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May Concentrated Growth Factor Affects Different Materials Osseointegration Potentials: An Experimental Sheep Study

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Abstract: This study aimed to assess whether peri-implant bone defects with zirconia (ZrO_2) and titanium (Ti) implants differ regarding osseointegration and bone regeneration following the application of concentrated growth factor (CGF). A total of three standardized box-shaped defects were created bilaterally in the iliac bones of six male sheep. Dental implants were placed in the center of each defect, and a control implant was placed in intact bone (Control group). After implant placement, three treatment modalities were randomly applied: i) CGF, ii) autogenous bone, and iii) no augmentation (Empty control group). ZrO_2 implants were placed on the right ilium and Ti implants on the left ilium of each animal. After an eight-week healing period, one central histological section from each site was prepared. Histomorphometric assessments were performed to evaluate new bone formation (NBF) percentages in the defect area and bone-implant contact (BIC) values. No statistically significant differences existed between BIC values for Ti and ZrO_2 implants in CGF, autogenous, and control groups. But in the empty control group, Ti implants had statistically higher BIC values than ZrO_2 ($p = 0.025$, $p < 0.05$). There were no statistically significant differences between NBF values for Ti and ZrO_2 implants in the CGF and autogenous groups. The empty control group's NBF values for both implant materials were significantly lower than other treatment modalities ($p = 0.002$, $p = 0.007$, $p < 0.005$). ZrO_2 and Ti implants have similar osseointegration capacity in grafted or intact bone areas, and the application of CGF to peri-implant bone defects positively affects bone regeneration.

Keywords: bone regeneration; dental implants; growth factors; zirconia.

1. Introduction

With the latest developments in implant dentistry and increased patient awareness, implant therapy has become an aesthetic treatment method. However, the main materials used for dental implants, titanium (Ti) and Ti alloys, can reflect from the gingiva, particularly at the anterior region where the buccal bone is thin. Since this situation is rather challenging to

compensate for, researchers have focused on finding new implant materials which are aesthetically compatible and an alternative to Ti in terms of osseointegration capacity [1, 2].

The clinical use of ceramic implants as an alternative to Ti is increasing in modern dental practice. Zirconium dioxide (zirconia [ZrO_2]) has made an impact as an implant material based on its biocompatibility and mechanical properties like fracture toughness and flexural strength [3-7].

The literature has revealed that ZrO_2 has an osseointegration capacity similar to Ti [8-11]. A recent systematic review analyzed 54 investigations with bone-implant contact (BIC) values, removal torque values, and push-in tests of ZrO_2 and Ti implants. The data concluded no significant differences between ZrO_2 and the 'gold standard' Ti [12,13]. In addition, some studies showed that ZrO_2 had significantly lower plaque affinity than Ti, reducing the risk of a peri-implant inflammatory tissue response [14].

Prosthetically correct implant placement is the key to long-term implant success [15]. Especially in immediate implantation cases, performing a hard tissue augmentation is often a clinical necessity. Autogenous bone grafts are still the gold standard based on their osteogenic capacity and osteoinductive and osteoconductive properties [16,17].

On the other hand, synthetic graft materials allow for obtaining the desired amount of graft along with simple storage conditions. But osteoinductive and osteoconductive properties are limited with these types of materials. Therefore, using these materials combined with autogenous platelet concentrations is an increasing trend in bone regeneration [17-19].

Platelet concentrations contain significant amounts of platelet-derived growth factor, transforming growth factor-beta (TGF-β), insulin-like growth factor, fibroblast growth factor, and bone morphogenetic proteins responsible for bone regeneration [21- 27]. These features of blood products enable the clinician to access graft material with the osteoinductive properties of products being investigated is concentrated growth factor (CGF), which has attracted considerable attention in tissue healing. Sacco introduced CGF in 2011 and showed that gelatin-like platelets could be obtained using a special centrifugation technique. The study revealed abundant vascular endothelial growth factor (VEGF), TGF-β1, and CD34 in CGF [28-31].

Although many studies in the literature compare ZrO_2 implants with Ti implants, clinical and preclinical studies comparing platelet concentrations with peri-implant defects are limited [4].

The present study aimed to assess whether CGF application to peri-implant bone defects differs between ZrO_2 and Ti implants regarding osseointegration. Moreover, ZrO_2 and Ti implants with autologous bone and CGF were compared regarding bone regeneration.

2. Materials and Methods

2.1. Animals.

A total of six-male (3-year-old) sheep weighing 50–70 kg were included in this trial. The study was approved by the Animal Research Ethics Committee of Istanbul University, Istanbul, Turkey (No: 2018/35). Animals were operated on and housed at the Department of Surgery at the Veterinary Hospital of Istanbul University.

2.2. Concentrated growth factor (CGF) preparation.

CGF was prepared by obtaining 9 mL of jugular venous blood from each animal. The blood samples were centrifuged for 12 min at 2400–2700 rpm (Medifuse Machine, Silfradent S.R.L., Italy), resulting in three separate layers. The top layer was acellular plasma, the middle was CGF, and the bottom consisted of red corpuscles separated by scissors from the CGF [32].

2.3. Surgical procedures.

The participating surgeons were experienced in dental implant placement and bone regeneration procedures.

All surgical procedures were performed under general anesthesia. Prior to general anesthesia, the animals were sedated with intramuscular xylazine (0.2 mg/kg; Rompun, Bayer, Switzerland) and intravenous ketamine hydrochloride (2 mg/kg; Ketalar, Pfizer, New York, NY, USA). Anesthesia was maintained during the entire operation with 2%–3% isoflurane (Aerrane, Eczacibasi-Baxter, Istanbul, Turkey) administered by inhalation. Animals were placed in the lateral rest position, after which the skin surface of the ilium was disinfected with povidone-iodine (Batikon, Istanbul, Turkey). After making skin and periosteal incisions, the pelvis was exposed, and the periosteum was dissected.

2.3.1. Defect preparation.

At the dorsal side of each animal's right and left ilium wing, three box-shaped defects (5 x 5 x 10 mm) were surgically prepared with a diamond disc (Frios MicroSaw, Dentsply, Charlotte, NC, USA) attached to a handpiece A section of intact bone (5 mm) was left between the defects. After the preparation of defects, one implant was placed in each defect, and a control implant was placed without preparing a defect. Thus, three of four implants were placed in defects, and one was placed in a defect-free area.

After implant placement, two of three bony defects were randomly filled with autogenous bone particles or CGF, and one defect was left empty. ZrO_2 implants were placed in each animal's right ilium and Ti implants in the left ilium. A total of 24 zirconium 4.0 mm x 8.0 mm (CERALOG®, HEXAGLOBE) and 24 Ti 3.8 mm x 9 mm (Camlog, SCREW-LINE) implants were placed (Figure 1). Flap closure was completed with No. 0 poliglecaprone suture (Monocryl, Ethicon, Istanbul, Turkey), and the skin was closed with No. 1 polypropylene suture (Medilen, Medeks, Istanbul, Turkey).

Ceftriaxone (Novasef 500 mg, 20 mg/kg IM, Zentiva, Istanbul, Turkey) was used for infection control, and meloxicam (Melox 0.1 mg/kg IM Nobel, Istanbul, Turkey) was administered for pain management preoperatively and postoperatively for five days.

2.3.2. Sacrification.

Eight weeks after surgery, following euthanasia performed according to the principles of the Ethical Committee, the pelvic bone was dissected, and specimens were retrieved.

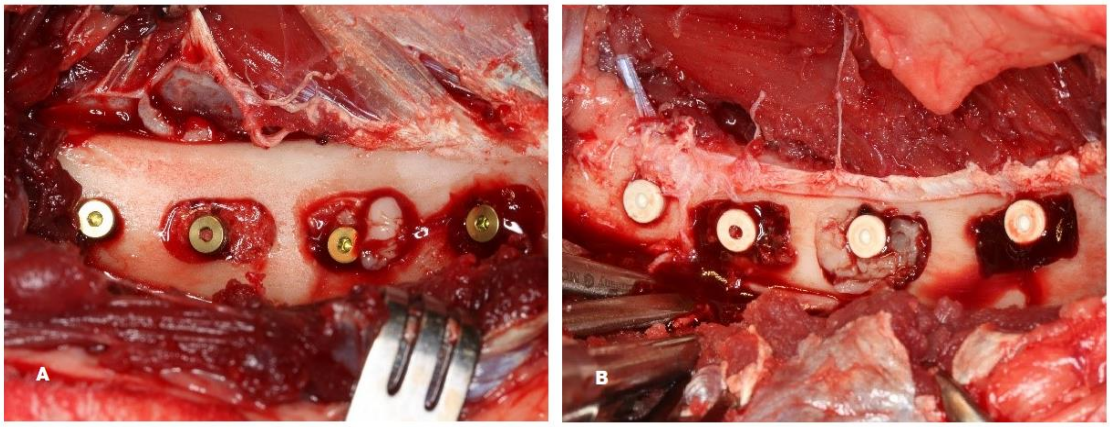

Figure 1. Surgically prepared peri-implant defects augmented with control implant, autogenous bone particles, CGF, and empty control **(A)** Titanium implants; **(B)** Zirconia implants).

2.4. Histological preparation.

The specimens were fixed in 10% buffered formalin for two days, then dehydrated in ethanol solutions of increasing concentrations (60%, 80%, 96%, and 100%) and subsequently embedded in methyl methacrylate resin (Technovit 7200 VLC; Heraeus Kulzer GmbH & Co. KG, Wehrheim, Germany).

A central section of each implant prepared using a special slicing system (Exact 300 CL; Exakt Apparatebau, Norderstedt, Germany) was cut into 300-µm-thick sections that were thinned to 40 µms and stained with toluidine blue.

2.5. Histomorphometric analysis.

All histomorphometric analyses were performed by two blinded examiners, and the mean measurement values were recorded as final.

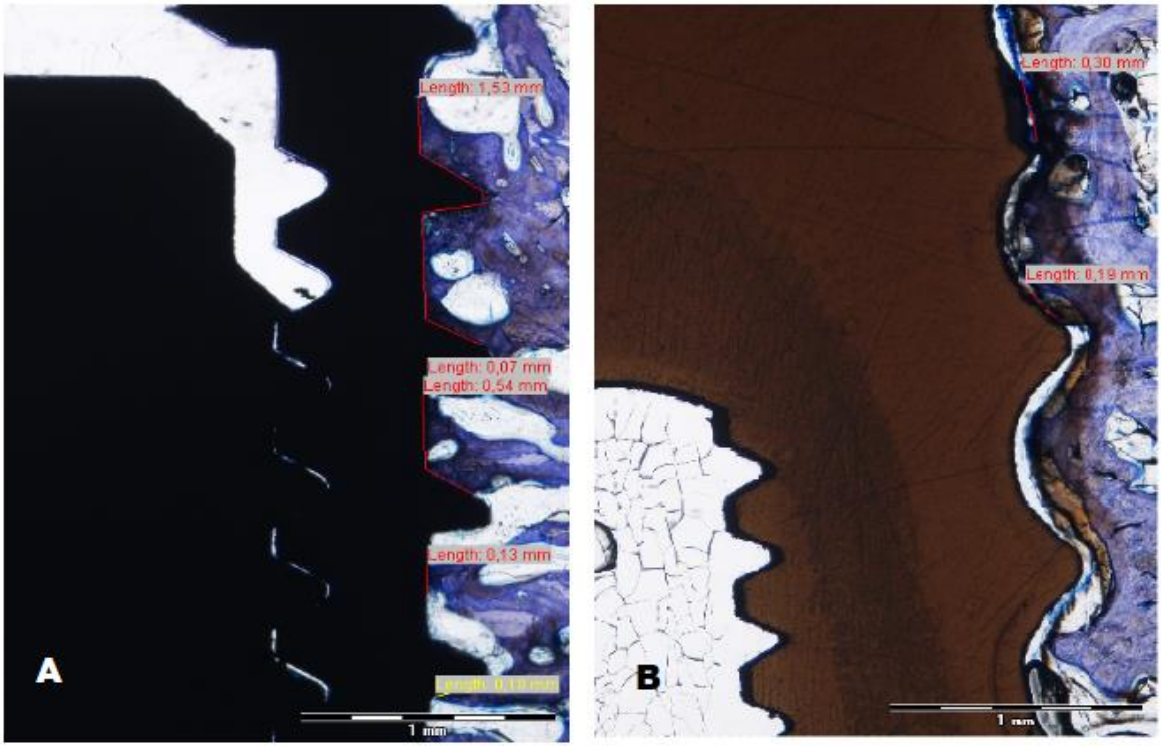

Figure 2. Histomorphometric analysis of bone-implant contact (BIC) **(A)** Titanium, **(B)** Zirconia) (Original magnification 40x; stained with toluidine blue).

A stereomicroscope (Olympus BX60, Tokyo, Japan) connected to a color video camera (Olympus DP 25, Olympus Optical Co. Ltd. Tokyo, Japan) was used for image capturing. All measurements were taken with Olympus image analysis software (Olympus Soft Imaging Solutions GmbH, Münster, Germany) for histomorphometric analysis.

The whole surfaces of all the implants were captured in four contiguous microscopic fields. The BIC values were determined by measuring the length of the attached bone structure to the implant surface divided by the whole surface perimeter at 100X magnification (Figure 2). The new bone formation (NBF) and soft tissue areas were measured separately at the bone-implant interface (Figure 3).

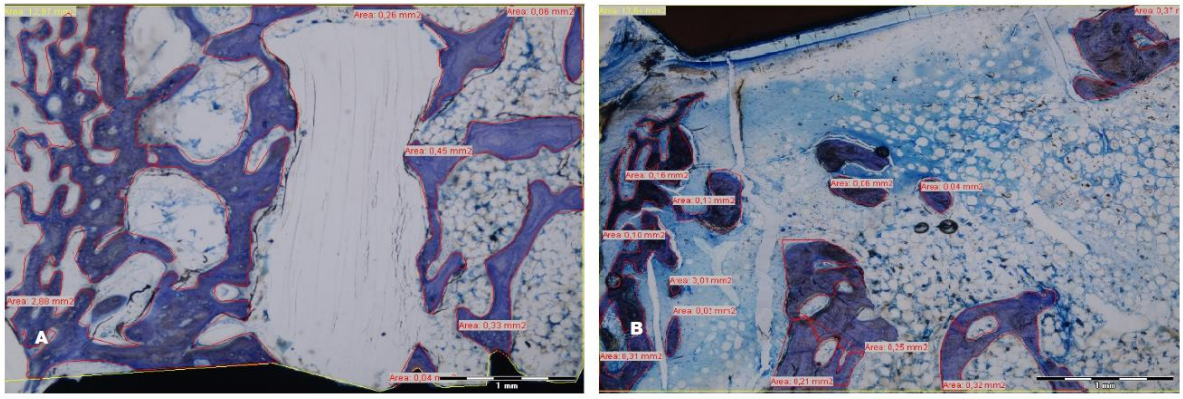

Figure 3. Histomorphometric analysis of new bone formation (NBF) at peri-implant defects **(A)** Titanium, **(B)** Zirconia) (original magnification 40x; stained with toluidine blue).

2.6. Statistical analysis.

Descriptive statistical data consisting of the mean, standard deviation (SD), median, interquartile range (IQR), range (minimum-maximum), and 95% confidence interval (CI) were calculated for NBF and BIC parameters using the Number Cruncher Statistical System (NCSS, Kaysville, UT, USA). A Kruskal–Wallis test was used to determine any significant differences between the experimental groups. Spearman correlation analysis was used to evaluate the relationship between NBF and BIC quantitative variables. Any P-value below 0.05 was accepted as statistically significant.

3. Results and Discussion

No animals presented signs of postoperative infection. Healing was uneventful during the osseointegration period, and no complications or adverse events occurred.

3.1. Histological observations.

There were no signs of inflammation or foreign body reaction in all implant sections, and osseointegration occurred. An active osteoid deposition was noticeable around zirconia and titanium implants. However, some gaps were appointed in zirconia in the CGF group. Primary osteons were more prominent in titanium implants, while bone healing seemed delayed in the zirconia implants.

Active remodeling of the osseointegrated bone interface was observable around both implant materials. An apparent demarcation line was visible between the host and the new bone. Compared to the autografts, an organized bone matrix containing primary osteon was found around the titanium implants, and lamellar bone deposition was observed.

3.2. Histomorphometric findings.

3.2.1. Bone-implant contact values.

BIC measured (Figure 4) 41.1 ± 15.2% for ZrO_2 + CGF, 53.1 ± 9.6% for Ti + CGF, 44 ± 15.1% for ZrO_2 + autogenous bone particles, 59.4 ± 22.5% for Ti + autogenous bone particles, 26.1 ± 9.8% for ZrO_2 empty control, 47.4 ± 13.4% for Ti empty control, 48.4 ± 16.9% for ZrO_2 control, 56.7 ± 23.4% for Ti control.

In the empty control group, BIC% values of Ti implants were statistically higher than ZrO_2 implants (P = 0.025). The differences in BIC% between the treatment modalities of both implant materials did not reach statistical significance (P > 0.05).

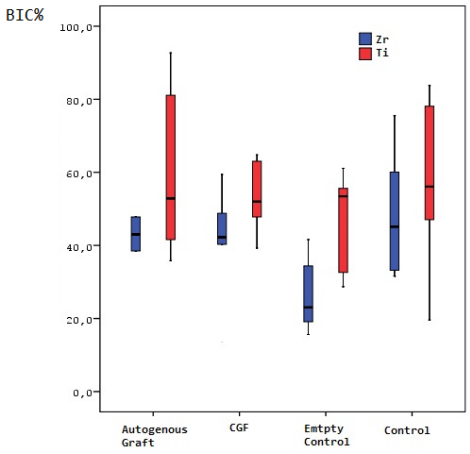

Figure 4. Box-whisker plot showing median, quartile, and outlier values for the bone-implant contact percentages (BIC%).

3.2.2. New bone formation values.

NBF values measured (Figure 5, Table 1) 30.5 ± 8.1% for ZrO_2 + CGF, 49.2 ± 19.2% for Ti + CGF, 54.1 ± 23.1% for ZrO_2 + autogenous bone particles, 53.2 ± 19.6% for Ti + autogenous bone particles, 17.7 ± 10.5% for ZrO_2 empty control, 21.9 ± 9.3% for Ti empty control, 63.3 ± 18.3% for ZrO_2 control, 67.4 ± 19.3% for Ti control.

The empty control group's NBF values for both implant materials were significantly lower than the other treatment modalities (P = 0.002, P = 0.007, P < 0.005).

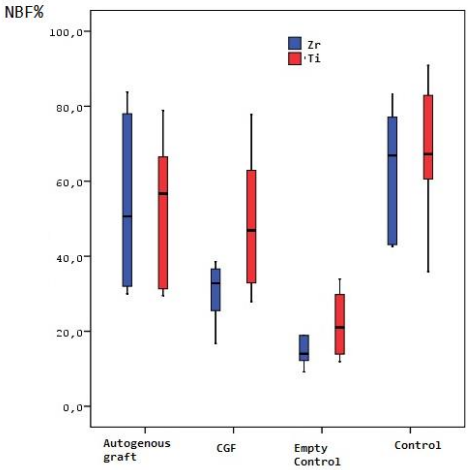

Figure 5. Box-whisker plot showing median, quartile, and outlier values for the new bone formation percentages (NBF%).

Table 1. Treatment modality.

Histomorphometrical analysis	Treatment Modality								Values	
	1. ZrO_2 +CGF (n = 6)	2. Ti+CGF (n = 6)	3. ZrO_2+Auto (n = 6)	4. Ti+Auto (n = 6)	5. ZrO_2 (No Augm) Empty Control (n = 6)	6. Ti (No Augm) Empty Control (n = 6)	7. ZrO_2 (No Defect) Control (n = 6)	8. Ti (No Defect) Control (n = 6)	P_2	
Parameter	Mean ± SD (Q1, median, Q3)								p ZrO2	p Ti
NBF%	30.5 ± 8.1 (23.3, 32.8, 37.1)	49.2 ± 19.2 (31.6, 46.9, 66.6)	54.1 ± 23.1 (31.5, 50.6, 79.4)	53.2 ± 19.6 (30.8, 56.7, 69.6)	17.7 ± 10.5 (11.4, 14.0, 23.7)	21.9 ± 9.3 (13.4, 21.0, 30.8)	63.3 ± 18.3 (42.9, 66.9, 78.75)	67.4 ± 19.3 (54.4, 67.2, 84.9)		
P_1	*0.078*		*0.873*		*0.522*		*0.748*		***0.002 ** **	***0.007 ** **
BIC%	41.1 ± 15.2 (33.6, 42.2, 51.4)	53.1 ± 9.6 (45.6, 52.0, 63.4)	44 ± 15.1 (34.6, 43.0, 53.1)	59.4 ± 22.5 (40.1, 52.8, 84.0)	26.1 ± 9.8 (18.2, 23.0, 36.2)	47.4 ± 13.4 (31.6, 53.4, 56.9)	48.4 ± 16.9 (32.8, 45.1, 63.9)	56.7 ± 23.4 (40.1, 56.1, 79.5)		
P_1	*0.150*		*0.240*		***0.025****		*0.337*		*0.107*	*0.914*

NBF%, new bone formation percentage; BIC%, bone-implant contact percentage; ZrO_2, zirconia; Ti, titanium; CGF, concentrated growth factor; Auto, autogenous bone particles; No Augm, no augmentation performed; P_1, Mann-Whitney U Test; P_2, Kruskal–Wallis test; SD, standard deviation; Q1, first quartile; Q3, third quartile
*Statistically significant

3.3. Discussion.

Tooth loss is a common issue in modern society. Increased patient awareness has led clinicians to use various implant materials to treat this condition. The use of ZrO_2 implants, which are more aesthetically successful, has become popular, rather than long hard tissue augmentations, especially in losses in the anterior region. Furthermore, autogenous blood products are frequently used to reconstruct defects in the alveolar bone. CGF, the latest generation autogenous blood product, is used in clinical applications due to properties such as tensile strength and high viscosity.

This study compared CGF or autogenous bone particle application to peri-implant bone defects in ZrO_2 and Ti implants for osseointegration and bone regeneration.

Several histological studies did not find significant differences in BIC values between ZrO_2 and Ti implants [4, 7, 10]. A recent systematic review of 54 animal studies evaluating the insertion torque, reversing torque, and BIC values of ZrO_2, ZrO_2 composite, and Ti implants found no significant difference between the materials [7]. The present study's findings regarding the osseointegration capacity of ZrO_2 and Ti are similar to previous investigations in the literature.

In terms of long-term implant health and aesthetics, marginal bone loss of the Ti implants and bacterial adhesion to these rough surfaces are clinical issues challenging to compensate for. The tooth-like color of ceramic implants is advantageous, especially when the implant body reflects from the soft tissues within thin gingival biotype cases [10]. There are also studies comparing ZrO_2 and Ti in terms of bacterial adhesion. In an in vitro study, Ti and ZrO_2 discs (with a polished or rough surface) were subjected to 72-hour incubation with a

combination of triple microorganisms, *Streptococcus sanguinis*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* bacteria prepared from human plaque biofilm. Human plaque biofilm thickness was significantly lower in ZrO_2 discs than in Ti discs. The investigators concluded that ZrO_2 could reduce the peri-implant inflammatory tissue response regarding long-term implant health [9]. In our study, both implant materials are unaffected by any bacterial infections during healing.

The BIC values of both implant materials in the control group were in accordance with most of the studies in the literature. In an earlier investigation, 96 implants, including polished surface ZrO_2, modified-surface ZrO_2, and oxidized Ti, were placed in rabbit tibias. After a six-week osseointegration period, measurements were made, including reverse torque and BIC values. No significant difference was found between Ti oxide and modified-surface ZrO_2 implants; however, it was observed that both types of implants had significantly higher values than the polished surface ZrO_2 implants. Thus, the authors concluded that the modified-surface ZrO_2 implants have similar osseointegration capacity as oxidized surface implants [3]. In the present study, six of each implant material were placed in intact bone sites (Figure 1 and Figure 2) to compare the implant materials' performance with previous study results. Similarly, in the control groups of our study, both ZrO_2 and Ti implant BIC values did not differ significantly.

The study results confirmed that ZrO_2 and Ti implants have similar osseointegration capacity in grafted or intact bone areas in terms of BIC values. Therefore, according to the literature and the findings of the present study, it can be concluded that ZrO_2 is a reliable alternative to Ti.

The most expected outcome of this trial was that the sites augmented with autogenous bone particles attained very high BIC values. These values are quite close to the mean BIC values of the implants placed in the intact bone areas with both materials (ZrO_2 = 43, Ti = 52.85). These findings reveal that autogenous bone still provides the most predictable results in augmentation procedures. The fact that autogenous bone is so effective in NBF is attributed to its osteoinductive properties [33].

On the other hand, various biomaterials have been used in bone regeneration procedures to enhance bone metabolism and accelerate osseointegration. The use of platelet products [34] is an increasing trend in tissue engineering, and the third generation of blood products is one of these biomaterials. CGF is a fibrin-rich organic matrix containing growth factors, platelets, leukocytes, and CD34+ cells participating in regeneration [35]. Rodella et al., in 2011, concluded that CGF demonstrates higher tensile strength, a greater amount of growth factors, higher viscosity, and higher strength than platelet-rich fibrin (PRF) [32].

There are studies in the literature that have investigated the osteogenic properties of CGF. In an animal experiment, Kim et al., in 2014, formed defects 10 mm x 15 mm in the parietal bone of 12 rabbits. They applied platelet-rich plasma (PRP), PRF, and CGF to the defects, leaving one defect in the control group empty. They analyzed bone mineral density and new bone volume at 6 and 12 weeks of bone healing with microscopic computed tomography and histomorphometric sections. The PRF group showed the highest grayscale value in the sixth postoperative week. The difference between the control and PRF groups was significant in the sixth and twelfth postoperative weeks. In the sixth week, the CGF group showed the lowest grayscale value ($P < 0.05$). Based on these results, it was concluded that using PRP, PRF, and CGF facilitates NBF in the early stage of bone graft healing. After 12 postoperative weeks, there was no significant difference in osteogenesis between growth

factors [25]. Similarly, in our study application of CGF to peri-implant defects with both implant materials resulted in higher NBF values than empty defects.

In an animal study, H.C. Park et al. prepared four defects in the right femur of six dogs and placed Ti implants in each defect. The defects were filled with PRF, CGF, and synthetic bone grafts, and one was left empty as a control. Two weeks after the first surgery, the same procedure was performed on the left femur, and two weeks after the second surgery, euthanasia was performed. Histological sections were evaluated under a light microscope, and the BIC% was measured. In addition, an ELISA test was applied to the CGF and PRF preparations obtained during surgery and analyzed for TGF-β1 and VEGF. In the two-week healing period, the CGF and synthetic graft's BIC% were significantly higher than the other groups. The VEGF amounts of CGF were significantly higher than PRF. The authors concluded that in the brief period of peri-implant defect healing, CGF was similar to the synthetic graft materials [16].

Similarly, in our study, the sites augmented with CGF around both implant materials resulted in proximate values with autogenous bone in terms of NBF% values; however, only the defects augmented with CGF around Ti implants were significantly higher than the empty control.

In a recent study, Benic et al. investigated the efficiency of guided bone regeneration of peri-implant defects with ZrO_2 and Ti implants. They created four semi-saddle bone defects in the maxillary premolar region of seven dogs. They placed ZrO_2 implants in three of four defects with different GBR materials and one Ti implant in the last defect. After three months, histomorphometric assessments were performed. The authors concluded that ZrO_2 and Ti implants and peri-implant defects did not differentiate regarding NBF and implant osseointegration. In the same study, the authors found that the bone growth along the exposed buccal surface of the Ti implants reached more coronal levels than ZrO_2; however, it was not statistically significant [36].

These findings are in accordance with the results of the present trial. The lowest mean value of NBF was measured in the empty control group around ZrO_2. While the NBF value of CGF around Ti implants was statistically higher than the empty control ($P = 0.016$), the same comparison around ZrO_2 implants did not reach statistical significance ($P = 0.055$). It can be concluded that the osteogenic properties of Ti are higher than ZrO_2.

To our knowledge, the present study is the first trial comparing platelet concentration effects on NBF around different implant materials. In the literature, there are studies comparing different platelet products, including CGF, around Ti implants.

The important limitation of the present trial is the lack of different euthanasia periods. The level of osseointegration and amount of NBF can change at different time points. Nevertheless, the present study provides valuable data on the effect of CGF on peri-implant defects with ZrO_2 and Ti implants. Controlled clinical trials are needed to compare the findings of the present preclinical study.

4. Conclusions

Within the limits of this study, ZrO_2 and Ti implants have similar osseointegration capacity in grafted or intact bone areas, and the application of CGF to peri-implant bone defects has a positive effect on bone regeneration.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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