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RESEARCH ARTICLE

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Comparison of the osseointegration of implants placed in areas grafted with HA/TCP and native bone

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Abstract

This study evaluated the osseointegration of implants in areas grafted with biphasic ceramic based on hydroxyapatite/β-tricalcium phosphate (HA/TCP) and in native bone (NB). Twenty-eight rats were randomly assigned into two groups of 14 animals each: HA/TCP group: implants installed in areas grafted with HA/TCP and NB group: implants installed in areas of native bone. Bone defects were made in both tibiae of the rats belonging to the HA/TCP group and then filled with this bone substitute. After 60 days, the rats were submitted to surgical procedures for implant placement in grafted areas in both tibiae in the HA/TCP group while the implants were installed directly in native bone in the NB group. The animals were euthanized 15 and 45 days, respectively, after the implant placement. Biomechanical (removal torque), microtomographic (volume of mineralized tissues around the implants), and histomorphometric (Bone-Implant contact—%BIC and bone area between the implant threads—%BBT) analyzes were conducted to assess the osseointegration process. The HA/TCP group showed lower values of removal torque, volume of mineralized tissue around the implants, lower %BIC, and %BBT compared to the NB group in both experimental periods. Osseointegration of implants placed in grafted areas with HA/TCP was lower compared to the osseointegration observed in native bone areas.

Research Highlights

- The areas grated with HA/TCP presented poor biological conditions.
- The reduced biological properties for bone formation impaired the osseointegration in HA/TCP grafted areas.

KEYWORDS
bone substitutes, dental implants, osseointegration

1 | INTRODUCTION

The therapy with dental implants has been commonly applied in the treatment of totally and partially edentulous patients promoting successful clinical outcomes in terms of restoring masticatory function, aesthetic, and quality of life (Jiang et al., 2020; Romanos et al., 2019). However, the installation of dental implants directly in native bone is not always possible since the tooth loss induced the resorption of the alveolar process which can result in dimensions changes of the alveolar ridge and in the formation of bone defects (Sculean et al., 2019;

Tan et al., 2012). In cases where the resorption process of the alveolar bone is advanced, it is necessary to apply guided bone regeneration techniques associated with bone substitutes. This procedure allows the increase of the bone tissue availability for subsequent implant placement in the proper position (Fernandez de Grado et al., 2018; Spin-Neto et al., 2015).

Alternative bone substitutes have been described instead of using autogenous bone graft and are indicated to increase bone availability prior to the installation of dental implants (Mourão et al., 2019; Uzeda et al., 2017), due to the limitations inherent to the use of the autografts such as the need for a second donor surgical site, high morbidity, longer surgical time, and limited availability (Nkenke & Neukam, 2014). Among the bone substitutes proposed to increase bone availability, the biphasic ceramic based on hydroxyapatite/β-tricalcium phosphate (HA/TCP) is noteworthy for presenting good outcomes on the success and survival of implants in clinical studies (Bouler et al., 2017; Cordaro et al., 2008; Mordenfeld et al., 2016).

Despite the good clinical outcomes presented by HA/TCP, the fact that this bone substitute presents only the biological property of bone formation by the osteoconduction means that the grafted area with HA/TCP can be biologically inferior to the areas grafted with autogenous bone or into the native bone (Antunes et al., 2013; Danesh-Sani et al., 2016), and it can induce less degree of osseointegration and less resistance to periimplantitis progression (Bouler et al., 2017). Indeed, some studies point to the grafted areas themselves as a possible risk factor for the unsuccess of the implant therapy with approximately five times greater chances of failure of implants placed in grafted area compared with implants placed in native bone (Pieri et al., 2017; Sesma et al., 2012).

In fact, the comparison of the quality of osseointegration in areas grafted with osteoconductive bone substitutes requires more information, since the most amount of the knowledge of the biological quality of the HA/TCP grated areas were provided in pre-clinical or clinical studies that assessed these areas without the implants (Cordaro et al., 2008; Danesh-Sani et al., 2016; Fabris et al., 2018; Frenken et al., 2010). So, the aim of this pre-clinical study was to evaluate the osseointegration in areas grafted with HA/TCP in comparison with the osseointegration in native bone.

2 | METHODOLOGY

2.1 | Ethical considerations

This study was submitted and approved by the Animal Ethics Committee of the School of Dentistry at Araraquara (CEUA: 26/2016). Twenty-eight rats (Rattus novergicus, Hotzman variation), 12-weeks old, weighing 250–300 g were used for this study. The animals were kept in an environment with temperature (21 ± 1°C), humidity (65%–70%), and controlled light cycles (12 h). The animals were offered water and *feed* ad libitum. This study was conducted according to the ARRIVE protocol for conducting preclinical studies.

2.2 | Groups

The animals were randomly assigned to two groups of 14 animals each, which were divided according to the type of biomaterial that was used to fill the bone defects: HA/TCP Group: Defect filled with Biphasic ceramics base on Tricalcium phosphate and hydroxyapatite (Bone Ceramic®, Straumann, Basel, Switzerland); NB Group: The implants were placed directly in the native bone of the tibia metaphysis. A surface machined implant was placed in the grafted and native bone at both groups (Neodent®, Curitiba, PR, Brazil). The HA/TCP presented 60% of HA and 40% of TCP, 90% of porosity and 0.5–1.0 mm of granules size (Antunes et al., 2013; Cordaro et al., 2008).

2.3 | Surgical procedure

The animals were anesthetized by a combination of Ketamine (Agener União Ltda, São Paulo, SP, Brazil) at a dosage of 0.08 ml/100 g body mass with Xylazine (Rompum, Bayer SA, São Paulo, SP, Brazil) at the dosage 0.04 ml/100 g body mass. Subsequently, they were undergoing a trichotomy of the internal region of the right and left hind paws and the antisepsis was performed.

An approximately 10-mm incision was made in planes over the tibial tuberosity. After delicate dissection, the bone tissue was submitted to osteotomy by means of a counter-mounted spherical drill with the aid of a 1200 rpm electric motor (BLM 600—Driller, São Paulo, SP, Brazil) under abundant solution irrigation sterile saline. A spherical drill was used to make perforations in the anterior cortical portion of the tibias. Then, these perforations were connected and a portion of the anterior cortical of the tibia was removed. The margins of the defects were flattened with the same drill. The defect formed had as final measurements 4 mm in length and width and 1.5 mm in depth. Then, the HA/TCP was hydrated with saline solution and gently accommodated into the bone defects. The tissue was sutured by planes internally with 5.0 resorbable thread (Vicryl Ethicon, Johnson & Johnson, São Jose dos Campos, Brazil) and externally with 4.0 silk thread (Ethicon, Johnson & Johnson, São Jose dos Campos, Brazil). The animals received a single dose of streptomycin-associated penicillin at a dosage of 0.1 ml/kg (Multibiotic Small, Vitalfarma, São Sebastião do Paraíso, MG, Brazil) and 0.1 ml/kg ketoprofen (Ketoflex; Mundo Animal, São Paulo, Brazil) intramuscularly.

After a period of 60 days, a second surgical intervention was performed in the region that received the biomaterials for implant placement in the HA/TCP group. An incision similar to the first procedure was made over the tibial tuberosity. The grafted region was prepared for implant placement by applying a progressive sequence of drills (spear drill; 2.0 mm spiral drill—Neodent®; Curitiba, PR, Brazil) to accommodate a machined surface implant with 4 mm high and 2.2 in diameter (Neodent®; Curitiba, PR, Brazil). All drilling was performed with the aid of an electric motor, adjusted to 1200 rpm, under abundant irrigation with sterile saline solution. The implant was installed with the aid of a digital key (1.2 mm hexagonal digital key—Neodent, Curitiba, PR, Brazil). The tissue suture and the postoperative drug protocol that was used were similar to those used in the first surgery. The animals of the native bone group were submitted only for the surgery for implant placement with the same surgical and post-surgical protocol.

At 15 and 45 days after the implant placement surgical procedure, the animals were euthanized by deepening the anesthetic dose. The tibias were separated according to the performed analyzes. The right tibia was used for microtomographic and histomorphometric analysis, while the left tibia was used for biomechanical analysis.

2.4 | Biomechanical evaluation

After euthanasia, the left tibias were stabilized in a small walrus. A hexagon wrench was attached to both the implant and torque wrench (Tohnichi, model ATG24CN-S, Tokyo, Japan) and a counterclockwise movement was performed to unscrew the implant. The maximum peak required to move the implant was noted as the removal torque value (Ncm).

2.5 | Microtomographic evaluation

The right tibias were fixed in 4% paraformaldehyde for 48 h and then stored in 70° alcohol. These samples were scanned by a microtomograph (Skyscan, Aatselaar, Belgium) with the following parameters: Camera pixel: 12.45; x-ray tube power: 65 kVP, x-ray intensity: 385 μA, integration time: 300 ms, filter: Al-1 mm and voxel size: 18 μm^3. The images were reconstructed, spatially repositioned, and analyzed by specific software (NRecon, Data Viewer, CTAnalyser, Aatselaar, Belgium). The region of interest (ROI) was defined as a 0.5 mm circular region around the entire diameter of the implant. This ROI was defined as total volume (0.5 mm margin around implants − 4.5 mm × 3.2 mm). As the implants placed did not receive the cover screw in some cases there was bone formation inside the prosthetic platform. To prevent this bone formation from interfering with the analysis of the volume of mineralized tissue around the implant, a second ROI was defined to remove the platform volume. With the results obtained in both ROIs, it was possible to define the bone formation volume using the formula: Total volume − Platform volume = Volume of mineralized tissues. The threshold used in the analysis was 25–90 shades of gray, and the volume values of mineralized tissue around the implants were obtained as a percentage (%BV/TV) (Freitas de Paula et al., 2018). A trained examiner blinded to the experimental groups performed this analysis.

2.6 | Histomorphometric evaluation

After scanning, the right tibias were dehydrated in a growing series of ethanols (60–100%) and infiltrated and polymerized into light-curable resin (Technovit 7200 VLC, Kultzer Heraus GmbH & CO, Wehrheim, Germany). The blocks containing the implant and bone tissue were cut at a central point using a wear and tear system (Exakt Apparatebeau, Hamburg, Germany). The final sections were approximately 45-μm thick and stained with Stevenel's blue associated with acid fuchsin and analyzed under an optical microscope (DIASTAR—Leica Reichert & Jung products, Wetzlar, Germany) at 100× magnification. The histomorphometric evaluation was performed using image analysis software (Image J, San Rafael, CA, USA). The percentages of bone-implant contact (%BIC) and bone area between implant turns (%BBT) were evaluated separately in the first six implant threads. These analyzes were performed by a blind and trained examiner.

2.7 | Statistical analysis

GraphPad Prism 6 software (San Diego, CA, USA) was used to perform the statistical analysis of this study. All the data were distributed according to the normality as shown by the Shapiro–Wilk Normality test. Then, all these data were analyzed using the parametric unpaired *t*-test. All tests in this study were applied with a significance level of 95%.

3 | RESULTS

All animals survived after the surgical procedures and were healthy throughout the experimental period. The sample size calculation was referenced to %BIC data from a previous study that evaluated the effect of an implant surface osseointegration in grafted areas in a similar experimental model and assessment as performed in this study (Pinotti et al., 2018). Considering that the smallest difference between the means in the groups where there were statistically significant differences was 19.29% with a standard deviation difference between these groups 6.59%, it was found that a sample of 7 animals per group/period was sufficient for the application of statistical tests with type α error set at .05 and β power of .90.

3.1 | Implants placed in HA/TCP grafted area presented low secondary stability

It was verified by the biomechanical analysis that implants placed in HA/TCP-grafted areas presented lower removal torque values than implants placed in native bone at both periods of evaluation (1.66 ± 0.51 Ncm vs. 7.33 ± 1.03 Ncm at 15 days and 2.83 ± 1.72 Ncm vs. 20.83 ± 2.40 Ncm at 45 days) ($p < .001$) (Table 1).

3.2 | HA/TCP grafted area presented low bone volume around the implants

It was verified by microtomographic analysis that implants installed in HA/TCP grafted areas presented lower BV/TV values than implants

TABLE 1 Mean and standard deviation of implant removal counter torque data in all experimental groups and periods

Groups/Period	15 days	45 days
HA/TCP	1.66 ± 0.51	2.83 ± 1.72
NB	7.33 ± 1.03***	20.83 ± 2.40***

****p* < .001—Higher value of implant removal counter torque compared to the HA/TCP group—Unpaired *t*-test.

TABLE 2 Mean and standard deviation of BV/VT data around implants in all groups and experimental periods

Groups/Period	15 days	45 days
HA/TCP	26.91 ± 3.62	37.54 ± 3.36
NB	48.35 ± 1.51***	70.06 ± 0.92***

****p* < .001—Higher BV/TV value compared to the HA/TCP Group—unpaired *t*-test.

installed in native bone area at both periods of evaluation (26.91 ± 3.62% vs. 48.35 ± 1.51% at 15 days and 37.54 ± 3.36% vs. 70.06 ± 0.92% at 45 days) ($p < .001$) (Table 2). Figure 1 shows the tomographic aspect of the implants placed in native bone and in the HA/TCP areas at the 45-day period.

3.3 | Implants placed in HA/TCP grafted areas presented low %BIC and %BBT

It was verified by histometric analysis that implants installed in HA/TCP grafted areas presented lower %BIC (8.40 ± 5.81% vs. 41.34 ± 4.97% at 15 days and 10.73 ± 7.34% vs. 82.36 ± 4.72% at 45 days) and %BBT values (10.56 ± 7.48% vs. 34.65 ± 5.59% at 15 days and 13.89 ± 8.02% vs. 81.31 ± 2.97% at 45 days) than implants installed in native bone areas at both periods of evaluation ($p < .001$) (Table 3). Figure 2 shows the representative images of the non-decalcified histological sections.

4 | DISCUSSION

In general, the results of this study showed that the osseointegration in areas grafted with HA/TCP presented a lower rate compared with the osseointegration in native bone. These findings can indicate that the implants placed in grafted areas with HA/TCP may require longer times of healing to enable the occlusal loads. Furthermore, these implants can be at a higher risk of biological complications.

The biomechanical analysis showed that the implants placed in a native bone showed 4–6 times higher removal torque values compared with the implants placed in HA/TCP grafted areas at both periods of evaluation. Although previous biomechanical studies have not been performed to compare mechanical properties of native bone with HA/TCP grafted areas, a previous preclinical study showed that segmental defects in rabbit tibias grafted with HA/TCP did not show adequate mechanical resistance to support the load bearing required for this type of defect and additional intramedullary retentions are necessary to improve the mechanical properties of the HA/TCP (Balçik et al., 2007). Another preclinical study that compared the autografts, allografts, HA/TCP, and HA/TCP associated with collagen that were used to fill the bone defects in the tibias of dogs demonstrated that there were no statistically significant differences between the groups in the mechanical traction analyzes, however, the defects treated with HA/TCP presented more empty spaces than defects treated with autografts and allografts (Hamson et al., 1995), and this finding may be related to the lower locking of implants installed in grafted areas with HA/TCP that was verified in this study.

The lower secondary stability of implants placed in areas grafted with HA/TCP may have been directly influenced by the outcomes of the histometric and microtomographic analyzes of this study. It was shown that the implants placed in areas grafted with HA/TCP presented lower BV/TV, %BIC, and %BBT in both assessment periods compared with the implants placed in native bone. It is possible that the lower bone formation in areas grated with osteoconductive bone substitutes may influence the outcomes of the osseointegration. In fact, previous preclinical studies have been showing that the bone healing in area grafted with HA/TCP was associated with less bone formation than bone defects filled with coagulum or autogenous bone graft (Fabris et al., 2018; Prisinoto et al., 2020). In addition, one clinical study showed that the amount of bone formation in biopsies harvested from the maxillary sinus before the implant's placement presented lower bone formation than the sinus grafted with autografts (Danesh-Sani et al., 2016).

Among the osteoconductive bone substitutes, the HA/TCP deserves highlight due to the good biological properties, inducing bone formation at even higher rates than that induced by deproteinized bovine bone, which is the most traditionally inquired osteoconductive bone substitute (Cordaro et al., 2008; Yip et al., 2015). This biomaterial has the mechanism of action based on the controlled resorption of βTCP that stimulates bone formation and the maintenance of HA that would maintain the volume of the grafted area (Antunes et al., 2013; De Coster et al., 2011). However, the volumetric reduction of HA/TCP can be around 20% (Favato et al., 2015) without necessarily being associated with the formation of bone tissue (Prisinoto et al., 2020), which may have influenced the smaller volume of mineralized tissues around the implant in the grafted areas with HA/TCP compared with the volume around implants placed in NB observed in this study. In addition, it is likely that the soft tissues constitute a large part of the newly formed tissues in the grafted area (de Coster et al., 2011; Gonçalves et al., 2020), and this may also have influenced the lower degree of osseointegration and biomechanical locking in implants installed in grafted areas with HA/TCP compared to implants installed in NB areas. In fact, previous preclinical and clinical studies have shown that the further away the grafts are from the bone walls, the smaller is the bone formation in the grafted area (Araújo & Lindhe, 2009; de Coster et al., 2011; Pignaton et al., 2020; Prisinoto et al., 2020). In

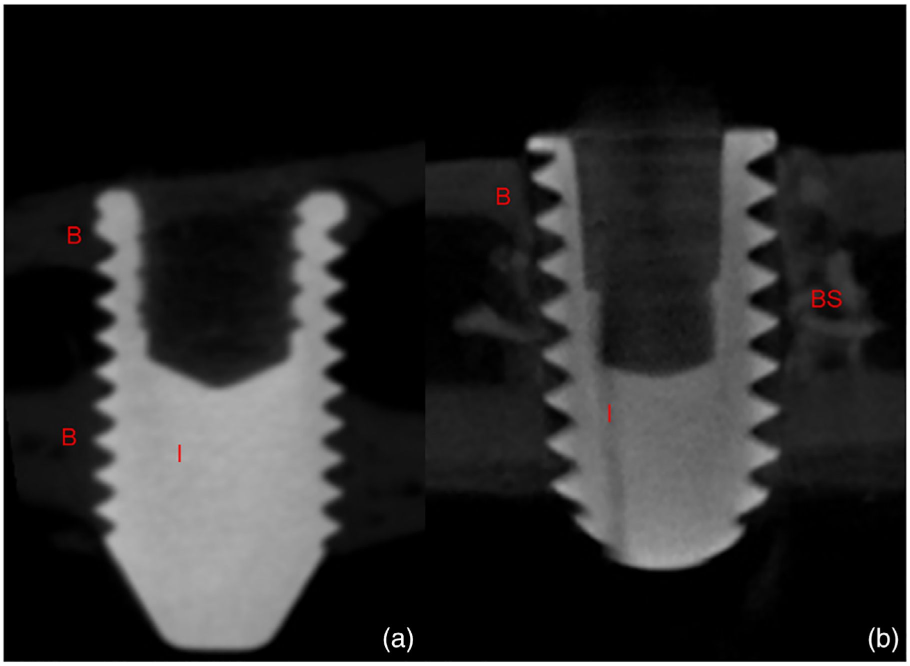

FIGURE 1 Representative microtomographic aspect of the implants placed in native bone (a) and in the HA/TCP grafted areas (b) at the 45-day period. It is possible to note that the amount of mineralized tissues close to the implants in HA/TCP grafted areas is lower than close to the implants placed in native bone. B, bone, BS, bone substitute; I, implant

TABLE 3 Mean and standard deviation of %BIC and %BBT data in all groups and experimental periods

Parameters	Groups/Period	15 days	45 days
%BIC	HA/TCP	8.40 ± 5.81	10.73 ± 7.34
	NB	41.34 ± 4.97***	82.36 ± 4.72***
%BBT	HA/TCP	10.56 ± 7.48	13.89 ± 8.02
	NB	34.65 ± 5.59***	81.31 ± 2.97***

***$p < .001$—Higher %BIC and %BBT value than the HA/TCP group—unpaired *t*-test.

the experimental model used in this study, the implants were installed in the middle of the bone defect, which would be the most critical area for bone formation.

The HA/TCP has been used clinically in order to obtain increased bone availability in different clinical conditions such as maxillary sinus lifting (Cordaro et al., 2008; Danesh-Sani et al., 2016), preserving of the volume of the post-extraction socket (de Coster et al., 2011; Uzeda et al., 2017), and in the treatment of periodontal defects (Hoffmann et al., 2016; Peres et al., 2013). However, a subject that has been less investigated is the success of implants placed in areas grafted with osteoconductive biomaterials. Clinical studies have been showing that implants placed in these areas have lower success rates than implants installed in NB (Mordenfeld et al., 2016; Pieri et al., 2017; Sesma et al., 2012), especially when the region of the implant platform is located within the grafted area (Pieri et al., 2017), a condition similar to the experimental model used in this study. Thus, the search for improvement in the quality of the areas grafted with osteoconductive bone substitutes is necessary to improve the predictability of the osseointegration process and the maintenance of the peri-implant bone in implants installed in these areas.

The evaluation of osseointegration in clinical studies have been usually performed indirectly through radiographic (da Silva et al., 2020), or by resonance frequency analysis (Barbosa et al., 2021). The evaluation of grafted areas occurs after the obtaining biopsies that are evaluated by microscopy or tomographic analysis (Danesh-Sani et al., 2016; Spin-Neto et al., 2015), however the evaluation of these biopsies occurs at the time of implant placement and the evaluation of osseointegration has been poorly explored (Cordaro et al., 2008; Danesh-Sani et al., 2016). In preclinical studies, the evaluation of osseointegration in areas of native bone by means of microscopy techniques are routinely applied (Freitas de Paula et al., 2018). However, assessment of osseointegration in grafted areas is less common, and has been investigated in larger animals (Antunes et al., 2013). In the model proposed in this study, it was possible to perform the evaluation of osseointegration in rat tibias, which reduces the operational complexity for this type of evaluation (Pinotti et al., 2018) Furthermore, the evaluation of osseointegration in grafted areas can be performed with microscopic methods similar to those used in evaluation in areas of native bone (de Oliveira et al., 2020; Pinotti et al., 2018), which allowed the comparisons proposed in this study. It is worth noting that the microscopic evaluation was considered the primary variable of this study due to the limitations of the microtomographic and biomechanical methods. The microtomographic methods presets artifacts produced by the metal of the implants and the radiopacity of the HA/TCP (Pinotti et al., 2018), while the biomechanical analysis has as a confounding factor the fact that the implants have been locked in the posterior cortical bone of the tibia (de Oliveira et al., 2020). It is possible that the stabilization of the implants at the cortical bone at the apex may have modified the values of the real imbrication of the implant body in the grafted areas.

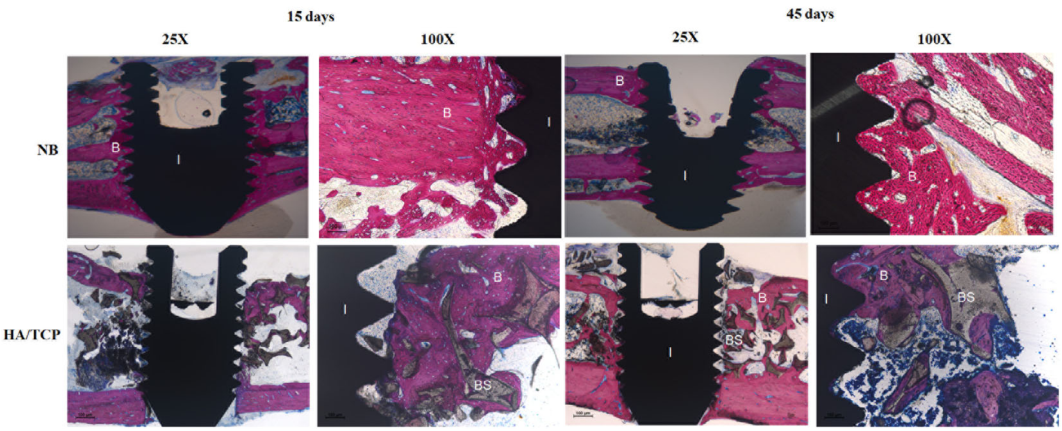

FIGURE 2 Representative images of the non-decalcified histological sections that showed lower osseointegration in the implants placed in HA/β-TCP compared with the implants placed in NB (25× and 100×—Stevenel's blue associated with acid fuchsin). B, bone; BS, bone substitute; I, implant

Therefore, although the results of the study were favorable to the osseointegration of implants in native bone, it is important to note that this study presented some drawbacks that limited clinical inferences. The animal model used has characteristics of good quality bone tissue (Freitas de Paula et al., 2018), which does not mimic clinical situations where normally the bone presents characteristics inferior to the model tested. In addition, important interference factors such as the influence of systemic risk factors, as well as occlusal loads, need to be assessed in implants placed in areas grafted with osteoconductive bone substitutes. Another relevant aspect to be considered is that the experimental model used in this study cannot necessarily represent the ideal healing time for grafted areas with HA/TCP, and the waiting period for implant placement in areas grafted with HA/TCP has not yet been clearly defined. Human studies have shown that HA/TCP-grafted maxillary sinuses associated with autogenous bone show greater bone formation at 9 months than at 6 months (28.6 ± 7.8% vs. 41.6 ± 8.3%) (Artzi et al., 2008). In addition, post-extraction sockets grafted with HA/TCP showed poor bone tissue formation and the presence of disorganized connective tissue up to 38° after the surgical procedure (de Coster et al., 2011). On the other hand, it seems that the influence of different experimental periods on the bone formation in grafted areas with HA/TCP in pre-clinical studies has less impact than the observed in clinical studies. For example, critical sized-calvarial defects grafted with HA/TCP did not present distinct bone formation between the periods of 14 and 42 days after the grating procedure (Fabris et al., 2018), and these results are in accordance with the findings of this study.

5 | CONCLUSION

Thus, according to the data presented, it can be concluded that implants installed in grafted areas with HA/TCP present a lower degree of osseointegration than in native bone.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are part of the MSc Dissertation of Julia Raulino Lima and it will be openly available at the UFU repository system in the beginning of 2024 at https://repositorio.ufu.br/handle/123456789/34106.

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