Bone Regeneration Using Adipose-Derived Stem Cells with Fibronectin in Dehiscence-Type Defects Associated with Dental Implants: An Experimental Study in a Dog Model

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Purpose: *To determine the bone regeneration potential of a ceramic biomaterial coated with fibronectin and adipose-derived stem cells covered in three-wall critical-size defects associated with dental implants.* ***Materials and Methods:*** *In a total of 18 dogs, four dehiscence-type and critical-size defects were created surgically in the edentulous alveolar ridge with the simultaneous placement of dental implants. Defects were randomly regenerated using biomaterials coated with particulate ß-tricalcium phosphate (ß-TCP), ß-TCP with fibronectin (Fn) (ß-TCP-Fn), and ß-TCP with a combination of Fn and autologous adipose-derived stem cells (ADSCs) (ß-TCP-Fn-ADSCs), leaving one defect as the control. The animals were divided into three groups according to the time of euthanasia (1, 2, or 3 months).* ***Results:*** *Statistically significant differences between the three study groups (ß-TCP, ß-TCP-Fn, ß-TCP-Fn-ADSCs) and the control group in the total area of bone regeneration and mineralized and nonmineralized tissue at 1, 2, and 3 months of healing were not observed. At 2 months, defects treated with ß-TCP-Fn-ADSCs showed a significant decrease in the percentage of bone-to-implant contact (BIC) as compared with the ß-TCP-Fn (P = .041) and control (P = .012) groups. At 3 months of healing, however, significant differences in BIC between the three study groups and controls were not found (P = .388).* ***Conclusion:*** *The use of ADSCs in the bone regeneration processes of dehiscence-type defects associated with simultaneous implant insertion does not seem to improve the area of bone regeneration or the percentage of BIC compared with other biomaterials or the control alveolar defect.* Int J Oral Maxillofac Implants 2017;32:e97–e106. doi: 10.11607/jomi.5169

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Traumatic tooth extraction, tooth infection, or alveolar bone resorption after tooth extraction cause morphologic defects in the edentulous alveolar ridge, which may compromise not only implant placement, but also esthetic rehabilitation results.[1,2] The vestibular cortical zone of the alveolar ridge is mostly affected by loss of teeth, and thus, regeneration procedures are frequently required before or at the time of the placement of dental implants.[3,4] Autografts are still the gold standard for this purpose, since they provide osteogenic cells, osteoinductive growth factors, and an osteoconductive scaffold, all essential for new bone growth and restitution ad integrum.[5–9] However, autogenous bone grafting carries the limitations of morbidity at the harvesting site and limited availability. Xenograft, allograft, and alloplast biomaterials have been developed as alternative options for enhancing good results for restoration of bone defects associated with implants.[10,11] Nevertheless, these bone substitutes lack osteoinductive capacity due to the absence of cellularity and growth factors, and become a simple space maintainer mechanism that may limit the ability to regenerate the bone defect.

Tissue engineering introduced in the last decade involves the morphogenesis of new tissue using biocompatible scaffolds and stem cells in combination with growth and differentiation factors. Adipose-derived stem cells (ADSCs) are an alternative source of bone marrow–derived mesenchymal stem cells for bioengineering, minimizing the inconveniences of bone marrow aspiration (lower yield and procedure-related morbidity).[12–15] Bone regeneration may be enhanced by other factors, such as fibronectin. Fibronectin is a glycoprotein of the extracellular matrix that in association with some biomaterials induces adhesion of stem cells or osteoblasts in bone regeneration procedures.[16–19] Also, fibronectin favors osseointegration of dental implants due to its properties of cell adherence, differentiation, and expansion.[17,20,21]

The selection of dogs for this research is due to the standardization of this type of experimental animal in the treatment study of dehiscence-type defects associated with implants, as evidenced by various publications[22–26] and a previous study by the present group of authors.[27] It is considered a critical defect with high prevalence in daily implant practice and easy to reproduce in the animal model for its study.

The objective was to assess bone regeneration potential in three-wall critical-size defects of vestibular cortical bone after tooth extraction and implant placement, using a ceramic biomaterial (β-tricalcium phosphate [β-TCP]) alone or coated with fibronectin or the combination of fibronectin and ADSCs compared with a control defect (without biomaterial filling). It was hypothesized that a higher total area of bone regeneration, new bone, and percentage of bone-to-implant contact (BIC) would be associated with the use of a particulate biomaterial coated with a combination of fibronectin and ADSCs (β-TCP-Fn-ADSCs) in regenerated alveolar defects.

MATERIALS AND METHODS

Material

Eighteen somatically homogenous female healthy Beagle dogs (mean age: 22.6 ± 6.4 months and mean weight: 12.3 ± 3.2 kg) were included in the study. The same sample was used to carry out two parallel studies simultaneously to reduce the number of dogs. Prior to inclusion, all animals completed a quarantine period. Each animal was identified with an ear tattoo and microchip implant and was kept in an individual cage and under control of environmental variables (temperature, ventilation, humidity, and light). Animals were divided into three study groups and sacrificed at 1 (T1), 2 (T2), and 3 (T3) months postoperatively. The study was approved by the Ethics Committee on Animal Research (CEEA 227-109) of the University of Barcelona.

Surgical Procedure

The surgical procedure had two phases: dental extraction (phase 1) and creation of critical bone defects and simultaneous implant insertion (phase 2).

In phase 1, the first, second, and third premolars and the first molar of both mandibular hemiarches were extracted under general anesthesia. Dogs were premedicated with acepromazine, 2.5 mg/10 kg subcutaneously (SC) (Pharmavet) and atropine sulphate, 0.05 mg/kg SC (John Martin Anesthesia was induced with sodium thiopental, 10 mg/kg intravenously (IV) (Pentovet, Richmond Vet Pharma) and maintained with inhaled 1.5% to 2% isoflurane (Sofloran, Pisa Agropecuaria) by endotracheal intubation. Local anesthetic infiltration, 1.8 mL per arch (articaine hydrochloride 4% and epinephrine 1:100,000, Ultracain, Normon) was also administered. Dental extractions were performed by odontosections to ensure preservation of the outer cortical bone, using round burs No. 6 tungsten carbide inserted in handpieces and under constant irrigation with sterile saline. The wound was sutured with 4-0 silk sutures on a curved needle (Aragó). The suture was removed 10 days later.

In phase 2, and after a healing period of 3 months, four cylindrical bone defects (7 × 7 × 7 mm) were prepared after elevation of a mucoperiosteal flap, using a trephine bur (7-mm outer diameter), causing complete destruction of the buccal cortical plate of the alveolar ridge. Four dental implants (Ø3.3 × 10 mm NobelSpeedy Groovy with TiUnite surface; Nobel Biocare) made of surgical-grade commercially pure titanium and with a moderately rough surface were inserted in each defect showing part of their buccal side exposed (approximately six implants). Surgically created critical bone defects were similar to those previously reported.[22–27] The position of the first premolar was assigned to the control group. The three remaining defects were filled at random (www.randomization.com) with *(1)* 0.25 to 1 mm of particulate β-TCP (KeraOs, Keramat), *(2)* particulate β-TCP coated with fibronectin (β-TCP-Fn), and *(3)* particulate β-TCP coated with a combination of fibronectin and ADSCs (β-TCP-Fn-ADSCs) (Figs 1a and 1b). Defects were then covered with 30 × 40-mm porcine collagen membrane (Bio-Gide, Laboratorios INIBSA), and the surgical field was closed by primary intent with 4-0 silk sutures (Aragó).

Postoperatively, animals were kept on a soft diet and treated with amoxicillin trihydrate (Clamoxyl, Pfizer), 15 mg/kg intramuscularly (IM) every 48 hours, starting 24 hours before surgery (five doses, total 10 days) and 0.2 mg/kg/day of meloxicam (Meloxicam Syntex,

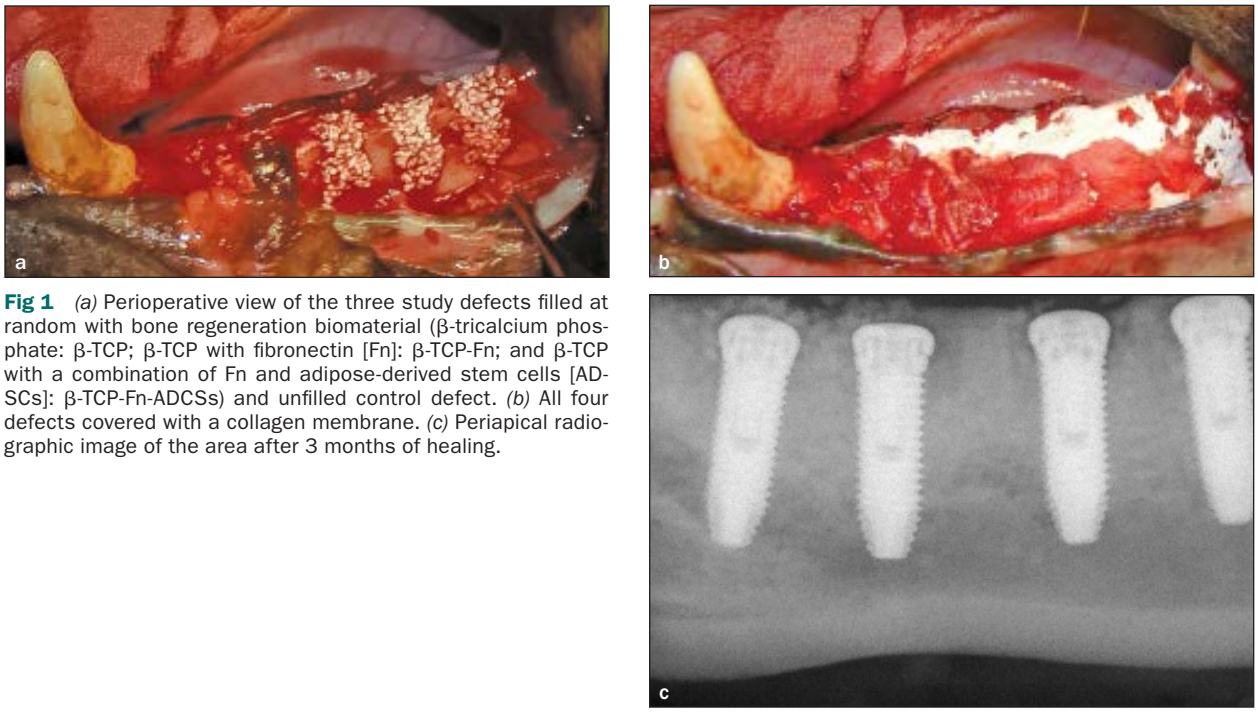

Fig 1 *(a)* Perioperative view of the three study defects filled at random with bone regeneration biomaterial (β-tricalcium phosphate: β-TCP; β-TCP with fibronectin [Fn]: β-TCP-Fn; and β-TCP with a combination of Fn and adipose-derived stem cells [ADSCs]: β-TCP-Fn-ADCSs) and unfilled control defect. *(b)* All four defects covered with a collagen membrane. *(c)* Periapical radiographic image of the area after 3 months of healing.

Syntex S.A.). Oral hygiene included daily brushing and irrigation with 0.2% aqueous solution of chlorhexidine. A periapical radiographic image of the area after 3 months of healing is shown in Fig 1c.

Animals were sacrificed by a lethal dose of thiopental at times T1, T2, and T3. Both hemimandibles were dissected and immersed in 40% formaldehyde solution in codified containers for histomorphometric analysis.

Bone Histomorphometry

All hemimandibles were fixed in 10% formaldehyde, and subsequently dried and cut into sections containing an implant with the surrounding tissue. Each fragment with the respective implant was referenced. Samples were dehydrated in graduated ethanol solutions progressing from 70% to 90% to 100% during 6 days at room temperature, rinsed in xylene, and dried in an incubator for 24 hours at 60°C. Individual samples were embedded on a low-temperature curing epoxy resin, section cut, grinded, and polished. A 50-µm-thick sample containing the implant and the surrounding material was then prepared with additional cutting, grinding, and polishing procedures, and stained with toluidine blue according to Schenk's protocol with minor modifications.[28,29] Images for individual samples were obtained through light transmission microscopy using a digital microphotography system (Nikon Kodak Ltd) and were processed with the Image_Pro Plus, Media Cybernetics image analysis system (Media Cybernetics).

A standardized study area was established for all samples, 4 mm axially from the margin of the implant neck and 2 mm radially to the vestibular cortical of the alveolar ridge (Fig 2). In each sample, the following measurements were taken: total area of bone regeneration (biomaterial and tissue surrounding the implant within the standardized study area), percentages of mineralized and nonmineralized tissue, percentage of ceramic particles within the surface area of bone regeneration, and percentage of bone-to-implant contact (BIC).

Canine Adipose-Derived Stem Cells

Canine adipose-derived stem cells (cADSCs) were obtained from abdominal subcutaneous adipose tissue following a modified method described by Zuk et al.[30] Samples (approximately 5 g of adipose tissue) were washed intensively with Dulbecco phosphate-buffered saline (H_2O, 0.1 mol/L NaCl, 2.6 mol/L KCl, 1.4 mol/L KH_2PO_4, 8 mmol/L Na_2HPO_4; pH 7.4), digested with type I collagenase (0.16 mg/mL, Sigma) at 37°C under shacking for 35 minutes, and centrifuged at 1,200 g for 10 minutes to separate the stromal cell fraction. The pellets were treated with red cell lysing buffer (KO 2HPO4 5.7 mmol/L, NH4CL 155 mmol/L, and ethylenediaminetetraacetic acid [EDTA] 0.1 mmol/L at pH 7.23) for 10 minutes at room temperature and centrifuged at 750 g for 10 minutes. The final pellet was resuspended in cADSC proliferative medium (PM) consisting of Dulbecco's modified Eagle's medium (Lonza) supplemented with 10% fetal bovine serum (FBS) (Lab

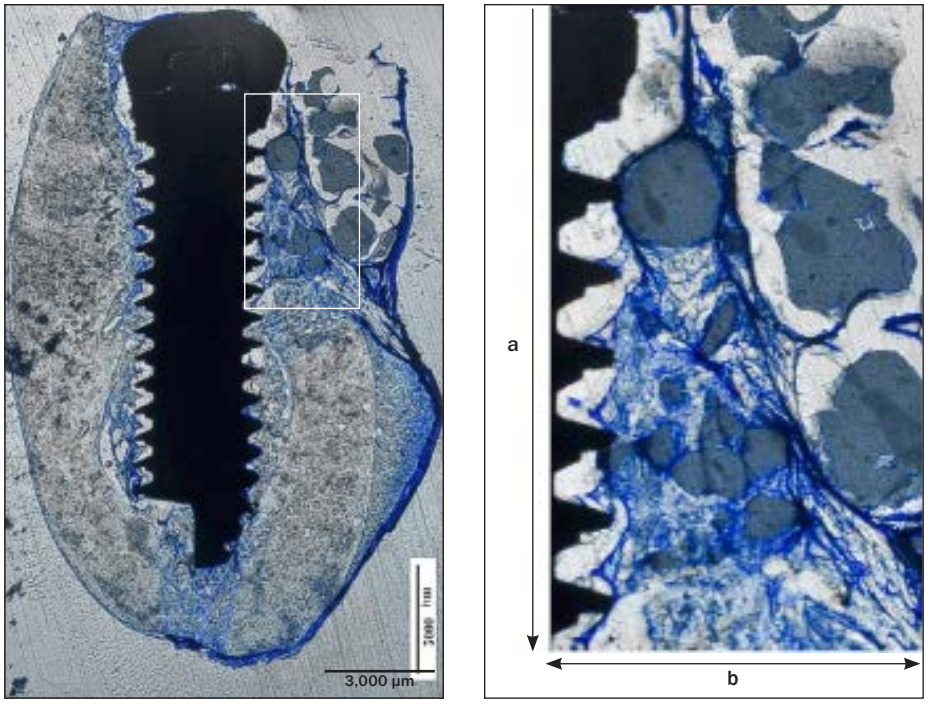

Fig 2 Standardized study area for all samples *(a)* 4 mm axially from the margin of the implant neck and *(b)* 2 mm radially to the vestibular cortical of the alveolar ridge.

Clinic), 2 mmol/L L-glutamine (Lonza), 10 mmol/L Hepes (Lonza), and antibiotics (Lonza). The cell suspension was filtered through a 100-µm mesh (Falcon). Finally, a portion of the cADSC cells were cryopreserved in cryopreservation medium (90% FBS-10% dimethyl sulfoxide [DMSO]), frozen at –80°C in an isopropanol-jacketed closed container, and stored in liquid nitrogen the next day. The other portion of the cells were resuspended in PM, plated at 1×10^5 cells/cm^2 in a T75 flask (Nunc), and incubated at 37°C in 5% CO_2. After 24 hours, samples were washed with DPBS to eliminate nonadhesive cells and kept in a PM. To obtain a large number of cells, cADSCs were further expanded on polystyrene culture flask with PM at a density of 7,000 cells/cm^2, and the medium changed three times a week. After one passage, when 80% confluence was achieved, cells were harvested with trypsin-EDTA (Sigma) and used for the characterization experiments.

Coating of β-TCP with Fibronectin (Fn) and ADSCs

One week before the surgery, the cADSCs were thawed and plated at 1×10^5 cells/cm^2 in a T75 flask in PM medium and incubated at 37°C in 5% CO_2. After 24 hours, the samples were washed with DPBS to eliminate nonadhesive cells and kept in a PM. The cells were maintained with PM medium, which was changed three times a week. Twenty-four hours before the surgery, 500 µL of fibronectin (BD Biosciences) solution (10 µg/mL in DMEM 1 g/L) was added per gram of TCP (KeraOs, Keramat) and incubated at 37°C for 24 hours. Finally, the coating solution was eliminated, and the grafts were washed with DPBS. On the day of surgery, cADSCs were harvested with trypsin-EDTA (Sigma) and seeded on β-TCP bone graft with or without fibronectin coating (5×10^5 cells/1 g β-TCP bone graft). To promote the adhesion, the cells seeded on the bone graft were maintained at 37°C and 5% CO_2 for 2 hours.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS; SPSS Inc), version 15.0 for Windows, was used for the analysis of data. Bone histomorphometric variables are expressed as mean and standard deviation (SD). Normality of data was confirmed by the Kolmogorov-Smirnov test. Differences of histomorphometric variables between the control group and the three study groups (β-TCP, β-TCP-Fn, and β-TCP-Fn-ADSCs) at T1, T2, and T3 were assessed with analysis of variance (ANOVA) and post hoc Bonferroni's correction when statistical significance was found. Differences between data at T1, T2, and T3 were analyzed with the paired Student *t* test. Statistical significance was set at $P < .05$.

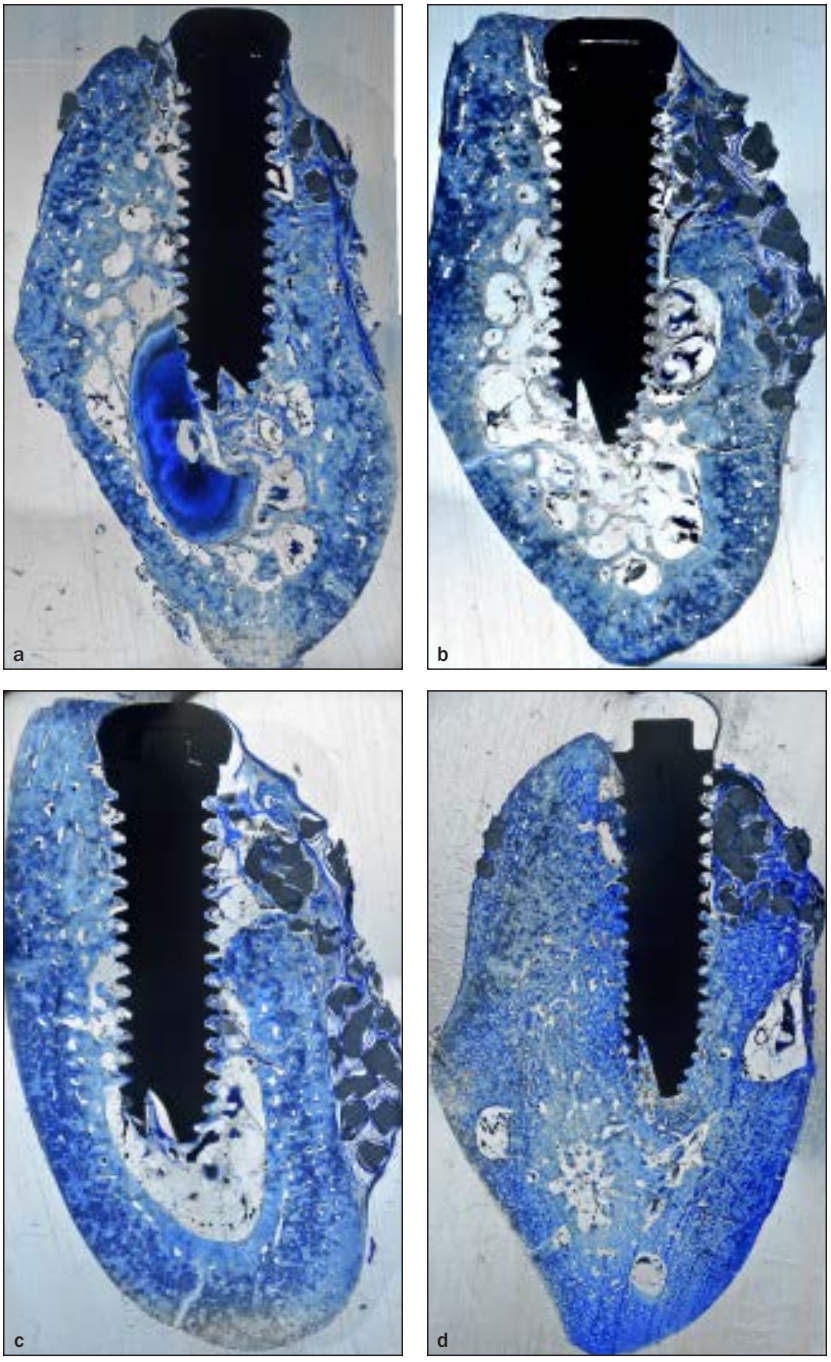

Fig 3 Histologic images of the defects at 3 months of healing treated with the Image_Pro Plus, Media Cybernetics image analysis system (Media Cybernetics). Samples were stained with toluidine blue based on Schenk's protocol with minor modifications. *(a)* Control; *(b)* β-tricalcium phosphate: β-TCP; *(c)* β-TCP with fibronectin: β-TCP-Fn; and *(d)* β-TCP with a combination of fibronectin and adipose-derived stem cells (ADSCs): β-TCP-Fn-ADCSs. Note contamination with particulate biomaterial of the control defect from the adjacent defect.

RESULTS

Of the 18 animals included in the study, two were excluded at the time of histomorphometric analysis because of dehiscence of the operated area with important loss of regeneration material. Small wound dehiscence occurred in another two animals, which were sutured immediately, minimizing the loss of biomaterial. Therefore, results of this experimental study are based on histomorphometric data from 16 Beagle dogs. Four histologic samples for each dog (control, β-TCP, β-TCP-Fn, and β-TCP-Fn-ADSCs) with a total of 64 samples were analyzed (Fig 3). Samples were grouped according to the euthanasia time (1 month, T1 [5 dogs, 20 implants]; 2 months, T2 [5 dogs, 20 implants]; 3 months, T3 [6 dogs, 24 implants]).

Particulate Biomaterial, Bone Regenerated Area, and Mineralized and Nonmineralized Tissue

The amount of particulate biomaterial in each of the critical-size defects (β-TCP, β-TCP-Fn, and β-TCP-Fn-ADSCs) did not show significant changes throughout

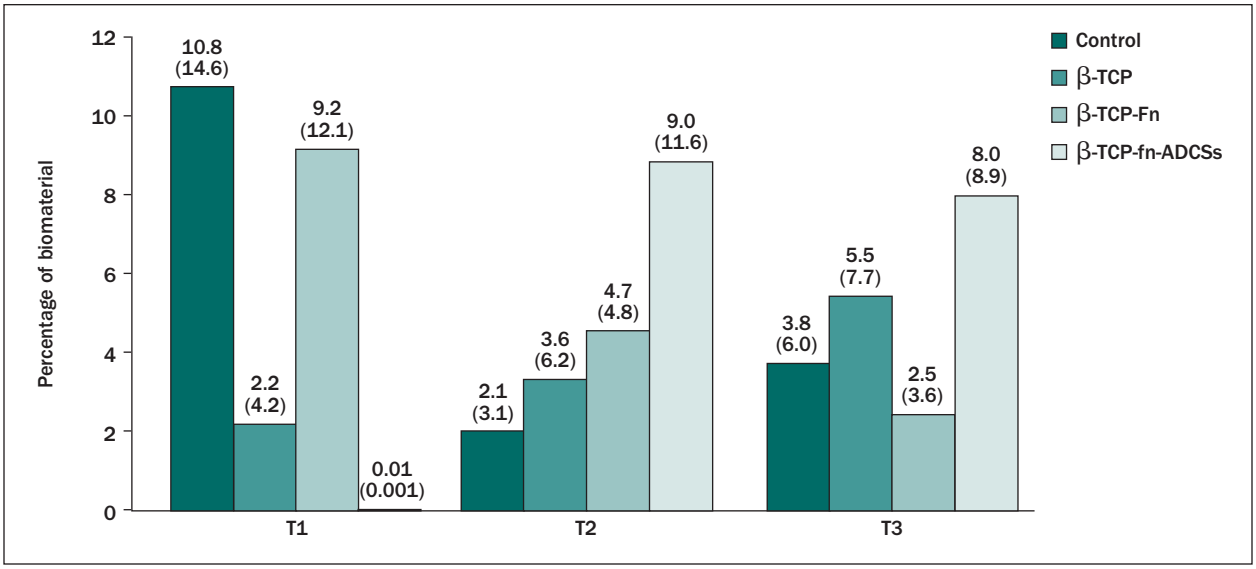

Fig 4 Percentage of biomaterial present in the study defects: β-tricalcium phosphate (β-TCP), β-TCP with fibronectin (β-TCP-Fn), and β-TCP with a combination of fibronectin and adipose-derived stem cells (ADSCs) (β-TCP-Fn-ADCSs). Control defects showed contamination by particles from the adjacent defects.

the study period ($P > .05$), remaining uniform in the three groups at each healing time (T1, $P = .166$; T2, $P = .557$; T3, $P = .419$). Control defects showed a mean contamination of the study area by particulate biomaterial of 5.6%, without significant differences in the three healing times ($P > .05$). Figure 4 shows the percentage of biomaterial present in the study defects and in the control defects, which were contaminated with particles from the neighboring defects.

The mean bone regeneration area and the amount of mineralized and nonmineralized tissue in each of the study groups are shown in Table 1. Significant differences at each healing time between the three study groups were not found. The nonmineralized area at T2 was significantly lower compared with T1 ($P = .033$) and T3 ($P = .012$) only in the control group.

Neoformed Bone in Contact with the Implant Surface

The percentage of BIC in the study groups at the three healing times is shown in Table 1. Defects treated with β-TCP-Fn-ADSCs at T2 showed a statistically significantly lower mean ± SD percentage of BIC compared with the control group (20.18 ± 9.21 vs 47.67 ± 17.23, $P = .012$) and the β-TCP group coated with fibronectin (β-TCP-Fn) (20.18 ± 9.21 vs 43.38 ± 12.89, $P = .041$). Also, differences in the percentage of BIC according to the healing times were statistically significant only in the control group and the β-TCP-Fn-ADSCs, although patterns were different. Whereas in the controls, the percentages of BIC were higher at T2 ($P = .017$) and at T3 ($P = .016$) compared with T1, in the β-TCP-Fn-ADSCs group, there was only an increase in the percentage of BIC at T3 compared with T2 (39.92 ± 16.51 vs 20.18 ± 9.21, $P = .042$). Also, this was the only group in which there was a decrease in the percentage of BIC at T2 compared with T1. In this group, the percentage of BIC at T3 was the highest of all study groups, although significant differences were not encountered.

DISCUSSION

The differentiation ability of mesenchymal stem cells into osteoblasts allows taking advantage of the osteoinduction and osteoconduction properties of autologous bone grafting for bone regeneration procedures.[31–33] The use of ADSCs has similar differentiation capabilities to bone marrow cells, with the advantage that they can be easily harvested and cultured.[12,32,34] Some studies have provided evidence confirming that tissue-engineered bone regeneration using ADSCs is an acceptable alternative to autologous bone graft.[12–15] However, the regenerative capacity of ADSCs does not seem to be superior to autologous bone or other bone substitutes in the same conditions,[35–37] which is consistent with the present findings. Differences in the bone regenerated area and mineralized tissue between defects treated with a ceramic biomaterial coated with ADSCs (β-TCP-Fn-ADSCs) compared with other particulate biomaterials (β-TCP or β-TCP-Fn) or control defects were not found.

Table 1 Surface Area of Bone Regeneration, Mineralized and Nonmineralized Tissue, and Percentage of BIC in the Study Groups According to the Time of Euthanasia

	Study groups								
	Control		β-TCP		β-TCP-Fn		β-TCP-Fn-ADSCs		
Data	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	*P* value
Bone regeneration area (mm^2)									
T1 (n = 5)	3.66 (1.10)	45.24	3.63 (1.41)	44.58	4.47 (1.16)	54.44	4.01 (0.86)	48.93	.640
T2 (n = 5)	4.45 (1.28)	54.84	4.63 (1.64)	56.78	4.84 (1.55)	60.38	4.41 (1.01)	54.32	.958
T3 (n = 6)	3.91 (1.74)	48.40	4.53 (1.14)	56.05	4.44 (1.59)	54.97	4.94 (0.74)	61.51	.636
Mineralized tissue (mm^2)									
T1 (n = 5)	2.20 (1.57)	27.46	2.16 (1.94)	26.66	2.42 (2.09)	29.60	2.99 (1.41)	36.60	.873
T2 (n = 5)	3.08 (1.51)	38.06	3.53 (1.75)	43.28	3.39 (1.68)	42.46	2.92 (0.99)	36.09	.915
T3 (n = 6)	2.93 (1.56)	36.20	2.96 (1.34)	36.53	3.28 (1.86)	40.73	3.22 (1.01)	40.14	.965
Nonmineralized tissue (mm^2)									
T1 (n = 5)	0.57 (0.42)	7.02	1.28 (0.78)	15.74	1.28 (0.72)	15.68	1.01 (0.64)	12.33	.308
T2 (n = 5)	1.19 (0.37)[a]	14.68	0.80 (0.50)	9.92	1.08 (1.10)	13.50	0.75 (0.0)	9.30	.698
T3 (n = 6)	0.68 (0.16)	8.40	1.12 (0.57)	14.01	0.95 (0.52)	11.77	1.07 (0.38)	13.33	.329
BIC (%)	**Mean (SD)**		**Mean (SD)**		**Mean (SD**		**Mean (SD)**		
T1 (n = 5)	19.99 (11.23)		23.22 (15.38)		26.20 (12.31)		27.67 (17.82)		.840
T2 (n = 5)	47.67 (17.23)[b]		30.39 (3.43)		43.38 (12.89)		20.18 (9.21)		.008
T3 (n = 6)	39.26 (10.70)		29.31 (13.46)		29.16 (15.53)		39.92 (16.51)[c]		.388

Differences of histomorphometric variables between the control group and the three study groups (β-TCP, β-TCP-Fn, and β-TCP-Fn-ADSCs) at T1, T2, and T3 were assessed with the analysis of variance (ANOVA) (*P* value).
[a]Control (NMT): $P = .033$ for the comparison of T2 vs T1 and $P = .012$ for T2 vs T3 (paired Student *t* test).
[b]Control (BIC): $P = .017$ for the comparison of T2 vs T1 and $P = .16$ for T3 vs T1 (paired Student *t* test).
[c]β-TCP-Fn-ADSCs (BIC): $P = .042$ for the comparison of T3 vs T2 (paired Student *t* test).

The use of agents as transfer vehicles of stem cells has a positive effect in the process of bone regeneration of critical defects. Tricalcium phosphate appears to be a biomaterial of choice for tissue-engineered bone regeneration because of its properties of biocompatibility, high conductivity, and lack of immunogenicity.[38–42] Also, the high resorption rate of tricalcium phosphate over time facilitates neoformed bone substitution in bone regeneration procedures.[43,44] In the present study, significant differences in the amount of particulate biomaterial and mineralized and nonmineralized tissue regarding the type of biomaterial (β-TCP, β-TCP-Fn, and β-TCP-Fn-ADSCs) or the healing time (T1, T2, and T3) were not observed. However, the mean amount of nonmineralized tissue in the control group showed significant differences according to the healing time, with an increase in animals sacrificed at 2 months. Because metabolism in dogs is more accelerated than in humans and given that control defects were left unfilled (despite the area being contaminated with particulate biomaterial [Fig 4]), the differences observed may be explained by autogenous bone remodeling occurring during the healing phases of the process.[43,45]

Long-term survival of osseointegrated implants in regenerated and nonregenerated bone appears to be similar.[46,47] In addition, regeneration of dehiscence-type defects associated with simultaneous implant insertion using different biomaterials and membranes has shown satisfactory results when the total area of neoformed bone and the percentage of BIC are analyzed.[23,25,43,48] Results of the present study showed that BIC values in dehiscence-type defects regenerated with a ceramic biomaterial coated with Fn-ADSCs were not superior to the other study groups. Besides, this group showed lower percentages of BIC compared with defects treated with fibronectin or controls at 2 months of healing. BIC values in defects treated with Fn-ADSCs were in general lower than those reported by other authors using autologous bone or other biomaterials for equivalent healing periods in similar experimental models.[23,25,43,48] However, the results of the present study were similar to those obtained in the treatment of peri-implant defects with autologous bone marrow–derived cells in canine models.[49,50] Ribeiro et al[50] found statistically higher new bone area and BIC in defects filled with bone marrow–derived cells compared with control defects (no treatment) at 3 months. Ito et al[49]

also reported significantly higher percentages of BIC at 4 and 8 weeks after implantation in the group of mesenchymal stem cells, platelet-rich plasma and fibrin in comparison with fibrin, mesenchymal stem cells and fibrin, and defects only (controls). In these studies, however, nonabsorbent titanium-reinforced membranes were used to cover the defect to ensure adequate space maintenance and stabilization of the cell-scaffold construct. The selection of the type of membrane does not seem to be a confounding factor in regeneration of dehiscence-type bone defects, as shown by Schwarz et al[51] in their study, in which differences in the percentages of BIC at 12 weeks using different types of barrier membranes were not found. It should be noted that the lack of data on the use of ADSCs in bone regeneration processes of critical defects associated with simultaneous implant placement does not allow a rigorous comparison of the present results.

The absence of a group treated only with β-TCP-ADSCs is a limitation of the study. However, the efficacy already demonstrated by ADSCs in bone regeneration processes,[12,13,15,27] as well as technical difficulties in placing sufficient defects in the same region, restricted the number of study groups. The groups selected were those considered to be of interest due to the lack of previous studies in an in vivo model. On the other hand, the use of fibronectin (a highly effective adhesive protein compared with other proteins of the extracellular matrix[52,53]) favors filling of the empty spaces between the filling material and the surface of the bone defect as well as between biomaterial particles and the surface of the implant, providing mechanical stability in the initial phases of osseointegration and bone regeneration, in which stability is a crucial factor; in addition, fibronectin accelerates cellular adhesion and differentiation through integrins and, therefore, the formation of new bone tissue surrounding dental implants and regeneration materials.[53,54]

Another limitation of the study is the use of particulate biomaterial, in which the size of the particles was 0.25 to 1 mm. The effect of carrier particle size on in vivo bone formation by human bone marrow stromal cells was evaluated by Mankani et al[38] using commercially available hydroxyapatite/tricalcium phosphate (HA/TCP) particles. These authors showed that transplants incorporating HA/TCP particles of 0.1- to 0.25-mm size demonstrated the greatest bone formation.[38] In this respect, the size of the particles of 0.25 to 1 mm used in the present study might compromise the bone formation capacity of stem cells and may be one of the reasons for the lack of statistical significance between defects treated with ADSCs combined with fibronectin and the remaining groups.

The lack of inherent plasticity of this biomaterial may prevent efficient adaptation to the morphology of dehiscence-type defects and may favor contamination of the adjacent defects.[10] For this reason, the use of biomaterials with some plasticity, such as fibrin glue or platelet-rich plasma, facilitates handling and adaptation to bone defects during tissue-engineered bone regeneration with stem cells.[55,56] Khoshzaban et al[57] argued that the material of the experimental defect got transferred to its adjacent empty defect from circulation and animal movement, especially at the operation area after periosteal approximation. Also, the lack of stabilization of the membrane covering the defects with some system of fixation may also be a factor compromising the amount of regenerated area as well as favoring displacement of the biomaterial.[58,59] Mir-Mari et al,[58] in a study of bone defects in pig mandibles, concluded that the stability of the bone substitute and collagen membrane was enhanced by the application of fixation pins and by the use of block bone substitute instead of particulated bone substitute.

Dehiscence-type defects may also have some capacity of spontaneous bone regeneration.[43,60] The confounding effect of this factor together with the fact that control defects were also coated with a collagen membrane may account for the lack of significant differences between the β-TCP-Fn-ADSCs and the other study groups in terms of the bone regenerated area and the amount of mineralized tissue, despite the critical-size design of the defects.[22–25]

The use of some extracellular matrix proteins to cover osteoconductive biomaterials has been shown to facilitate not only the adhesion capacity of cells in regenerative processes, but also to intervene directly in the differentiation capacity of stem cells.[52] Fibronectin in combination with a xenograft or a ceramic biomaterial (calcium phosphate) have a favorable effect on cell adhesion, especially within the first hours after culture.[61] The adhesion-promoting property of fibronectin is particularly relevant in regeneration procedures of bone defects favoring adhesion of stem cells or osteoblasts when combined with some biomaterials.[16–19] It is important to consider that the lack of studies in in vivo models with biomaterial covered with fibronectin makes the comparison of the results of the present study difficult.

The results of this study reveal that the use of a particulate β-TCP biomaterial coated with fibronectin combined with ADSCs was not associated with an improvement of bone regenerated area or mineralized tissue compared with the other study groups (β-TCP, β-TCP-Fn) or the control defects in the three healing times analyzed. Moreover, although the percentages of BIC were similar to the remaining groups at 3 months of healing, significantly lower values compared with defects coated only with fibronectin at 2 months were found. Accordingly, the advantages of

the combination of fibronectin and ADSCs in bone regeneration procedures of dehiscence-type defects remain unclear.

CONCLUSIONS

The use of ADSCs in bone regeneration processes of dehiscence-type defects associated with placement of dental implants does not seem to improve the amount of bone regenerated area or new bone in contact with the implant compared with other osseous substitutes or the control defect during the same period of healing. Further studies are needed to assess the efficacy of ADSCs as well as the use of different vehicles in the reconstruction.

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