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ORIGINAL ARTICLE

Deproteinized bovine bone derived with collagen improves soft and bone tissue outcomes in flapless immediate implant approach and immediate provisionalization: a randomized clinical trial

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Abstract

Objectives This study aimed at evaluating soft and hard tissue dimensions after immediate implant placement and immediate temporization with or without alveolar preservation at the maxillary anterior region.

Materials and methods Twenty-two patients needing maxillary incisor extraction and with the possibility of immediate implant placement were randomly assigned to the following groups: test ($n = 11$): immediate implant placement + deproteinized bovine bone derived with collagen inserted into the alveolus or control ($n = 11$): immediate implant placement without biomaterial. All soft tissue measurements were evaluated at baseline, 3 months, and 6 months after implant therapy. Cone beam tomography was performed at baseline and at 6 months after implant placement to evaluate hard tissue dimension.

Results The test group presented higher height of soft tissue at mesiobuccal and distobuccal sites at 3 months and 6 months when compared to the control group ($p < 0.05$). Regarding the bone tissue, the test group showed higher buccolingual ridge dimension at 6 months when compared to the control group ($p < 0.05$).

Conclusions It can be concluded that the use of deproteinized bovine bone derived with collagen together with immediate dental implants results in better soft and bone tissue outcomes than immediate implants alone.

Clinical relevance The use of deproteinized bovine bone derived with collagen may enhance the results regarding soft and bone tissue in combination with immediate implant and temporization.

Keywords Biomaterial · Extraction socket · Grafting · Healing · Immediate implant · Tomographic evaluation

Introduction

Dental implant therapy is a successful and reliable technique for replacing missing teeth [1, 2]. Although the success rate of this technique is high, the aesthetic achievement and preservation in the anterior region is difficult [3]. Considering the importance of improving aesthetic results and preservation of bone volume, different approaches, such as the flapless technique [4], immediate provisionalization [5, 6], and ridge preservation, have been proposed [7–10].

The flap approach with total mucoperiosteal flap elevation denuding the buccal bone promotes additional resorption after tooth extraction. Animal studies have shown the negative effect of surgical trauma due to flap elevation on soft and hard tissue [11–13], even with socket preservation techniques. Additionally, clinical studies have confirmed the above findings with resorption observed after surgical exposure of the alveolar bone [14–16]. Reduced vertical bone loss was observed with the flapless approach [4].

Immediate provisionalization is also an important aspect. A randomized clinical trial compared the effect of immediate and delayed provisionalization, and the author concluded that implants should be instantly provisionalized to limit the amount of midfacial soft tissue loss [17]. Besides, a pre-clinical study concluded that immediate provisionalization had a positive effect on the soft tissue position [18].

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Ridge preservation is closely related to the achievement of aesthetic teeth replacement. It is well known that when an extraction occurs, bone tissue suffers a resorption process (40% height and 60% width) that leads to functional and aesthetic problems, especially in the anterior region [13, 19, 20]. The reduction in bone volume is severe at the buccal walls due to its thickness and the presence of bundle bone, which is more susceptible to dimensional loss. The preservation of this volume is clinically important for the long-term success of the implant and for the maintenance of aesthetic outcomes [21].

Immediate implant placement results in a void between the buccal bone wall and inserted implant. This gap has a high percentage of spontaneous filling [22], even though the filling of the gap with biomaterial is recommended to achieve better aesthetic results and improve healing [23, 24]. Araújo et al. [25], in an animal model, showed that a larger gap enhanced the bone formation and bone-to-implant contact after 4 weeks of repair. However, concomitantly, the buccal and lingual bone wall presented high resorption, and the height of the buccal bone wall reduced significantly. Pre-clinical studies have shown that an extensive gap can result in soft tissue invasion and impaired healing [26–28].

Given this scenario, aiming at overcoming bone morphological modification, bone volume loss, and aesthetic problems, different treatment strategies have been tested, with the combination of immediate implant placement and associated alveolar ridge preservation techniques. For this, different materials (e.g., autogenous bone, graft material, and biological agents) have been tested in order to obtain ridge preservation, and they presented superior clinical results when compared to the natural healing of the alveolus [29–33]. Araújo et al. [32] showed, in an animal model, the maintenance of the buccal wall dimensions with immediate implants and deproteinized bovine bone derived with collagen placement, and this may be important for the maintenance of soft tissue height.

Some clinical studies have tested the association of immediate implant placement and deproteinized bovine bone derived with collagen, showing positive results regarding soft and bone tissue levels [6, 34, 35]. Despite these observations, there are some differences in the clinical protocol adopted by the authors, such as the association of other biomaterials, use of the flap approach, and absence of immediate provisionalization. Therefore, this randomized clinical trial (RCT) aimed at evaluating the impact of the use of deproteinized bovine bone derived with collagen associated with immediate implants in the maxillary anterior region, with a flapless approach and immediate provisionalization, on bone and soft tissue dimensions, by means of clinical and tomographic analysis.

Material and methods

Patient population

The population of this prospective, randomized, double-blind, controlled clinical trial was recruited from patients referred to Paulista University between January 2015 and December 2016. The clinical procedures and evaluations were carried out between October 2015 and January 2017. Twenty-two patients were selected (4 males and 18 females) ranging in age from 21 to 58 years. This study was approved by the university's Ethics Committee (56342516.8.0000.5512) and has been performed in accordance with Declaration of Helsinki ethical standards.

Inclusion and exclusion criteria

The inclusion criteria for this study were as follows: patients needing maxillary incisor extraction and possibility of immediate implant placement in the same region, presence of teeth adjacent to the extraction area (periodontally healthy and no attachment loss), absence of acute infection at the site of implant placement, presence of buccal alveolar wall, absence of periodontal disease, and age between 18 and 65 years. Immediate implants were indicated when the placement was possible immediately after tooth extraction in an ideal tridimensional position considering provisionalization and primary stability.

The exclusion criteria were as follows: presence of systemic diseases that may interfere with bone repair (including diabetes, arthritis, hypothyroidism, hyperparathyroidism, and osteoporosis), use of medications that contraindicated the placement or that are known to alter implant osseointegration (including anti-inflammatory and bisphosphonates) up to 6 months before surgery, treatment with radiation or chemotherapy (within a 5-year period), bone grafts and history of previous regenerative procedures in the area of the implant, insufficient bone for implant insertion, pregnant or breastfeeding women, and smokers or ex-smokers.

All eligible patients were thoroughly informed of the nature, potential risks, and benefits of their participation in the study, and they each signed an informed consent document.

Experimental groups

The selected patients went through the extraction procedure and received one immediate implant with immediate provisionalization. Using a computer-generated list (managed by FRC), the patients were randomly assigned to the following groups: test ($n = 11$): immediate implant placement + deproteinized bovine bone derived with collagen (Bio-Oss Collagen—Geistlich Pharma, Wolhusen, Switzerland) inserted into the alveolus or control ($n = 11$): immediate implant placement + without biomaterial.

Implant therapy

The surgeries and all postoperative follow-up appointments were performed at the Dental Clinic of Paulista University. Flapless tooth extraction was performed with the use of a periotome and elevators, and all patients received one dental implant (diameter of 4.1 mm) with internal hexagon connections (Biomet 3i Full Osseotite Tapered Certain—Palm Beach Gardens, FL). One surgeon (HSF) with clinical experience in implant dentistry performed all surgical procedures. Briefly, the surgical area was anesthetized, and the site preparation was carried out with a flapless approach in a more palatal position with the implant shoulder placed 3 mm apical to the buccogingival margin of the adjacent teeth. After the implant placement, a space between the buccal wall and the implant shoulder and part of the implant body was observed. The test group had the space (mesial, distal, and buccal gap) filled with deproteinized bovine bone derived with collagen with no compression, while the control group did not receive any graft material. All patients were blinded to the group that they were included.

Sutures were performed with interrupted sutures using absorbable polyglactin 910 (Vicryl, Ethicon, Somerville, NJ). Amoxicillin (2 g administered 1 h before the procedure), sodium dipyrone (500 mg every 6 h for 2 days postoperatively), and 0.12% chlorhexidine mouthwash (every 12 h for 7 days) were indicated.

After the implant placement, it was used a temporary abutment for 3 months, and at the time of fixed prosthesis installation, a final stock abutment was used.

Evaluation of dimensional alterations of the soft and bone tissue

All soft tissue measurements were evaluated at baseline (surgical procedure and provisionalization) and at 3 months (fixed prosthesis installation) and 6 months after implant placement. An individual stent was made to standardize the location of the periodontal probe (Hu-Friedy, NC) to assess the soft tissue height. For this, the measurement from the stent incisal surface to the gingival margin was standardized in three points: mesiobuccal, buccal, and distobuccal (Fig. 1).

Evaluation of bone tissue was performed using cone beam computed tomography (KODAK 9000 C3D) and a specific measurement program (CS 3D Imaging Carestream Dental). Tomography was done in the preoperative period and 6 months after the surgical procedure, and standardization was accomplished by using a tomographic positioner during the exam. Tomographic cuts for bone measurement of the buccolingual distance were obtained by calculating the average distance between the incisal surfaces of the teeth adjacent to the study site, standardizing the tomographic cut used at baseline and at 6 months. The measurement of the buccal and lingual bone plate thicknesses was performed 1 mm (Fig. 2), 3 mm (Fig. 3), and 5 mm (Fig. 4) apical to the bone crest [30]. The distance between the buccal bone surface and implant platform (GAP) was also measured at baseline using a millimeter probe (Hu-Friedy, NC) and at 6 months by tomographic analysis.

Fig. 1 Individual stent made to standardize the location of periodontal probe for measurement of soft tissue

All the measurements were performed by the same calibrated and blind examiner (FFG). The designated examiner measured the buccolingual distance and the soft tissue height of 10 patients twice within 24 h with an interval of ≥ 1 h between examinations. The intraclass correlation was calculated for each parameter, resulting in 98% reproducibility for bone measurement and 94% for soft tissue height.

Data analysis

Firstly, the data were analyzed for homogeneity using the Shapiro-Wilk test. Afterwards, the data were compared between groups by analysis of variance with Tukey's post hoc test. All analyses were performed using SAS 9.1 (SAS

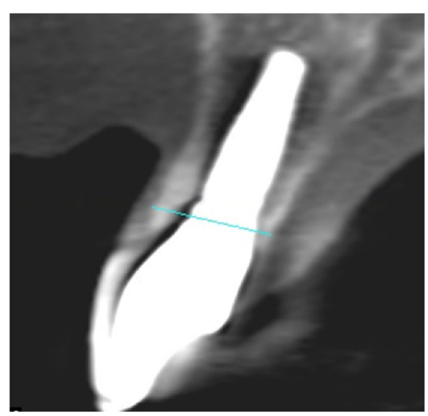

Fig. 2 Measurement of hard tissue dimension 1 mm apical to the bone crest. The measurement was performed using cone beam tomography 1 mm apical to the bone crest. Solid light blue line represents the distance between the buccal and lingual bone surface

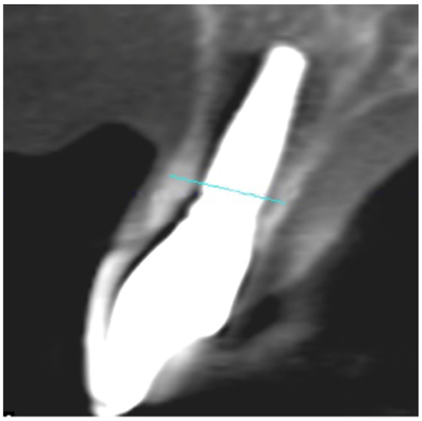

Fig. 3 Measurement of hard tissue dimension 3 mm apical to the bone crest. The measurement was performed using cone beam tomography 3 mm apical to the bone crest. Solid light blue line represents the distance between the buccal and lingual bone surface

Institute Inc., Cary, NC, USA), considering each patient as an experimental unit and $\alpha = 5\%$.

Results

Initially, 42 patients were selected, but 12 of these were excluded, as they did not meet the inclusion criteria. Thus, 30 patients were included in the study; eight were lost due to inadequate insertion torque values or implant loss, and 22 completed the follow-up (Fig. 5).

Considering the height of soft tissue at the buccal site, there was no significant difference for the measurement between the groups and throughout the time ($p > 0.05$) (Table 1). When the mesiobuccal site was analyzed, the test group presented lower height reduction at 3 months (8.36 ± 1.75 mm) and 6 months (8.36 ± 1.75 mm) when compared to the control group (9.55 ± 1.57 mm and 9.55 ± 1.57 mm at 3 months and 6 months, respectively) ($p < 0.05$) (Table 2). Additionally, the test group presented higher soft tissue preservation at the distobuccal site, with lower values at 3 months (8.36 ± 1.54 mm) and 6 months (8.36 ± 1.54 mm) when compared to the control group (9.35 ± 1.43 mm and 9.35 ± 1.43 mm at 3 months and 6 months, respectively) ($p < 0.05$) (Table 3). There was no significant difference in the intragroup analysis for the mesiobuccal and distobuccal surfaces for both groups ($p > 0.05$).

Fig. 4 Measurement of hard tissue dimension 5 mm apical to the bone crest. The measurement was performed using cone beam tomography 5 mm apical to the bone crest. Solid light blue line represents the distance between the buccal and lingual bone surface

Regarding the bone tissue, when the measurement was performed 1 mm apical to the bone crest, a higher width for the test group (6.57 ± 0.45 mm) was observed at 6 months when compared to the control group (6.07 ± 0.24 mm) ($p < 0.05$) (Table 4). Intragroup analysis revealed a significant difference between baseline (7.04 ± 0.49 mm and 6.75 ± 0.27 mm for the test and control groups, respectively) and 6 months (6.57 ± 0.45 mm and 6.07 ± 0.24 mm for the test and control groups, respectively) for both groups ($p < 0.05$), with a reduction in bone width at 6 months (Table 4). Considering the measurement 3 mm apical to the bone crest, the test group showed higher width (6.65 ± 0.45 mm) when compared to the control group (6.15 ± 0.24 mm) at 6 months ($p < 0.05$). Intragroup analysis detected a reduction in the width from baseline (7.12 ± 0.49 mm and 6.83 ± 0.28 mm for the test and control groups, respectively) to 6 months (6.65 ± 0.45 mm and 6.15 ± 0.24 mm for the test and control groups, respectively) for both groups ($p < 0.05$) (Table 4). For the measurement of the buccal and palatal bone plate thicknesses performed 5 mm apical to the bone crest, intergroup difference was also observed with lower bone reduction in the test group (6.69 ± 0.45 mm) compared to the control group (6.18 ± 0.24 mm) at 6 months ($p < 0.05$) (Table 4). The comparison between baseline (7.15 ± 0.49 mm and 6.86 ± 0.27 mm for the test and control groups, respectively) and 6 months (6.69 ± 0.45 mm and 6.18 ± 0.24 mm for the test and control groups, respectively) showed reduction in bone volume for both groups ($p < 0.05$) (Table 4).

Considering GAP measurement, there was no significant difference between the test and control groups ($p > 0.05$). The intragroup analysis showed a significant difference between baseline and at 6 months, with reduction in the GAP at 6 months for both groups ($p < 0.05$) (Table 5).

Discussion

Better outcomes regarding bone remodeling with the combined use of regenerative techniques and immediate implants have been reported [36]. Deproteinized bovine bone derived with collagen has shown positive results when used for ridge volume preservation [7–10]. Besides, the association of different graft biomaterials and immediate implants reduces the tissue loss over time [30–32, 34]. This study clinically evaluated the dimensions of soft and bone tissues in the anterior

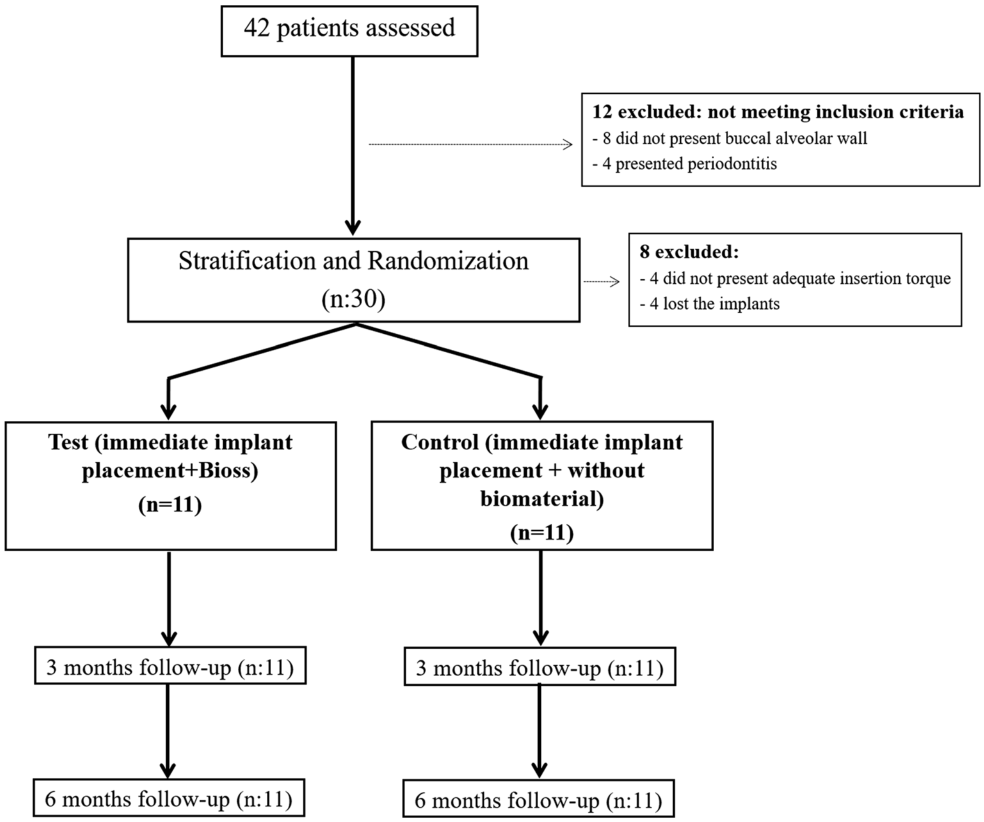

Fig. 5 Flowchart of the study

maxilla with flapless immediate implant placement associated with deproteinized bovine bone derived with collagen and immediate provisionalization, showing higher preservation of both tissues when the biomaterial was applied.

The present study used deproteinized bovine bone derived with collagen alone to fill the void between the buccal bone wall and inserted implant. Deproteinized bovine bone derived with collagen is a deproteinized bovine bone mineral combined with 10% collagen from porcine origin. Animal studies have demonstrated the potential of deproteinized bovine bone derived with collagen in promoting bone formation in fresh extraction sockets [20, 37]. Remarkably, Araújo et al. [32] related the maintenance of the buccal wall dimensions in immediate implants and placement of deproteinized bovine bone derived with collagen.

Our results show higher buccolingual thickness for the deproteinized bovine bone derived with collagen (test) group when compared to the control group. This result agrees with the results of studies where immediate implants were combined with collagen graft materials in clinical [6, 34, 35] and animal studies [32], reducing the bone loss expected after tooth removal. Although the studies of Sans et al. [34] and Cardaropoli et al. [6, 35] have used deproteinized bovine bone derived with collagen with immediate implants, there are some differences when compared to our study. Cardaropoli et al. [35] compared the placement of immediate implants with or without the use of deproteinized bovine bone derived with collagen and collagen membrane with a flapless approach in a RCT. However, the association of a barrier membrane to the biomaterial may enhance the tissue preservation and the cost of the procedure, and the effect of the lone use of deproteinized bovine bone derived with collagen could not be evaluated. The study of Cardaropoli et al. [6] evaluated the soft tissue changes in immediate implants with placement

Table 1 Mean ± standard deviation of tissue height at buccal site (mm)

	Baseline	3 months	6 months
Test	11.82 ± 2.32	11.82 ± 2.32	11.82 ± 2.32
Control	10.82 ± 1.54	11.82 ± 1.54	11.82 ± 1.54

There is no difference between the groups (two-way ANOVA/Tukey, $p > 0.05$). There is no difference in intragroup analysis (two-way ANOVA/Tukey, $p > 0.05$)

Table 2 Mean ± standard deviation of soft tissue height at mesiobuccal site (mm)

	Baseline	3 months	6 months
Test	8.45 ± 1.81	8.36 ± 1.75	8.36 ± 1.75
Control	8.55 ± 1.57	9.55 ± 1.57*	9.55 ± 1.57*

*A statistically significant difference between the groups (two-way ANOVA/Tukey, $p < 0.05$). There is no difference in intragroup analysis (two-way ANOVA/Tukey, $p > 0.05$)

Table 3 Mean ± standard deviation of soft tissue height at distobuccal site (mm)

	Baseline	3 months	6 months
Test	8.45 ± 1.75	8.36 ± 1.54	8.35 ± 1.54
Control	8.36 ± 1.57	9.35 ± 1.43*	9.35 ± 1.43*

*A statistically significant difference between the groups (two-way ANOVA/Tukey, $p < 0.05$). There is no difference in intragroup analysis (two-way ANOVA/Tukey, $p > 0.05$)

Table 5 Mean ± standard deviation of buccal GAP (mm)

	Baseline	6 months
Test	2.55 ± 0.52*	2.29 ± 0.48
Control	2.45 ± 0.52*	2.17 ± 0.46

There is no difference between the groups (two-way ANOVA/Tukey, $p > 0.05$)

*A statistically significant difference between the periods of evaluation within each group (two-way ANOVA/Tukey, $p < 0.05$)

of deproteinized bovine bone derived with collagen and immediate provisionalization, showing maintenance of the soft tissue contour and aesthetics. Nevertheless, this was a cohort about the effect of this treatment. Sans et al. [34] conducted a RCT evaluating the immediate implant placement with or without deproteinized bovine bone derived with collagen in a significant number of patients (43 patients in each group). Deproteinized bovine bone derived with collagen treatment reduced horizontal bone resorption in the buccal bone, even with a flap approach and no immediate provisionalization.

Soft tissue stability is an important outcome, and only a few studies have evaluated this feature. Roccuzzo et al. [9] evaluated the stability of soft tissues following alveolar ridge preservation with deproteinized bovine bone derived with collagen and late implant placement, observing positive results with less than 1 mm of gingival recession after 10 years. However, the authors combined deproteinized bovine bone derived with collagen with a collagen barrier or polylactide polymer. As previously discussed, Cardaropoli et al. [6], in a cohort study, showed achievement of tissue contour and favorable aesthetics by using immediate implants, deproteinized bovine bone derived with collagen, and immediate provisionalization. Accordingly, our results concerning the levels of soft tissue demonstrate higher height when the biomaterial was applied, even without barrier placement or association with another biomaterial. The preservation of bone tissue volume observed in this trial after using the graft material may be directly related to the height of soft tissue.

Another aspect to be pointed out is the flapless approach used in this study. Pre-clinical studies have shown that mucoperiosteal flap elevation contributes to additional resorption after tooth extraction. Araújo et al. [12] histologically evaluated the effect of surgical trauma on soft and hard tissue remodeling during the flap approach and observed attachment and bone loss. In extracted areas exposed by mucoperiosteal flap, osteoclasts were observed and signs of resorption were seen [13]. Fickl et al. [11] compared the effect of the flap and flapless approaches on the resorption rate after tooth extraction. The author found that the flapless approach caused lower resorption rates with or without socket preservation techniques, showing the importance of maintaining the periosteum in place. In the same way, clinical studies have confirmed the above findings with resorption observed after surgical exposure of the alveolar bone [14–16, 38]. Barone et al. [4] observed less vertical bone resorption without flap elevation. On the other hand, a systematic review [39] was inconclusive about the effect of a flap or flapless approach on the horizontal bone dimension following immediate implant placement without grafting. A clinical trial [5] compared a flapless and a flap approach with immediate implant placement and provisionalization in the aesthetic zone, observing comparable remodeling of the peri-implant mucosa, interproximal bone, and buccal ridge at 6 months and 12 months. However, differently from our study, Stoupel's [5] trial did not associate any biomaterial to the immediate implant placement, and this may be a decisive factor for soft tissue dimension preservation.

Recently, some studies have studied the effect of compression during the placement of biomaterial into the sockets on bone regeneration, showing more bone formation in this situation [40, 41]. Notwithstanding the observed positive effect of this approach, it is important to say that the discussed studies were conducted in animal models and used biomaterial (60% hydroxyapatite and 40% beta-tricalcium phosphate) that was

Table 4 Mean ± standard deviation of buccolingual measurement 1 mm, 3 mm, and 5 mm apical to the bone crest (mm)

	1 mm		3 mm		5 mm	
	Baseline	6 months	Baseline	6 months	Baseline	6 months
Test	7.04 ± 0.49**	6.57 ± 0.45*	7.12 ± 0.49**	6.65 ± 0.45*	7.15 ± 0.49**	6.69 ± 0.45*
Control	6.75 ± 0.27**	6.07 ± 0.24	6.83 ± 0.28**	6.15 ± 0.24	6.86 ± 0.27**	6.18 ± 0.24

*A statistically significant difference between the groups (two-way ANOVA/Tukey, $p < 0.05$)

**A statistically significant difference between the periods of evaluation within each group (two-way ANOVA/Tukey, $p < 0.05$)

different from what was used in our study (deproteinized bovine bone derived with collagen). Besides, as the present study was conducted clinically with immediate implant placement, the authors followed the protocol described by van Steenberghe et al. [42], in which the biomaterial (deproteinized bovine bone) was inserted with no compression in patients who received immediate implant placement.

Immediate provisionalization used in the present study is an important point to be considered when the involved area is the anterior region of maxilla, due to the aesthetic aspect required. The association of flapless immediate implant surgery, deproteinized bovine bone derived with collagen placement, and immediate provisionalization warranted soft and bone tissue preservation. Likewise, a systematic review showed that flapless implant surgery with immediate provisionalization presents lower risk of gingival recession when applied in areas with intact bone and thick gingival tissue [43]. In line, a RCT evidenced higher midfacial recession (2.4–3 times more) with delayed restoration in comparison with immediate restoration. Additionally, delayed restoration resulted in 0.75 mm of additional loss after 1 year [17].

The GAP measurement did not present significant difference between groups in this trial. Our results are in accordance with Assaf's [30] results, where it was compared with the use of bone ceramic (Straumann) and no graft material in immediate implants. Indeed, the gap did not influence the distance between the buccal and palatal bones, and the preservation of the bone volume in the test group was due to the use of deproteinized bovine bone derived with collagen. It is known that this void can repair spontaneously in a significant percentage of patients [22]. However, the use of biomaterial to fill the gap is still recommended to assure higher aesthetic outcomes and to avoid soft tissue invasion [26–28, 44]. Besides, a more palatal implant position was used in the present study, creating a larger gap that could enhance bone formation and bone-to-implant contact as demonstrated by Araújo et al. [25]. Animal studies have shown a different healing pattern with deproteinized bovine bone derived with collagen placement with a slower rate of repair [20, 37]. The particles act as a scaffold for bone formation, and some of them (the smaller ones) integrate into the woven bone. Nonetheless, there is a concomitant resorption of the buccal and lingual walls, and this could be the reason for the absence of difference between the groups in our trial.

A cone beam tomographic evaluation was adopted in this trial for bone thickness measurements. This is a non-invasive scanning that offers high spatial resolution and reduced radiation dose and costs when compared to conventional computed tomography [45, 46]. This type of analysis allows volumetric and exact evaluation, once the measures are performed by computer software. However, one limitation is the incapability of differentiation between newly formed bone and biomaterial.

The outcomes from this investigation suggest a positive effect in associating flapless immediate implants with socket preservation using deproteinized bovine bone derived with collagen and immediate provisionalization. The reduced bone volume loss and preservation of soft tissue height are important aspects to warrant the long-term functional and aesthetic success of dental implant therapy, especially in the anterior region. The results of this study provide a basis for future studies comparing other graft materials during immediate implant techniques in the aesthetic zone. This information could be used to determine the best treatment strategy and method of ridge preservation to benefit implant outcomes and lead to favorable prosthesis rehabilitation. Long-term follow-up is necessary to confirm the positive effect of the used clinical approach. Considering the discussed points, it can be concluded that the use of deproteinized bovine bone derived with collagen with flapless immediate dental implants and provisionalization was more effective in preserving soft and bone tissue.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the local ethical committee of Paulista University (56342516.8.0000.5512).

Informed consent Informed consent was obtained from all individual participants included in the study.

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