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ORIGINAL ARTICLE

Immediate placement of one-piece zirconia implants with or without xenograft into the buccal gap. Soft tissues as secondary outcomes of an experimental in vivo study

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Abstract

Objective: To histologically evaluate soft tissue healing following immediately placed one-piece zirconia implant and grafting a xenograft into the buccal gap.

Materials and Methods: The third and fourth premolars (PM3 and PM4) in both quadrants of the mandible of nine dogs were used for this experiment. Those teeth were removed flapless and implants were placed into the distal sockets in a lingual position. In one side of the jaw, the gap between the implant and the socket walls was grafted (test) while no grafting was performed in the contralateral side (control), randomly selected. After 6 months of healing, biopsies were obtained and prepared for histological analysis. Soft tissue measures like supracrestal soft tissue height (STH), length of barrier epithelium (BE), and connective tissue (CTC) were measured at buccal and lingual surfaces.

Results: The marginal mucosa was in a coronal position on the test side compared with the control side. At the buccal surface, the BE was longer in the test side than in the control side, while the CTC was longer in the control side than in the test side. For the STH (BE + CTC), the difference between the groups was not statistically significant.

Conclusion: The placement of a xenograft into the gap between a 1-piece zirconia implant and the buccal wall in dogs modified the process of soft tissue healing, providing less soft tissue recession. The gap size seems to have a modifying effect on the application of this protocol.

KEYWORDS
barrier epithelium, bone graft, connective tissue, dental implant, soft tissue, zirconia

1 | INTRODUCTION

In recent years, zirconia dental implants have emerged as an alternative to titanium implants (Özkurt & Kazazoğlu, 2011) and it is known that the integration of the ZrO2 ceramic implant into bone tissue does not differ from a titanium implant (Liñares et al., 2016). Zirconia implants, such as titanium implants, demonstrate a soft and hard tissue integration capacity but titanium tended to show a faster

initial osseointegration process compared to zirconia (Roehling et al., 2019). However, a 1-piece zirconia implant has proven to have the same survival rate as titanium implants in single-unit restorations and three-unit fixed dental prostheses over a mid-term period (Balmer et al., 2020; Bormann et al., 2018). Despite the fact that zirconia can be used as an alternative implant material, many questions remain unanswered regarding ideal material composition, long-term stability, implant design, the implant-abutment interface, implant-restorative complex, and soft tissue responses (Nishihara et al., 2018). With regard to titanium implants, it is known that flapless immediate implant surgery produces a significant reduction in the vestibular biologic width and a minor reduction in buccal bone plate resorption (Blanco et al., 2008).

As far as gap grafting, Favero, Lang, et al. (2013) found that the use of deproteinized bovine bone mineral (DBBM) particles to fill buccal defects of 2.5 mm or more at titanium implants installed immediately into alveolar extraction sockets did not preserve the buccal bony wall. This author had results contrary to those found by Araújo et al. (2011) in dogs and Sanz et al. (2017) in humans, that found that placing a demineralized bovine bone mineral with 10% collagen (DBBM-C) in the gap significantly reduced the horizontal bone resorptive changes occurring in the buccal bone after the immediate implantation in fresh extraction sockets. Regarding zirconia implants, it is known that the placement of DBBM-C in the gap between a 1-piece zirconia implant and the buccal wall in dogs modified the process of hard tissue healing providing an additional amount of hard tissue (Alves et al., 2021), that is, similar results to those found by Araújo et al. (2011) and Sanz et al. (2017) with titanium implants. Benic et al. (2017) have concluded that zirconia and titanium implants grafted with DBBM granules and covered with a collagen membrane did not perform differently regarding the augmented ridge contour, the new bone formation and the implant osseointegration. This author also concluded that for peri-implant defects at zirconia implants, the application of DBBM granules and collagen membranes revealed the most favorable results regarding the augmented ridge contour.

Preclinical studies on the behavior of soft tissues around zirconia implants presenting measures of barrier epithelium (BE), connective tissue (CTC), and supracrestal soft tissue height (STH) are few and there are no published studies on soft tissues around immediate one-piece zirconia implants with or without gap grafting. With regard to quantitative and qualitative peri-implant soft tissue dimensions Roehling et al. (2019) included only six studies using three different animal models (one with pigs, four with canines, and one with monkeys) in their preclinical review and meta-analysis to evaluate if zirconia implants demonstrate differences in hard and soft tissue integration compared to titanium implants. In the four studies using canines one presents BE, CTC, and STH (Igarashi et al., 2015), one presents BE and CTC (Thoma et al., 2015), one just presents STH (Delgado-Ruiz et al., 2014) and another just presents BE (Koch et al., 2013). Regarding peri-implant soft tissues, qualitatively and quantitatively similar soft tissue integration was reported by Roehling et al. (2019) for zirconia compared to titanium implants.

Bienz et al. (2021) revealed similar clinical outcomes for zirconia and titanium dental implants under healthy conditions. Lower plaque and bleeding scores were found around zirconia implants under experimental mucositis conditions. No significant differences between groups were found for the majority of the histological results, including the number of inflammatory cells and the length of the barrier epithelium.

The objective of the present experimental study was to histologically evaluate soft tissue healing following grafting a xenogenous bone substitute into the buccal gap around the immediately placed one-piece zirconia implant.

2 | MATERIALS AND METHODS

This publication reports the soft tissue outcomes derived from the publication: Immediate one-piece zirconia implants with/without xenograft in the buccal gap: a 6-month pre-clinical study (Alves et al., 2021). Since it is the same study, the Materials and Methods are the same except for the points observed in the histological examination. Therefore, in this publication, the materials and methods are presented in a summarized form.

2.1 | Animals and ethical statement

All in vivo procedures in nine healthy adult female Mongrel Hound dogs were approved by the Ethical Committee of the Rof Codina Foundation (reference number: 01/17/LU – 001) before the initiation of the trial. The animals were subjected to surgeries and housed in the Animal Experimental Facility of the Rof Codina Foundation (Cebiovet). This paper was written following the ARRIVE guidelines (Kilkenny et al., 2010).

2.2 | Study design and randomization

This study follows the same design as the original study, which was designed as a randomized controlled trial for the comparison of two treatment procedures in one healing period. The study was performed in one surgical phase including flapless tooth extraction of third and fourth lower premolars and immediate zirconia implant placement in distal alveoli with spontaneous healing (control; Figure 1) or ridge preservation with simultaneous grafting of alveolar buccal gaps (test; Figure 2), using a xenogenic bone substitute material containing 10% collagen (DBBM-C, Bio-Oss® Collagen, Geistlich Pharma AG).

2.3 | Surgical procedures

All surgical procedures were carried out under sterile conditions, in an animal operating theatre. The PM3 and PM4 in both quadrants

of the mandible were carefully extracted. The recipient sites were prepared for implant installation and a total of 36 one-piece Pure Ceramic Implants (Straumann® Dental Implant System; Narrow Implant, 3.3 mm wide and 8 mm long) were installed by a single dentist with experience in implantology. Each implant was placed without raising a flap in the distal socket and a lingual position. Hence, a <2 mm wide (PM3) or ≥2 mm wide (PM4) and ≥3 mm deep buccal void similar to a three-wall bone defect was established. The marginal level of the ZLA-coated surface of all implants was located flush with or slightly apical to the buccal bone crest. On one side of the mandible, the gaps were filled with Bio-Oss® Collagen (Geistlich Pharma AB; test group), while no grafting was performed on the contralateral side. Treatment allocation was concealed by means of sealed envelopes that were opened after the implant installation.

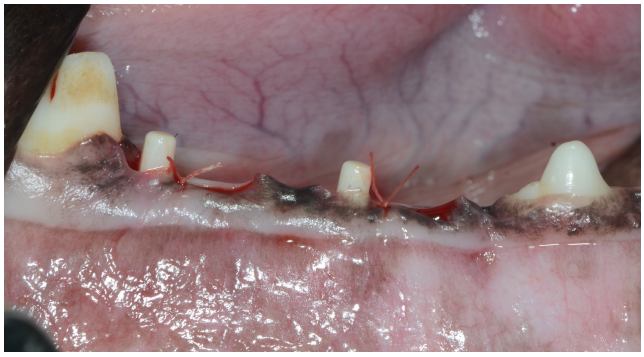

FIGURE 1 Clinical image illustrating the ridge after surgery in control side.

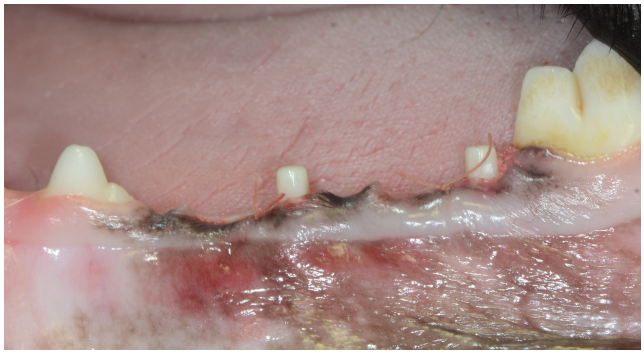

FIGURE 2 Clinical image illustrating the ridge after surgery in test side.

2.4 | Retrieval of specimens and histological preparation

After 6 months of healing, the dogs were euthanized. Subsequently, the lower jaws were dissected and fixed in buffered 10% formaldehyde solution at a temperature of 4°C for a week.

2.5 | Histological examination

The blocks containing the implant and the hard and soft tissues around the implant were obtained using an oscillating saw and identified. These blocks were dehydrated in different graded ethanol series and infiltrated with different graded mixtures of ethanol and glycometacrylate following previously published guidelines (Donath & Breuner, 1982). The samples were subsequently polymerized and heated at 37°C for 24 h to guarantee a complete polymerization. Longitudinal sections of approximately 40 µm were obtained. The slides were stained using the Levai–Laczkó method (Levai & Laczkó, 1975). The images were captured using a motorized stage transmission light microscope and a PC-based capture system and measurements were done using PC-based image analysis software. All reference points in the histologic

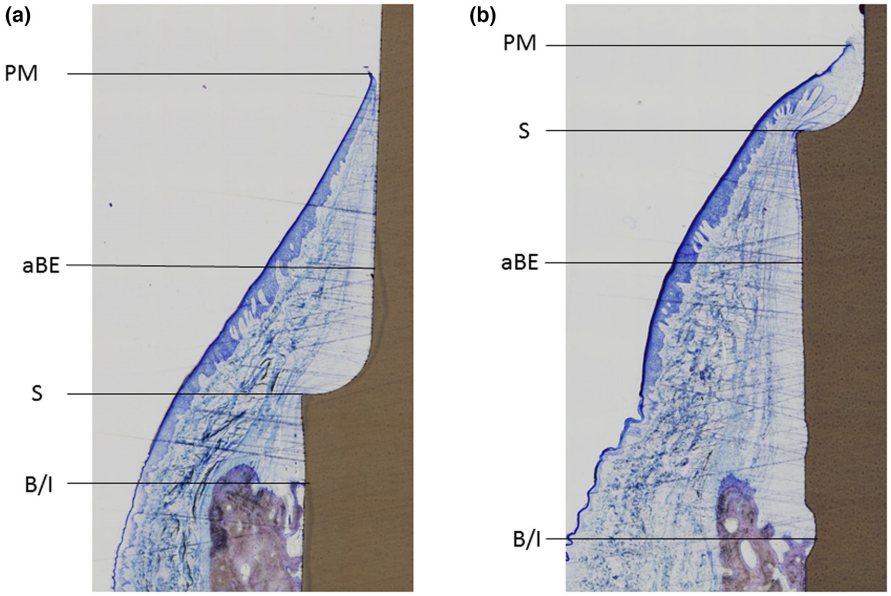

FIGURE 3 Histologic image representing the test (a) and the control (b) sites of the same dog, PM3, gap <2 mm, with landmarks of interest. PM—peri-implant mucosal margin; aBE—apical end of the epithelial attachment; S—Shoulder; B/I—the most coronal point of contact between bone and implant.

sections were independently marked by two experimented examiners and thereafter compared and discussed to aim for congruence. Measurements were then obtained. The points of interest (Figure 3) were identified from the digital histological images in order to subsequently measure the distances, which were expressed in microns.

The following landmarks were identified on both the buccal and lingual sides:

- (PM): peri-implant mucosal margin.
- (S): shoulder of the implant.
- (aBE): apical end of the barrier epithelium.
- (B/I): the most coronal point of contact between bone and implant.

The following linear measurements were made, in both groupsto determine the distance between these landmarks:

- PM-S: distance from the peri-implant mucosal margin to the implant shoulder.
- S-B/I: distance between the shoulder and the first bone–implant contact.
- PM-aBE: distance from the peri-implant margin to the apical end of the barrier epithelium, barrier epithelium length (BE).
- aBE-B/I: distance from the apical end of the barrier epithelium to the first bone implant contact, connective tissue contact length (CTC).
- PM-B/I (PM-aBE + aBE-B/I): distance from the peri-implant mucosal margin to the first bone to implant contact, supracrestal soft tissue height (STH).

2.6 | Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics for Windows (V.25.0). The primary outcome was the bone loss (ZLA--B/I), that is, the amount of bone lost during the healing period, presented in the first publication (Alves et al., 2021). The soft tissue outcomes were the variables PM-S, S-B/I, PM-aBE, aBE-B/I, and PM--B/I. Not all measures were *normally* distributed, so for the descriptive statistical analysis, the median and the interquartile ranges were applied to all outcomes (median; IQR). Before using the parametric tests for paired samples, the *Normality* assumption for the differences was checked by the Shapiro–Wilk test and it was rejected in multiple outcomes. Then, the Wilcoxon rank test was performed to evaluate the differences between the test and control sides (alternative hypothesis) followed by the estimate of the median confidence interval for the differences (Difference = Test side—Control side). In order to evaluate the modifying effect caused by the size of the gap, the same test was applied to take into account the type of gap, gap <2 mm (PM3) and gap ≥2 mm (PM4). A *p*-value with Bonferroni correction for multiple comparisons <.05 was considered to indicate statistical significance.

3 | RESULTS

Healing following a tooth extraction, implant placement, and grafting led to an event, that was a loss of an implant in a test site PM3. This implant was lost in the sixth week after implant placement due to a lack of osseointegration. The microscopic examination of the sections revealed that 35 out of the original 36 implants were well-osseintegrated. The surrounding bone tissue was comprised of mineralized bone and marrow. The mucosa surrounding all 35 implants was virtually free of clinical signs of inflammation either on the test (defect filled with Bio-Oss® Collagen) or control sides (defect not filled).

3.1 | Histometric measurements

The values for the principal outcome for soft tissue (PM-S) are shown in Table 1. Negative values mean that the peri-implant mucosal margin (PM) was coronal to the implant shoulder (S). The descriptive results are expressed in Median; Interquartile Range. For PM-S it was found that the median of the PM on the test side was at a more coronal level (−2.29; 2.17 mm) than on the control side (−1.24; 1.86 mm) with a statistically significant difference (Wilcoxon rank test $Z = -3.574$; $p < .005$). For this measurement, the difference (test-control side) was $CI_{0.95}$: −0.96; −0.36 (Table 2). Also in the measurement S-B/I, in the buccal surface, it was found that the median of the most coronal point of contact between bone and implant (B/I) on the test side was at a more coronal level (1.85; 1.01 mm) than on the control side (3.23; 1.38 mm) with a statistically significant difference (Wilcoxon rank test $Z = -3.621$; $p < .005$). For this measurement, the difference (test-control side) was $CI_{0.95}$: −1.58; −0.72 (Table 2).

In the test group for the barrier epithelium length (PM-aBE) in the buccal surface, it was registered at 2.15; 1.06 mm and the difference to the control group (1.61; 0.72 mm) was statistically significant (Wilcoxon Rank test $Z = -3.621$; $p < .005$). For this measurement, the difference (test-control side) was $CI_{0.95}$: 0.52; 1.19. For the connective tissue (CTC) in the buccal surface of the test group, it was registered 1.35; 0.74 mm and the difference to the control group (2.94; 1.09 mm) was also statistically significant (Wilcoxon Rank test $Z = -3.195$; $p = .005$). For this measurement, the difference (test-control side) was $CI_{0.95}$: −1.92; −0.97 (Table 3). However, in the test group, for the distance from the peri-implant mucosal margin to the bone crest (PM-B/I = PM-aBE + aBE-B/I) in buccal surface, it was registered 3.89; 0.94 mm and the difference to the control group (4.45; 1.35 mm) was not statistically significant (Wilcoxon Rank test $Z = -2.107$; $p = .175$). For this measurement, the difference (test-control side) was $CI_{0.95}$: −1.14; −0.15 (Table 3).

When comparing the type of the socket, PM3 and PM4, the difference between the test and control sides for PM-S was not statistically significant at the buccal aspect in PM3 (Wilcoxon Rank test $Z = -2.38$, $p = .085$) but statistically significant (Wilcoxon Rank test

TABLE 1 Database of histometric measurements (mm), PM-S (principal outcome for soft tissues), by experimental sides and type of premolar socket in buccal and lingual surfaces.

		PM-S (mm)			
		Test/grafted (n = 17)		Control/nongrafted (n = 18)	
Dog	Site	Buccal	Lingual	Buccal	Lingual
1	PM3	−0.06	−0.11	0.00	−1.24
	PM4	−1.22	−1.22	−0.53	−1.02
2	PM3	−3.43	−2.25	0.71	−1.83
	PM4	−2.96	−2.23	−1.95	−2.26
3	PM3	−0.50	−1.53	−0.50	−1.21
	PM4	−3.69	−2.12	−2.67	−1.71
4	PM3	No implant	No implant	−1.01	−0.80
	PM4	−2.53	−1.69	−2.52	−1.31
5	PM3	−0.94	0.55	0.00	−1.28
	PM4	−2.22	−2.58	−1.85	−1.98
6	PM3	−2.80	−2.08	−0.90	−0.70
	PM4	−2.29	−1.43	−1.93	−1.70
7	PM3	−1.70	−0.79	−0.85	−0.97
	PM4	−3.10	−2.21	−2.14	−1.80
8	PM3	−0.34	−0.59	0.19	−0.92
	PM4	−3.40	−2.78	−2.68	−1.61
9	PM3	−1.89	−1.66	−1.48	−1.20
	PM4	−3.71	−1.88	−3.51	−3.10
Median; IQR		−2.29; 2.17	−1.70; 1.21	−1.24; 1.86	−1.30; 0.80

Note: Negative values indicate that PM was coronal to S.
Abbreviation: IQR, Interquartile range.

TABLE 2 Histometric measurements (mm) PM-S and S-B/I, by experimental sides in buccal and lingual surfaces.

	PM-S		S-B/I	
	Buccal	Lingual	Buccal	Lingual
Test (n = 17)	**−2.29; 2.17**	−1.69; 1.21	**1.85; 1.01**	1.41; 1.52
Control (n = 18)	**−1.24; 1.86**	−1.30; 0.80	**3.23; 1.38**	1.99; 1.34
p (2-tailed)[a]	**<.005**		**<.005**	
Lower bound 95% CI[b]	**−0.96**		**−1.58**	
Upper bound 95% CI[b]	**−0.36**		**−0.72**	

Note: Results expressed in median; interquartile range (mm). Bold values show statistical significance for p-value ≤.05. Negative values indicate that PM or B/I was coronal to S.
[a]Wilcoxon Signed Ranks Test with Bonferroni correction for five comparisons (n = 17).
[b]Confidence interval (95%) for the median of the differences (n = 17).

Z = −2.666, p = .04) in PM4. The difference between the test side and the control side in PM3 and PM4 was 95% CI: −1.90, −0.06 and 95% CI: −1.02, −0.21, respectively (Table 4).

The difference between the test and control sides for S-B/I followed the same pattern, which was not statistically significant at the buccal aspect in PM3 (Wilcoxon Rank test Z = −2.521, p = .06) but statistically significant (Wilcoxon Rank test Z = −2.666, p = .04) in PM4. Furthermore, the difference between the test side and the control side in PM3 and PM4 was 95% CI: −1.66, −0.59 and 95% CI: −1.71, −0.70, respectively (Table 4).

Also for PM-aBE, the difference between the test and control sides was not statistically significant at the buccal aspect in PM3 (Wilcoxon Rank test Z = −2.521, p = .06) but statistically significant (Wilcoxon Rank test Z = −2.666, p = .04) in PM4. The difference between the test side and the control side in PM3 and PM4 was 95% CI: 0.52; 1.19 and 95% CI: 0.46; 1.27, respectively (Table 5), and the same pattern occurred in aBE-B/I, the difference between the test and control sides was not statistically significant at the buccal aspect in PM3 (Wilcoxon Rank test Z = −1.820, p = .345), but statistically significant (Wilcoxon Rank test Z = −2.666, p = .04) in PM4. The difference between the test

TABLE 3 Histometric measurements (mm) PM-aBE, aBE-B/I, and PM-B/I by experimental sides in buccal and lingual surfaces.

	PM-aBE		aBE-B/I		PM-B/I	
	Buccal	**Lingual**	**Buccal**	**Lingual**	**Buccal**	**Lingual**
Test (n = 17)	**2.15; 1.06**	1.11; 0.74	**1.35; 0.74**	1.72; 0.72	3.89; 0.94	2.86; 0.91
Control (n = 18)	**1.61; 0.72**	1.56; 0.61	**2.94; 1.09**	1.59; 0.93	4.45; 1.35	3.29; 0.67
p (2-tailed)[a]	**<.005**		**.005**		.175	
Lower bound 95% CI[b]	**0.52**		**−1.92**		−1.14	
Upper bound 95% CI[b]	**1.19**		**−0.97**		−0.15	

Note: Results expressed in median; interquartile range (mm).Bold values show statistical significance for p-value ≤.05.

[a]Wilcoxon Signed Ranks Test with Bonferroni correction for five comparisons (n = 17).

[b]Confidence interval (95%) for the median of the differences (n = 17).

TABLE 4 Histometric measurements (mm) PM-S and S-B/I, by experimental sides and type of premolar socket in buccal and lingual surfaces.

	PM-S		S-B/I	
	Buccal	**Lingual**	**Buccal**	**Lingual**
PM3				
Test (n = 8)	−1.32; 2.20	−1.16; 1.74	2.16; 1.90	2.01; 1.66
Control (n = 9)	−0.50; 1.05	−1.20; 0.40	3.47; 1.40	2.20; 0.92
p (2-tailed)[a]	.085		.06	
Lower bound 95% CI[b]	−1.90		−1.66	
Upper bound 95% CI[b]	−0.06		−0.59	
PM4				
Test (n = 9)	**−2.96; 1.29**	−2.12; 0.84	**1.69; 0.74**	1.10; 1.21
Control (n = 9)	**−2.14; 0.78**	−1.71; 0.66	**2.84; 1.35**	1.70; 1.04
p (2-tailed)[c]	**.04**		**.04**	
Lower bound 95% CI[d]	**−1.02**		**−1.71**	
Upper bound 95% CI[d]	**−0.21**		**−0.70**	

Note: Results expressed in median; interquartile range (mm). Bold values show statistical significance for p-value ≤.05. Negative values indicate that PM or B/I was coronal to S.

[a]Wilcoxon Signed Ranks Test with Bonferroni correction for 5 comparisons (n = 8).

[b]Wilcoxon Signed Ranks Test with Bonferroni correction for 5 comparisons (n = 9).

[c]Confidence interval (95%) for the median of the differences (n = 8).

[d]Confidence interval (95%) for the median of the differences (n = 9).

side and the control side in PM3 and PM4 was 95% CI: −2.09; −0.52 and 95% CI: −2.03; −0.97, respectively (Table 5).

For PM-B/I, the difference between the test and control sides was not statistically significant at the buccal aspect in PM3 (Wilcoxon Rank test Z = −0.840, p = 1) neither in PM4 (Wilcoxon Rank test Z = −2.192, p = 0.14) and the difference between the test side and the control side in PM3 and PM4 was 95% CI: −1.41; 0.67 and 95% CI: −1.3; 0.02, respectively (Table 5).

4 | DISCUSSION

The present experiment demonstrated that the placement of xenograft with 10% collagen into the buccal gap between a one-piece zirconia implant and bone walls of a fresh socket of dogs modified the process of soft tissue healing, providing a more coronal position of the peri-implant mucosal margin. This publication is the continuation of the study of Alves et al. (2021), and, therefore, the results are correlated.

Liñares et al. (2016) demonstrate a higher degree of soft tissue integration around the zirconia implant compared to the titanium because of a shorter sulcular epithelium and a higher grade of collagen organization in the zirconia implant. However, Igarashi et al. (2015) suggested that Ce-TZP/Al2O3 implants are comparable to titanium and Y-TZP implants in hard and soft tissue integration. Kohal et al. (2004) and Koch et al. (2013) suggest that the soft tissue configuration of uncoated zirconia implants appears to be similar to that of titanium implants. When a dehiscence is present in the moment of implant placement Thoma et al. (2019) observed that despite concomitant hard tissue changes in both zirconia and titanium implants a significant loss of the height of peri-implant mucosa was only observed for titanium implants.

TABLE 5 Histometric measurements (mm) PM-aBE, aBE-B/I, and PM-B/I, by experimental sides and type of premolar socket in buccal and lingual surfaces.

	PM-aBE		aBE-B/I		PM-B/I	
	Buccal	**Lingual**	**Buccal**	**Lingual**	**Buccal**	**Lingual**
PM3						
Test ($n = 8$)	1.93; 0.72	0.87; 0.74	1.24; 0.98	1.92; 1.10	3.64; 1.16	2.82; 0.72
Control ($n = 9$)	1.26; 0.64	1.42; 1.35	3.01; 1.49	1.70; 1.31	4.28; 1.75	3.26; 0.75
p (2-tailed)[a]	.06		.345		1.0	
Lower bound 95% CI[b]	0.52		−2.09		−1.41	
Upper bound 95% CI[b]	1.19		−0.52		0.67	
PM4						
Test ($n = 9$)	**2.91; 1.20**	1,26; 0,70	**1.65; 0.68**	1.72; 0.64	4.22; 0.83	3.08; 0.93
Control ($n = 9$)	**1.94; 0.44**	1,65; 0,45	**2.87; 1.46**	1.57; 0.82	4.81; 1.24	3.36; 0.92
p (2-tailed)[c]	**.04**		**.04**		.14	
Lower bound 95% CI[d]	**0.46**		**−2.03**		−1.30	
Upper bound 95% CI[d]	**1.27**		**−0.97**		0.02	

Note: Results expressed in median; interquartile range (mm). Bold values show statistical significance for p-value ≤.05.

[a]Wilcoxon Signed Ranks Test with Bonferroni correction for 5 comparisons ($n = 8$).

[b]Wilcoxon Signed Ranks Test with Bonferroni correction for 5 comparisons ($n = 9$).

[c]Confidence interval (95%) for the median of the differences ($n = 8$).

[d]Confidence interval (95%) for the median of the differences ($n = 9$).

Araújo et al. (2011), with a socket preservation protocol using tissue-level titanium implants, found the margin of the peri-implant mucosa (PM) in both groups (with/without gap preservation) coronal to the implant shoulder (S) but more coronal in the test group than in the control group, 1.80 ± 0.8 mm versus 0.80 ± 0.6 mm. In the present experiment, using one-piece tissue-level zirconia implants, similar results were found, PM was 2.29; 2.17 mm coronal to S in the test group versus 1.24; 1.86 mm in the control group, with a statistically significant difference, so the placement of Bio-Oss® Collagen in the buccal gap between a one-piece zirconia implant and bone walls of a fresh socket in the dog provided a coronal migration of the peri-implant mucosal margin. It is important to note that since no abutments were placed soft tissue could migrate over the implant shoulder.

The distance between the shoulder (S) and the most coronal point of contact between bone and implant (B/I) was 3.23; 1.38 mm in the control group and 1.85; 1.01 mm in the test group, with statistically significant difference. These results were very similar to the results found by Araújo et al. (2011) which were 3.1 ± 0.7 mm in the control group and 1.9 ± 0.5 mm in the test group. Was expected and it was observed that coronal migration of the bone induces coronal migration of the peri-implant mucosal margin.

For the supracrestal soft tissue height (STH: PM-B/I) the results were 3.89; 0.94 mm for the test group and 4.45; 1,35 mm for the control group, without statistically significant differences. As stated in the previous paragraph, the position of B/I is directly related to the position of PM. These measures followed the trend of those found by Araújo et al. (2011) using titanium implants in a gap preservation model.

For the barrier epithelium (BE: PM-aBE) the present investigation found longer BE in the test group (2.15; 1.06 mm) in relation to control group (1.61; 0.72 mm). Similar results were found by Araújo et al. (2011) using titanium implants. Regarding the connective tissue length, this study found longer CTC (aBE-B/I) in the control group (2.94; 1.09 mm) in relation to the test group (1.35; 0.74 mm). In contrast, the paper by Araújo et al. (2011) found similar lengths in both groups. In summary, the supracrestal soft tissue length seems to be similar when gap preservation is performed as when gap preservation is not performed but the soft tissue is in a coronal position and there is more epithelial tissue lengths when gap preservation is performed.

When looking at the study of Liñares et al. (2016) it is observed that one-piece zirconia implants placed in healed sites in a dog model presented the median measures for STH (3.84 mm), BE (1.93 mm), and CTC (1.58 mm) similar of what it is described at the present study: STH (3.89 mm), BE (2.15 mm), and CTC (1.35 mm). The same is observed in the study of Igarashi et al. (2015) with one-piece zirconia implants placed in healed sites in a dog model finding measures of STH (3.28 mm), BE (2.19 mm), and CTC (1.09 mm) and also to the results reported by Lim et al. (2018). Although there is no direct comparison, it seems that the placement of Bio-Oss® Collagen in the buccal gap between an one-piece zirconia implant and bone walls of a fresh socket results in a similar soft tissue configuration that the placement of an one-piece zirconia implant in a healed site.

When the gaps are distinguished for the measures PM-S and S--B/I statistically significant differences were found only for PM4. So, for this protocol, there seems to be less advantage in placing a xenograft in the buccal gap with <2 mm than when the gap has 2 mm or

more. Even though, the values in the test group for PM3 are better than the values in the control group.

For the measures BE (PM-aBE) and CTC (aBE-B/I) occurs the same, statistically significant differences were found for PM4 but not for PM3. It seems that in narrower gaps the placement of a xenograft does not produce such strong effects.

It is important to mention that in the present study adjacent teeth were completely extracted with implant placement in the distal roots and the mesial alveolus was left untouched. On the contrary, Araújo et al., 2011 only extracted one root and made the endodontic treatment of the adjacent root. For evaluation of soft tissues, this is an important issue, indeed the extraction of teeth adjacent to a socket into which implants were installed immediately after tooth extraction caused more alveolar bone resorption both for the buccolingual and at the mesio-distal aspects compared with sites adjacent to a maintained tooth (Favero, Botticelli, et al., 2013). It is important to highlight that in the present experiment this limitation affected all study groups, thus did not change the comparisons between them. On the contrary, in a split-mouth design as adopted, the Wilcoxon Rank test for evaluation of the treatment effect does not allow to assess of the possible site effect. Moreover, the potential carry-across effect has not been assessed. However, it is not believable that there can be a leakage effect between the two sides.

Another limitation of this experiment is that it was not possible to precisely determine the position of the implant in the apical-coronal direction, since it was a flapless surgery what we can say is that the implants were located flush with or slightly apical to the buccal bone crest. Also because of flapless surgery, which makes it very difficult to measure the bone without detaching the periosteum, this study lacks data about the initial size of the alveoli. The only exception was to measure the gap size after implant placement.

In vivo experiments on soft tissue integration to implants can provide an indication that such results may be applicable to the clinical setting but they need to be confirmed and validated in clinical trials in humans. Therefore, this study should be seen as a proof of concept.

5 | CONCLUSION

Within the limitations of this study, it can be concluded that the placement of DBBM-C into the gap between immediate one-piece zirconia implants and the buccal wall in dogs modified the process of soft tissue healing, providing less mucosal recession due to less bone recession. There are also changes in the length of the barrier epithelium and connective tissue.

AUTHOR CONTRIBUTIONS

Álvaro Azevedo: Data curation (equal); formal analysis (equal); methodology (equal); supervision (equal); writing – original draft (equal). **Antonio Liñares:** Conceptualization (equal); methodology (equal); validation (equal); visualization (equal). **Daniel Alves:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (lead); investigation (lead); methodology (equal); project administration (lead); resources (lead); software (equal); validation (equal); visualization (equal); writing – original draft (lead); writing – review and editing (lead). **Fernando MuÑoz:** Data curation (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing – original draft (equal). **Juan Blanco-Carrión:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal). **Ricardo Faria e Almeida:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal).

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DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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