA, tricalcium phosphate, and calcium sulfate), in addition to superior stability and osteogenic properties, compared to autologous bone grafts.[12] In experimental and clinical research, another study demonstrated the osteoconductive capacity of this type of graft material (ceramic-derived bone graft biomaterial) in a GBR procedure for the treatment of bone tissue defects.[13]

The aim of the present study was to compare the effects of HA-, DPB-, HALG-, and CAP-derived bone graft biomaterials used with titanium barriers on bone augmentation in a peri-implant GBR procedure in rat calvarium.

Methodology

The study consisted of 32 female Sprague-Dawley rats, which were provided by the Experimental Research Center of Firat University. The experimental protocol and procedures in this study were approved by the Animal Experimental Ethics Committee of Firat University (Elazig, Turkey). The welfare and care of the experimental animals complied with the guidelines of the Helsinki Declaration. Throughout the experiment, the rats were kept in standard cages and fed a standard diet, with access to drinking water *ad libitum*. The 32 rats were divided into four graft groups: HA, DPB, HALG, and CAP.

Rigid dome-shaped titanium barriers with a hole in the top were constructed, and the top was covered with a Teflon™ cap to produce a hermetic seal. Prior to the surgical procedures, all the titanium barriers were cleaned and sterilized. All the surgical procedures were performed under sterile conditions. General anesthesia was induced with 10 mg/kg of xylazine and 40 mg/kg of ketamine. After the induction of general anesthesia and before surgery, the skull skin was shaved, and the skin was washed with povidone iodine. A skin incision was made over the linea media on the skull. To reach the skull bones, the flap and periosteum were lifted using a periosteal elevator. Nine holes were then created using a standard steel burr of 1 mm diameter, with water irrigation to prevent burning of the bone. After this procedure, an implant cavity 2 mm long × 2 mm wide was created using a steel burr. Then, 4 mm long × 2 mm wide titanium implants with a machined surface were placed in the center of the grafted area. The titanium barriers were placed around the implants and holes. The edges of the titanium barriers were fixed to the skull bone tissue using the adhesive N-butyl-2-cyanoacrylate.

After these procedures, the grafts were embedded in the holes in the titanium barriers, and the holes were then covered with Teflon™ caps. The skull skin and soft tissue were sutured using resorbable sutures. An antibiotic and analgesic were injected intramuscularly in all animals once a day for the first three postoperative days. After 3 months, all the rats were sacrificed by carbon dioxide inhalation. Following sacrifice, the titanium barriers were removed, and the calvarial bones containing the implants were harvested for histomorphometric and immunohistochemical analyses.

The original grafted bone tissue was used for histomorphometric and immunohistochemical analyses. The bone tissue samples were fixed in 10% formaldehyde solution for 72 h and demineralized in 10% formic acid solution. The implants were then gently removed from the samples. After decalcification, bone tissue samples were dehydrated, embedded in a paraffin wax block, and sectioned for hematoxylin and eosin (HE) and Masson's trichrome (MT) staining and microscopic analyses.

Sections 6 µm thick corresponding to the bone augmentation area were evaluated via light microscopy. New bone formation was determined by calculating the amount of regenerated new bone area as a percentage of the total grafted area in the peri-implant bone tissues using an image analysis program. All images of histological samples were taken with a digital camera attached to a light microscope, and the images were transferred to a computer at the original magnification.[14] An Olympus DP71 (Tokyo, Japan) software imaging system was used for histomorphometric analysis.

The bone specimens were fixed by perfusion, decalcified, and embedded in paraffin as previously described. The sections were incubated for 10 min in an oven at 60°C, and then cut into 4 µm longitudinal sections. The sections were then transferred to an automatic staining machine for VEGF immunohistochemical staining. After the primary antibody procedure, the sections were washed with water and stored in ultramount.

In the immunohistochemical analysis, the staining ratio (%) of VEGF in regenerated new bone areas was calculated using an image analysis program.[14] All the images of histological samples were taken with a digital camera attached to a light microscope and transferred to a computer at the original magnification. An Olympus DP71 software imaging system was used for immunohistochemical analysis.

Statistical analysis

SPSS 22 software was used for statistical analysis. The data were analyzed using one-way ANOVA and Tukey's HSD tests. A value of $p < 0.05$ was accepted as denoting a statistically significant difference.

Results

No fatal or nonfatal complications (such as wound infection) were encountered during the experiment.

New bone formation ratios were 45.38 ± 4.24%, 43.63 ± 6.30%, 45.25 ± 6.71% and 42.75 ± 5.7% in the HA, DPB, HALG and CAP groups, respectively. There was no statistically significant difference between the four groups (one way ANOVA, $p > 0.05$) (Table, Figures 1 and 2).

VEGF immunohistochemical staining ratios were 40.75 ± 3.96, 41 ± 3.46%, 36.75 ± 4.27% and 38.38 ± 4.21 % in the HA, DPB, HALG and CAP groups, respectively, and no statistically significant difference was found between the four groups (one way ANOVA, $p > 0.05$) (Table 1, Figures 2 and 3).

No evidence of inflammatory activity was detected microscopically.

Discussion

The bone formation capacity of bone graft materials differed widely, and bone regeneration capacity influenced the integration of implanted bone grafts.[8,9,10] Although much progress has been made in recent years in oral implantology, autogenous bone grafts remain the gold standard in GBR procedures.[8,10,11] They have a major advantage in that they supply not only bone volume but also osteogenic cells, which are capable of quickly laying down new bone. However, they also have various drawbacks, including increased patient morbidity, limited bone graft availability, and additional surgical time/costs.[8,9,10,11] Thus, studies aimed at identifying substitutes have been conducted.

Previous studies of an experimental animal bone defect grafting model reported that 3 months was a sufficient time to induce healing and the emergence of angiogenesis and new bone formation.[9,10,15] In the present study, we observed marked histological changes in the grafted bone defects 3 months after the grafting procedure in all four groups.

Table. New bone formation (NBF) and vascular endothelial growth factor (VEGF) percentage of the groups (one way ANOVA, $p > 0.05$).

Parameters	Groups	n	Mean(%)	Standart deviation	p
NBF	HA	8	45.38	4.24	0.764
	DPB	8	43.63	6.30	
	HALG	8	45.25	6.71	
	CAP	8	42.75	5.73	
VEGF	HA	8	40.75	3.96	0.127
	DPB	8	41	3.46	
	HALG	8	36.75	4.27	
	CAP	8	38.38	4.21	

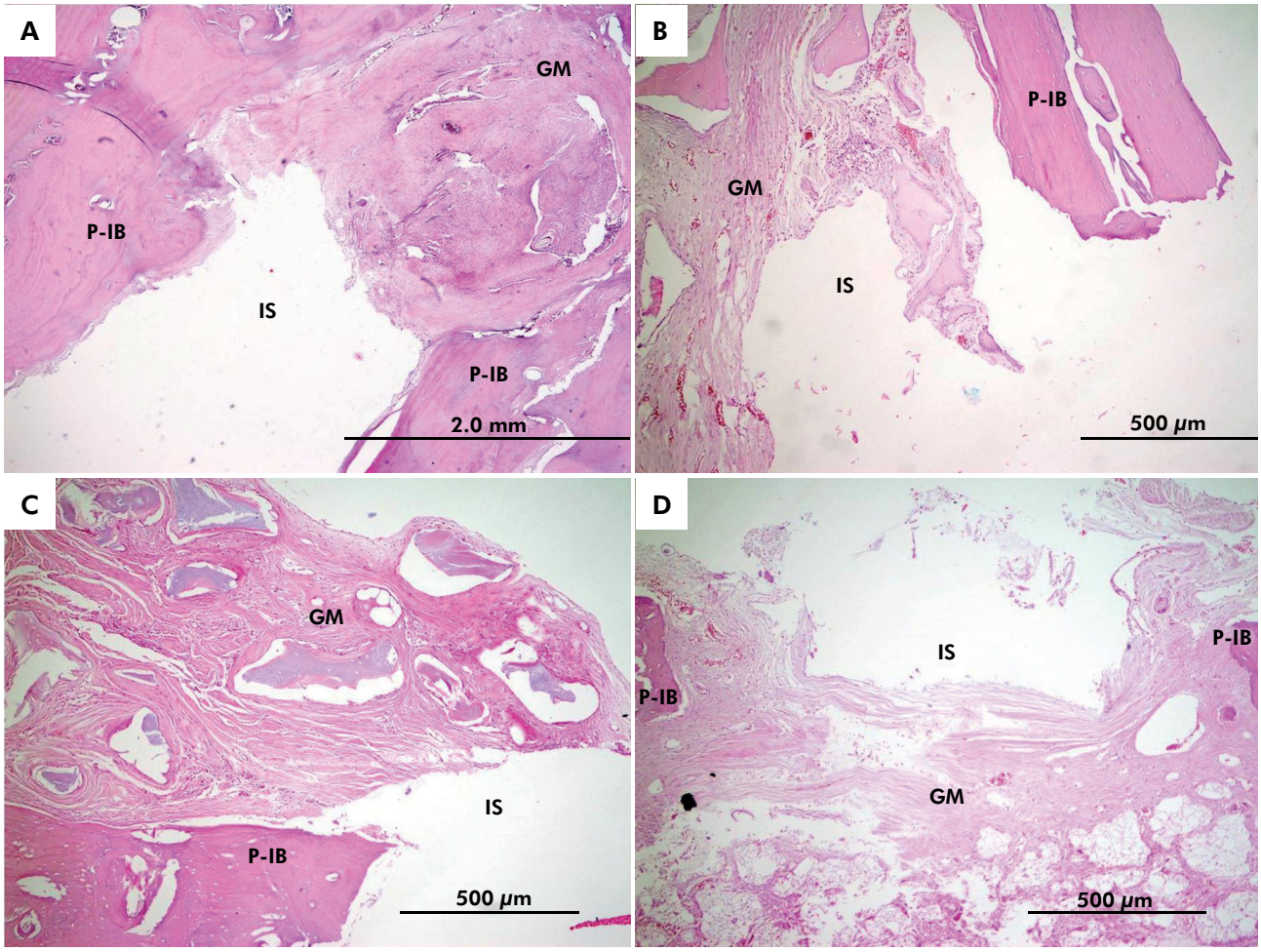

Figure 1. Histological images of a) calcium sulfate, b) deproteinized bovine, c) human-derived allogenic, and d) hydroxyapatite bone grafts. Hematoxylin and eosin staining.

The findings of the present study showed that bone formation beyond the skeletal system should occur in a similar way to that observed in previous studies. In an experimental animal study, Manfro et al.,[16] and Maréchal et al.[17] reported that new bone regeneration beyond the normal anatomic limits of a rabbit's skull bone occurred with autogenous blood application. Min et al.[7] demonstrated that new bone regeneration occurred after decortication of calvarial bone. In another experimental study, Ezirganli et al.[9] reported that new bone formation took place with different bone grafts and decortication of the calvarium.

The most common grafts used today are autografts, allografts, demineralized bone matrix, xenograft (bovine), and substitute bone grafts (calcium sulfate, calcium phosphate and HA).[17] To determine which graft is most appropriate for a given condition, an understanding of the biological function (osteogenesis, osteoinduction, and osteoconduction) of each graft is necessary. Furthermore, stable conditions in the host are essential for the incorporation of any graft material. Despite their drawbacks, autogenous bone autografts remain the gold standard to which every substitute must be compared.

The results of the present study were in accordance with those of previous studies of experimental applications of xenografts, human allografts, HA, and calcium sulfate grafts. There are a few reports in the literature on xenograft bone substitutes. Some studies showed good results in animal models and clinical research, whereas others demonstrated slower integration using xenograft bone substitutes compared with human allografts or lower bone union

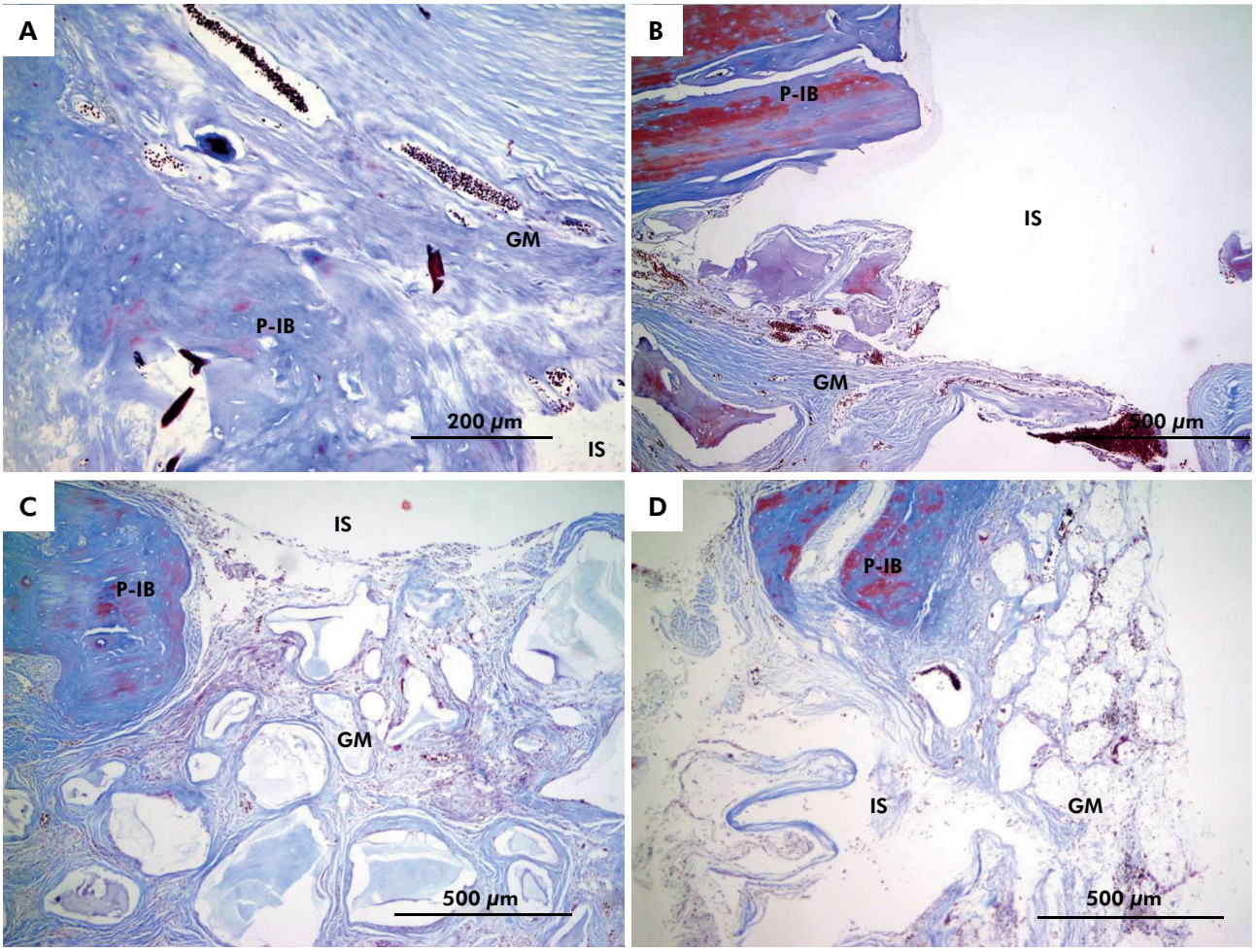

Figure 2. Histological images of a) calcium sulfate, b) deproteinized bovine, c) human-derived allogenic, and d) hydroxyapatite bone grafts. Masson's trichrome staining.

rates, with persistent radiolucent lines and local complications.[17,18,19] Calcium sulfate has been used many times as a bone void filler.[18,19] Recently, surgical grade calcium sulfate has been employed as a bone graft substitute.[17,18,19,20] Multicenter clinical studies demonstrated that trabecular bone filling in autografts was qualitatively similar to that seen in calcium sulfate grafts.[17,18,19,20,21] They also showed that surgical grade calcium sulfate was a host friendly and environmentally friendly biomaterial, which induced satisfactory bone production.[17,18,19,20] Researchers also demonstrated that the histological grade score for calcium sulfate was similar to that of other graft substitutes.[17,18,19,20,21]

Alloplastic bone graft materials should be biocompatible and not antigenic or trigger the inflammatory process.[20,22,23,24] A previous study revealed that HA-derived synthetic bone grafts stimulated new bone tissue formation and had high osteogenic potential.[21] HA-derived synthetic bone grafts, when compared with autogenous bone, were shown to encourage new bone formation in experimental animal studies, with excellent stability and new bone regenerative properties. Due to their content and structure, HA bone grafts dissolved slowly and were displaced gradually by bone tissue.[18]

Demineralized human bone allografts are thought to have osteoinductive capabilities and fast resorption, with bone ingrowth.[17] Demineralized freeze-dried bone allografts are extensively utilized in regenerative oral implantology, as they possess excellent osteoconductive potential.[25,26,27]

Angiogenesis is the most important pathophysiological process in bone repair and formation. VEGF has

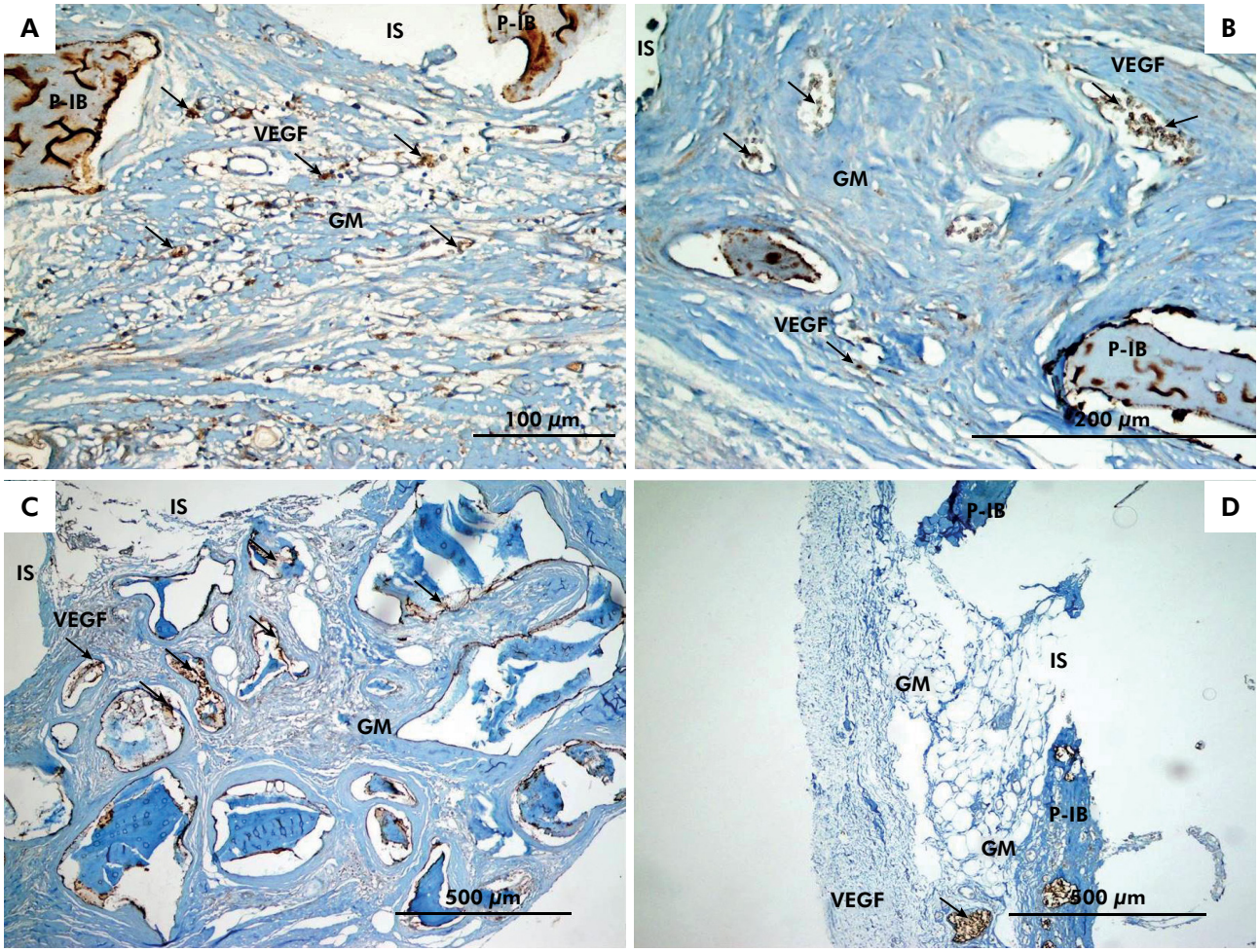

Figure 3. Immunohistochemical staining of VEGF in the a) calcium sulfate, b) deproteinized bovine, c) human-derived allogenic, and d) hydroxyapatite bone graft groups.

an important role in angiogenesis and induces angiopoiesis over vascular endothelial cells. VEGF, which is expressed by endothelial cells, is one of the most important cytokines in angiogenesis and is associated with bone formation, mesenchymal condensation, cartilage formation, cartilage resorption, and blood vessel invasion.[28] In immunohistochemical examination, VEGF expression was detected in all groups at similar levels with no significant difference between the groups.

In the present study, new bone tissue regeneration was evident in all the groups three months after implantation, with no statistically significant between-group differences. The histological findings indicated that all four graft materials (HA, DPB, HALG, and CAP) exhibited osteoconductive properties.

Conclusion

The present study compared the histological properties of several bone graft substitutes, which are widely utilized today. According to the results, none of the grafts showed superiority with respect to new bone formation. Although a number of studies in the field of oral implantology have examined the effects of different graft materials on peri-implant bone repair and regeneration, it is still unclear how these graft materials work or under which conditions they should be used.[3-5,13] Additional studies are needed to define the indications, specifications, limitations, and contraindications of GBR in the treatment of peri-implant bone defects.

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