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Hard and soft tissue healing around teeth prepared with the biologically oriented preparation technique and restored with provisional crowns: An in vivo experimental investigation

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Abstract

Aim: To evaluate the hard and soft tissues healing around teeth prepared with the biologically oriented preparation technique (BOPT) versus the chamfer technique versus non-prepared teeth.

Materials and Methods: Thirty-two teeth in eight beagle dogs were randomly prepared with the BOPT (test = 16) or chamfer (control = 16) techniques and covered with polymethylmethacrylate crowns as provisional restorations. Sixteen negative controls (non-prepared teeth) were also used for comparison. Histological description and histomorphometrical measurements of the periodontal tissues were collected at 4 and 12 weeks in 7 out of 8 dogs, including the soft tissue height and thickness, and the horizontal and vertical bone dimensions.

Results: When compared with negative controls, test and control preparation techniques exhibited a more apical location of the free gingival margin with respect to the cement-enamel junction ($\Delta = 1.1$ mm for both groups at 4 weeks ($p < .05$), 0.99 mm for the test group ($p = .043$) and 0.20 mm for control group ($p = 1.000$) at 12 weeks). There were no significant differences between test and control groups with respect to vertical and horizontal histometric measurements.

Conclusions: The BOPT and chamfer tooth preparation protocols induced similar qualitative and quantitative changes in the healing of the supra-crestal soft tissue complex, when compared with non-prepared teeth. Despite the limited amount of power, it appeared that differences between the tested preparation techniques were not statistically significant.

KEYWORDS
animal model, dental prosthesis, dental restoration, temporary, histology, comparative

Clinical Relevance

Scientific rationale for study: There is a lack of knowledge regarding how different preparation geometries affect the healing and structural organization of the periodontal tissues.

Principal findings: Both preparation techniques were associated with (1) a mild inflammatory infiltrate located in the most coronal part of the soft tissues; (2) the development of a soft tissue profile that followed the emergence profile of the provisional crown and (3) a more apical gingival margin as compared with unprepared teeth.

Practical implications: Understanding the healing characteristics elicited by different preparation protocols would be useful for both clinical practice and for the design of future clinical studies.

1 | INTRODUCTION

The biologically oriented preparation technique (BOPT) (Loi & Di Felice, 2013) is a prosthetic rehabilitation protocol based on the following co-interventions:

a. Delivery of a vertical tooth preparation extended within the space of the gingival sulcus, which eliminates the emergence profile of the cement-enamel junction (CEJ);
b. Creation of a horizontal space within the sulcus, between the abutment surface and the sulcular epithelium, as a consequence of the subgingival vertical tooth preparation;
c. Sealing of this space and provision of mechanical support through the emergence profile of a direct provisional crown, which is delivered right at the end of the tooth preparation, and places the prosthetic margin coronal to the bottom of the preparation.

The combined effect of these interventions aims to increase the availability of horizontal space for the supra-crestal soft tissue complex, in the area between the bottom of the preparation and the margin of the provisional crown, thus promoting, during healing, a thickening of the phenotype in the soft tissues surrounding the newly placed restoration.

In the last years, the use of the BOPT protocol in prosthetic dentistry has been supported by prospective case series (Agustin-Panadero et al., 2021; Serra-Pastor et al., 2019) and clinical trials comparing it to the standard of care chamfer technique (Agustin-Panadero et al., 2021; Paniz et al., 2016).

Although these clinical studies reported stable gingival margins and absence of complications when adopting the BOPT protocol, there is still lack of knowledge regarding how this technique affects the healing pattern, the morphology and the structural organization of the periodontium of the treated teeth.

It was therefore the objective of the present in vivo preclinical investigation, to assess the short-term morphological and dimensional changes that occur within the periodontium of teeth rehabilitated with the BOPT (test) or the chamfer technique (positive control), as compared with unprepared teeth (negative control). Specifically, the primary outcome was the position and dimension of the supra-crestal soft tissues with respect to the CEJ, whereas the secondary outcomes assessed the hard tissue changes.

This study represents the second part of a larger project, which following the triple R concept optimized the adoption of an experimental in vivo model also to assess the hard and soft tissue healing at implants with a modified neck configuration compared with conventional bone-level implants. Results from this first investigation have been recently published in an independent report (Palombo et al., 2021).

2 | MATERIALS AND METHODS

2.1 | Experimental design

The present study was designed as a preclinical in vivo investigation, where each animal provided the test and the corresponding controls in two different healing times (early at 4 weeks and delayed at 12 weeks after tooth preparation). This study was designed following the modified Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for reporting experimental preclinical investigations (Vignoletti & Abrahamsson, 2012) and in compliance with the current Spanish and European Union norms (European Communities Council Directive 86/609/EEC) regulating in vivo experimentation. The experimental phase of this investigation was conducted at the 'Centro de Cirugía de Mínima Invasión Jesús Usón' in Cáceres, Spain, once the implants and teeth study protocols had been approved by the local Ethical Committee (REGA code: ES 100370001499). Test and control teeth were prepared in both hemi-mandibles using a randomized group distribution. Randomization was generated using a computerized random block design, where the hemi-mandible side (left or right) and tooth position (mesial or distal) were introduced as balancing factors, assuring equal conditions for experimental and control groups.

2.2 | Sample and facilities

Eight adult beagle dogs between 1.5 and 2 years old and with a weight ranging between 10 and 20 kg were housed in purpose-designed kennels in a 12:12 light/dark cycle, at a temperature of 22–21°C, and fed on a soft pellet diet. Every animal received an identification code labelled in a sub-cutaneous RFID chip. Experienced veterinary doctors monitored these animals during the entire course

of the experimental study. Due to the descriptive nature of the study, sample size was not determined based on expectance of statistical power, but using a conventional number of eight experimental animals.

2.3 | Surgical and prosthetic procedures

2.3.1 | Intervention 1: Root extractions

Using a computer-generated random allocation sequence (Figure A1), in one hemi-mandible of each experimental animal, the teeth 1M1, 4P4, 3P3 and 2P2 were hemisected and the mesial root of 1M1 and 3P3 and the distal of 4P4 and 2P2 were extracted (Figure 1). This protocol provided three single-rooted experimental sites for tooth preparation, which were endodontically treated and the pulp chambers were filled with a composite material (Filtek One Bulk, 3M ESPE, USA). The adjacent sockets were left to heal spontaneously, providing two edentulous areas in each hemi-mandible.

2.3.2 | Intervention 2: Root extractions, tooth preparation and provisional restoration

Eight weeks after the first surgical intervention, the contralateral hemi-mandible received the same protocol including extractions, root canal treatment and maintenance of single-rooted teeth. In the same surgical intervention, the residual mesial root of 4P4 and the distal root of 3P3, in the contralateral hemi-mandible, were randomly assigned using computer-generated codes, to be prepared with either the BOPT (test) or the chamfer (control) technique, with subsequent insertion of a provisional crown.

The BOPT preparation technique was aimed to eliminate the natural emergence profile of the tooth by creating a vertically shaped preparation extended 1.5 mm subgingivally, using a series of flame-shaped diamond burs with a decreasing grit from 125 to 20 µm, specifically designed for this BOPT protocol (BOPT preparation drills; Sweden and Martina, Italy). Once the tooth was prepared, a provisional restoration was fabricated with heat-polymerizing polymethylmethacrylate (PMMA) acrylic resin (C&B V Dentine; Major Prodotti Dentari, Moncalieri, Italy) and relined with auto-polymerizing PMMA acrylic resin (Jet; Lang Dental Mfg Co, Wheeling, IL, USA) in order to place the restorative margin 0.5 mm below the gingival margin, This restoration was aimed to restore a new prosthetic emergence profile replicating the original tooth morphology, using as a guide the preoperative silicon impressions taken at baseline.

In the control group, the tooth preparation achieved a standard chamfer finishing line with a 1 mm axial reduction located 0.5 mm below the gingival margin, using chamfer diamond burs with a decreasing grit from 151 to 25 µm (856 series chamfer burs, Komet—Gebr. Brasseler GmbH & Co. KG, Germany). Similarly, PMMA

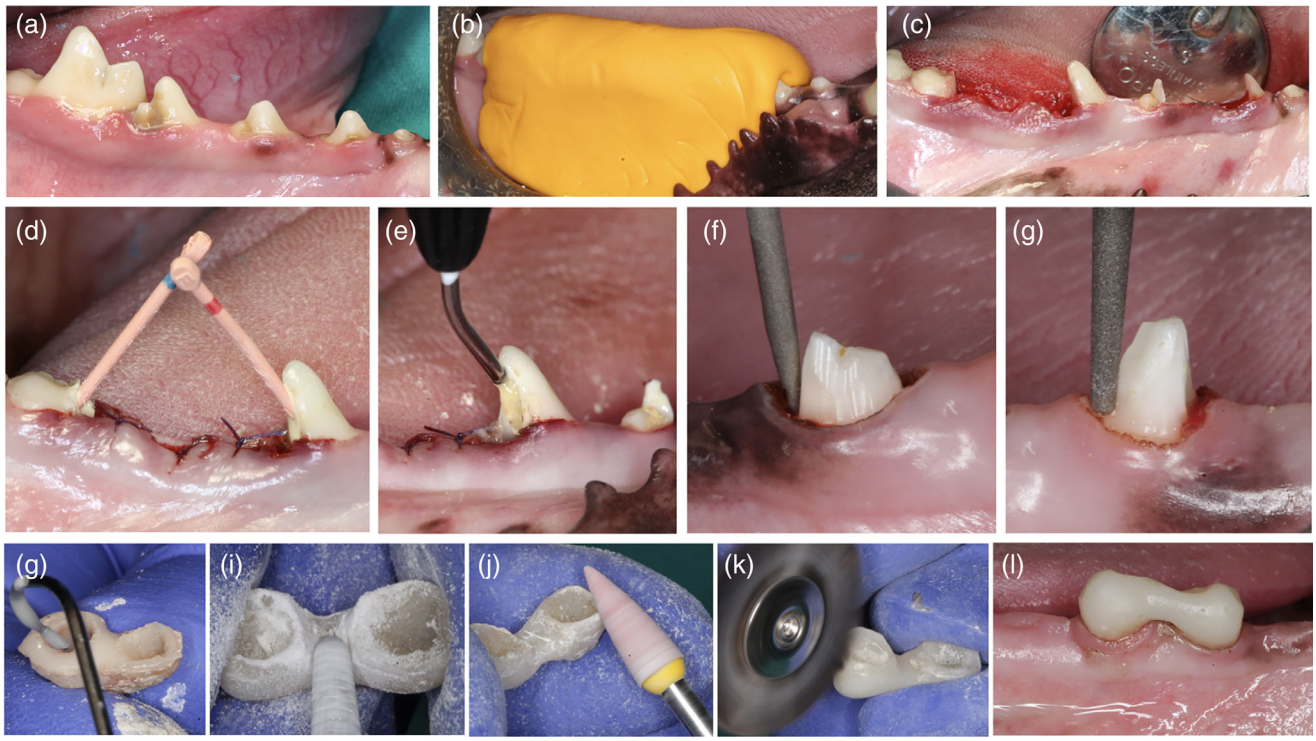

FIGURE 1 Sequence of clinical interventions performed at the first surgical session: (a) Baseline; (b) preoperative silicon impression to guide the fabrication of the provisional crowns; (c) teeth hemisection and extraction of the mesial root of 1M1 and 3P3 and the distal of 4P4 and 2P2; (d) endodontic treatment of the residual roots; (e) sealing of the endodontically treated roots with composite material (Filtek One Bulk, 3M ESPE, USA); (f) biologically oriented preparation technique preparation (test); (g) chamfer preparation (active control); (h–k) fabrication of the acrylic provisional crowns; and (l) provisional crowns cemented in situ.

provisional crowns were constructed using a silicon impression to reproduce the original tooth morphology and were delivered by locating the restorative margin at the finishing line. All the preparations were performed under 4.5× magnification using a 40.000-rpm hand piece. In both groups, a 1.5 mm incisal reduction was performed during the preparation and provisional crowns were cemented with a temporary cement (Temp Bond Clear, Kerr Dental, Orange, CA, USA). The provisional restorations were splinted in the occlusal third of the crown to increase their retention and thus to avoid its loss during healing.

2.4 | Intervention 3

Eight weeks after the second intervention, the same tooth preparation and provisional manufacturing protocols were replicated in the contralateral hemi-mandibles.

2.5 | Post-surgical care

Post-operatively, analgesic and antibiotic medications were administered. Animals were fed with a soft diet and plaque control was assured using a solution of chlorhexidine 0.12% and CPC 0.05% (PerioAid Tratamiento, Laboratorios Dentaid. Barcelona, Spain) sprayed on both hemi-mandibles 2 days per week. Once a week, the surgical areas were brushed using a conventional manual toothbrush and a chlorhexidine solution. At these weekly visits, the status of the periodontal and peri-implant tissues was assessed and if inflammation was present it was documented.

2.6 | Euthanasia

Animals were sacrificed 4 weeks after the third intervention through an overdose of sodium pentothal (40–60 mg/kg/i.v., Dolethal, Vetoquinol, France). Each animal provided two hemi-mandibles (4- and 12-week healing times), which were freed from their attached tissues and sectioned between the central incisors in two halves. Then they were placed into a sealable container containing a 4% formalin solution and stored in a secure area at a constant temperature (5°C) from the time of collection until the histological processing. From each of these specimens, three tissue blocks containing 1 BOPT-prepared tooth, 1 chamfer-prepared tooth and 1 unprepared tooth were used in this investigation.

2.7 | Histological processing

Tissue blocks from seven out of eight animals were processed by ground sectioning following the methods described by Donath and Breuner (1982), first through dehydration in a graded series of ethanol and then by embedding the blocks in methyl methacrylate. The resulting blocks were cut in a bucco-lingual plane and the central section was further grounded and polished until reaching a final thickness of approximately 30 µm (Exakt, Hamburg-Norderstedt, Germany). These sections were then stained using the Levai Laczko method (Jeno & Geza, 1975; Figure 2). The tissue blocks from one randomly selected animal (#7) were processed by decalcification following a modification of the 'fracture technique' (Berglundh et al., 1994) and its results will be reported in an independent report. The present manuscript focuses on the descriptive histology and histometric measurements performed on the sections from the non-decalcified blocks.

2.8 | Histological analysis

High-resolution images of the ground sections were acquired using an automated slide scanner system (Axio Scan Z1, Carl Zeiss Microscopy) and evaluated and measured by histomorphometry in duplicate by two independent and calibrated examiners (DP and MR) using a dedicated image analysis software (Zen lite Blue software, Carl Zeiss Microscopy). Intra-class correlation coefficients were generated to estimate the intra- and inter-examiner reproducibility, based on repeated measurements performed by the two examiners on animal #1. In order to avoid observer bias, repeated measurements were performed a week apart and the mean from the duplicate measurements was used for the statistical analysis.

2.8.1 | Histomorphometry of the hard and soft tissues

The following landmarks were used in the analysis:

- cement-enamel junction (CEJ);
- free gingival margin (FGM);
- apical border of the barrier epithelium (aBE);
- apical border of the provisional restoration (Prov);
- apical border of the preparation (Prep);
- bone crest (Bc).

The following vertical and horizontal measurements (expressed in millimetres) were evaluated on the buccal and lingual aspects of each tooth (Figure 3).

a. Hard tissues
   - bone crest relative to the CEJ (CEJ-Bc);
   - bone crest relative to the apical border of the provisional (Bc-Prov);
   - bone crest relative to the apical border of the preparation (Bc-Prep);
   - width of the bone crest 1, 2 and 3 mm apically to the peak of the crest (Bcw 1, 2 and 3).
b. Soft tissues
   - height of the supra-crestal soft tissues (FGM-B);

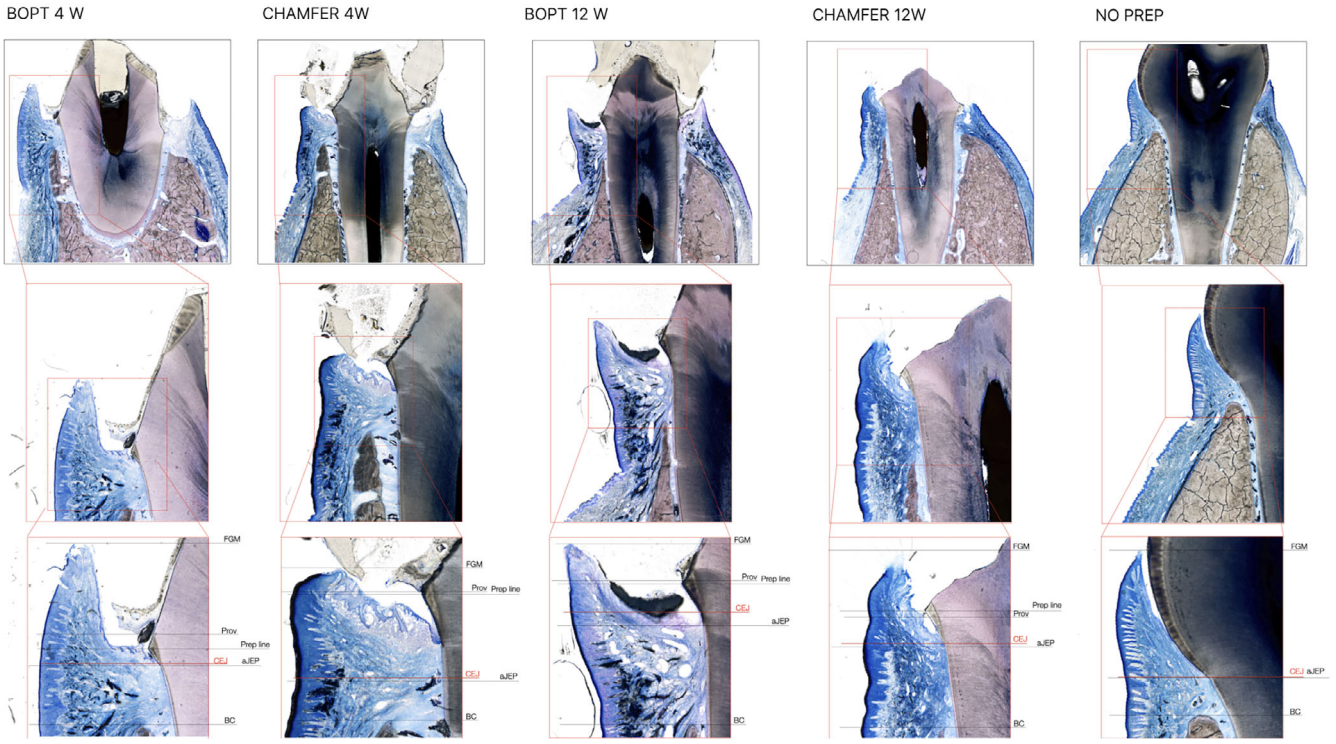

FIGURE 2 Incremental magnifications of the bucco-lingual histologic ground sections representing: (a) Test abutments at 4 weeks; (b) control abutments at 4 weeks; (c) test abutments at 12 weeks; (d) control abutments at 12 weeks; and (e) non-prepared teeth. BC, bone crest; CEJ, cement-enamel junction; FGM, free gingival margin; Prep line, apical border of the preparation; Prov, apical border of the provisional restoration.

- height of the barrier epithelium (FGm-aBE);
- height of the connective tissue attachment (aBE-B);
- gingival margin relative to the CEJ (FGM-CEJ);
- gingival margin relative to the apical border of the provisional (FGM-Prov);
- gingival margin relative to the apical border of the preparation (FGM-Prep);
- width of the gingiva at the level of the CEJ (Gth-CEJ);
- width of the gingiva at the level of the apical border of the provisional (Gth-Prov);
- width of the gingiva at the level of the apical border of the preparation (Gth-Prep);
- width of the gingiva 1, 2 and 3 mm apically to the FGM (Gth 1, 2 and 3).

All vertical soft tissue measurements were performed in duplicate: (i) first a conventional linear, point-to-point measurement, to assess the vertical dimension of the supra-crestal soft tissues; (ii) then a continuous line measurement tracing the profile of the supra-crestal soft tissues, thus assessing the true profile dimension (Tomasi et al., 2014).

2.9 | Statistical analysis

Outcome measurements were expressed as means and standard deviations (±SDs), considering the animal as the experimental unit of analysis. After performing normality tests (Shapiro–Wilk test), if data followed a normal distribution, the one-way ANOVA test with Bonferroni correction was used to assess the differences between the test and control teeth. When data did not follow a normal distribution, the non-parametric test of Kruskal–Wallis was used. Differences were considered as statistically significant when p was <.05. The statistical analysis was performed using the software SPSS 24.0 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Clinical outcomes

Healing was uneventful in seven out of eight animals; however, in one animal (#5), the advent of an endometriosis during the study caused its death. In the rest, their behaviour as well as their eating and drinking habits remained normal throughout the course of the study. Moreover, all teeth and implants were retained during the experimental period, as well as the provisional restorations inserted on the prepared teeth (Figure A2).

3.2 | Descriptive histology

At 4 weeks of healing, the overall histological picture was very similar among test, control and negative control sites, resembling the image of a healthy periodontium. When looking at the supra-crestal soft tissues,

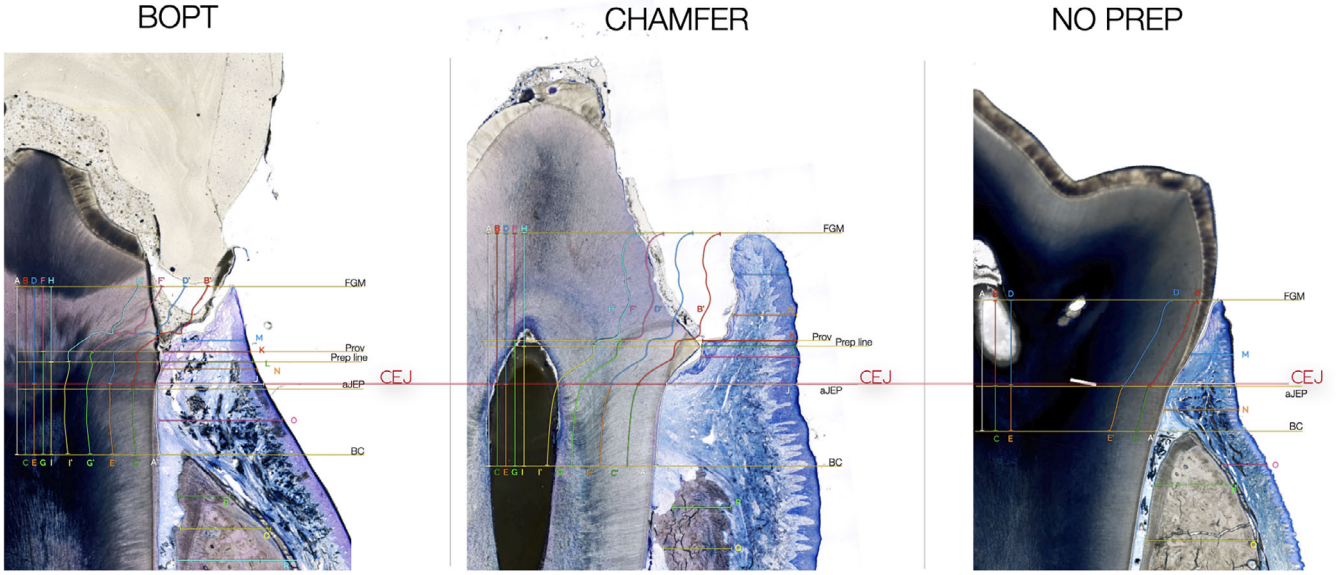

FIGURE 3 Vertical linear/continuous and horizontal measurements of hard and soft tissues at test, positive control and negative control sites: (a) Height of the supra-crestal soft tissues (FGM-B)—linear measurement; (a′) height of the supra-crestal soft tissues (FGM-B)—continuous line; (b) height of the barrier epithelium (FGm-aJE)—linear measurement; (b′) height of the barrier epithelium (FGm-aJE)—continuous line; (c) height of the connective tissue attachment (aJE-B)—linear measurement; (c′) height of the connective tissue attachment (aJE-B)—continuous line; (d) gingival margin relative to the cement-enamel junction (CEJ) (FGM-CEJ)—linear measurement; (d) gingival margin relative to the CEJ (FGM-CEJ)—continuous line; (e) bone crest relative to the CEJ (CEJ-Bc)—linear measurement; (e) bone crest relative to the CEJ (CEJ-Bc)—continuous line; (f) gingival margin relative to the apical border of the provisional (FGM-Pv)—linear measurement; (f′) Gingival margin relative to the apical border of the provisional (FGM-Pv)—continuous line; (g) bone crest relative to the apical border of the provisional (B-Pv)—linear measurement; (g′) bone crest relative to the apical border of the provisional (B-Pv)—continuous line; (h) bone crest relative to the apical border of the preparation (B-Prep)—linear measurement; (h′) bone crest relative to the apical border of the preparation (B-Prep)—continuous line; (i) gingival margin relative to the apical border of the preparation (FGM-Prep)—linear measurement; (i′) gingival margin relative to the apical border of the preparation (FGM-Prep)—continuous line; (j) width of the gingiva at CEJ (Gth-CEJ); (k) Width of the gingiva at provisional (Gth-Pv); (l) width of the gingiva at prep line (Gth-Prep); (m) width of the gingiva 1 mm apically to free gingival margin (FGM) (Gth 1); (n) width of the gingiva 2 mm apically to FGM (Gth 2); (o) width of the gingiva 3 mm apically to FGM (Gth 3); (p) bone thickness 1 mm apical to BC (Bcw1); (q) bone thickness 2 mm apical to BC (Bcw2); and (r) bone thickness 3 mm apical to BC (Bcw3).

the gingiva presented similar characteristics among the three groups with a keratinized, stratified squamous oral epithelium organized in rete ridges. The sulcular and junctional epithelium organization and structure were also similar, although a higher number and deeper rete ridges were observed in the sulcular epithelium at test and control sites. When looking at the lamina propria, an inflammatory infiltrate, however, was frequently present limited to the connective tissue adjacent to the barrier epithelium in test sites, in areas in close vicinity with the provisional restorations. Signs of inflammation were similarly detected in control sites, but to a minor extent and not in all sections. No inflammatory infiltrate was observed at negative control sites. On the other hand, the organization and structure of the supra-crestal connective tissue attachment were very similar among the three groups.

At 12 weeks, the histological picture was very similar with a small inflammatory infiltrate that was consistently present at both test and control sites, in the area neighbouring the provisional restoration.

The supra-crestal soft tissues were oriented in both test and control teeth following the emergence profile of the provisional restorations. In the test group, since these provisional restorations were horizontally over-contoured with respect to the profile of the tooth, the resulting supra-crestal tissue complex presented a more horizontal orientation. Conversely, in the control group, where the chamfer preparation did not completely erase the natural tooth emergence profile, the organization of the supra-crestal tissues followed a smoother profile, without presenting sudden changes in the fibre orientation, hence presenting a connective tissue orientation more similar to that found in non-prepared teeth (Figure 2).

3.3 | Histometric measurements

The results from the histometric comparisons are presented in Tables 1 and 2, while mean values and SD of all measurements are presented in Tables A1 and A2. The intra-examiner intra-class correlation coefficient was 0.995 (95% confidence intervals (CIs): 0.974–0.999) for DP and the inter-examiner intra-class correlation coefficient between DP and MR was 0.859 (95% CIs: 0.629–0.946).

3.3.1 | Height of the supra-crestal soft tissues (FGM-B)

At 4 weeks, the height of the supra-crestal soft tissues was similar between test and control sites (Figure A3). When compared with the

TABLE 1 Comparative statistics of the buccal and lingual measurements at 4 weeks.

	Buccal														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
Variable[a]	I-J (μm)	95% CI INF	95% CI SUP	SD (μm)	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
1. Soft tissue height LINEAR	−3.67	−861.79	854.45	536.62	1.00	−971.50	−1672.15	−270.85	489.86	.00	−967.83	−1724.63	−211.04	473.25	.01
2. Barrier Epithelium LINEAR	78.68	−764.68	922.03	527.39	1.00	−978.07	−1666.66	−289.47	481.43	.00	−1056.74	−1800.51	−312.97	465.11	.00
3. Connective tissue attachment LINEAR	−81.72	−987.48	824.03	566.41	1.00	6.57	−732.97	746.12	517.06	1.00	88.30	−710.50	887.09	499.52	1.00
4. Soft tissue margin relative to CEJ LINEAR	75.78	−923.23	1074.79	624.72	1.00	−1103.13	−1918.82	−287.44	570.29	.00	−1178.91	−2059.96	−297.87	550.95	.00
5. Bone Crest relative to CEJ LINEAR	−78.05	−1025.50	869.40	592.48	1.00	134.20	−639.39	907.79	540.86	1.00	212.25	−623.32	1047.83	522.52	1.00
6. Soft tissue margin relative to provisional LINEAR	271.17	−294.51	836.85	548.52	.30										
7. Bone Crest relative to provisional LINEAR	−392.11	−1074.28	290.06	648.59	.22										
8. Soft tissue margin relative to prep line LINEAR	225.44	−375.67	826.55	582.88	.41										
9. Bone Crest relative to prep line LINEAR	−357.61	−979.96	264.74	603.48	.22										
1B. Soft tissue height CONTINUOUS LINE	10.64	−995.60	1196.88	685.52	1.00	−567.03	−1462.11	328.04	625.79	.78	−667.67	−1634.46	299.12	604.57	.53
2B. Barrier Epithelium CONTINUOUS LINE	180.89	−946.51	1308.28	705.01	1.00	−494.62	−1415.14	425.90	643.58	1.00	−675.51	−1669.78	318.77	621.76	.57
3B. Connective tissue attachment CONTINUOUS LINE	−80.25	−1022.20	861.71	589.05	1.00	−72.41	−841.52	696.69	537.72	1.00	7.83	−822.90	838.56	519.49	1.00
4B. Soft tissue margin relative to CEJ CONTINUOUS LINE	132.98	−1127.70	1393.65	788.36	1.00	−639.94	−1669.28	389.40	719.67	.84	−772.91	−1884.73	338.90	695.26	.51
5B. Bone crest relative to CEJ CONTINUOUS LINE	−32.39	−1077.81	1013.03	653.75	1.00	72.91	−780.68	926.49	596.79	1.00	105.29	−816.68	1027.27	576.55	1.00
6B. Soft tissue margin relative to provisional CONTINUOUS LINE	934.91	309.41	1560.42	606.54	.01										
7B. Bone crest relative to provisional CONTINUOUS LINE	−1008.07	−1821.14	−194.99	788.42	.02										
8B. Soft tissue margin relative to prep line CONTINUOUS LINE	46.63	−1038.57	1131.83	1052.29	.92										
9B. Bone crest relative to prep line CONTINUOUS LINE	−119.78	−1194.59	955.02	1042.21	.80										
10. Soft tissue thickness 1 mm apical to gingival margin	−161.55	−699.08	375.99	335.08	1.00	374.81	−64.08	813.71	305.88	.16	536.36	62.30	1010.42	295.51	.02
11. Soft tissue thickness 2 mm apical to gingival margin	−133.01	−708.87	442.85	358.97	1.00	71.20	−398.99	541.38	327.69	1.00	204.21	−303.65	712.06	316.58	1.00
12. Soft tissue thickness 3 mm apical to gingival margin	−19.56	−562.83	523.72	338.66	1.00	−423.26	−866.84	20.33	309.15	.07	−403.70	−882.82	75.42	298.67	.17
13. Soft tissue thickness at CEJ	−238.94	−688.23	210.34	280.07	1.00	−158.88	−525.72	207.96	255.67	1.00	80.06	−316.17	476.30	247.00	1.00
14. Soft tissue thickness at provisional margin	156.08	−340.48	652.64	481.50	.49										
15. Soft tissue thickness at preparation line	261.45	−279.23	802.13	511.29	.29										

(Continues)

TABLE 1 (Continued)

Variable[a]	Buccal														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
	I-J (μm)	95% CI INF	95% CI SUP	SD (μm)	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
16. Bone width 1 mm apical to bone crest	−159.80	−665.25	345.66	314.00	1.00	−357.77	−770.47	54.93	286.65	.14	−197.97	−643.74	247.80	276.93	1.00
17. Bone width 2 mm apical to bone crest	−462.84	−1394.87	469.19	574.57	1.00	−727.18	−1519.25	64.89	545.93	.10	−264.34	−1115.16	586.48	524.51	1.00
18. Bone width 3 mm apical to bone crest	−198.92	−1530.74	1132.90	707.95	1.00	−870.90	−1910.55	168.75	713.46	.18	−671.98	−1906.61	562.65	656.29	1.00
Variable	**Lingual**														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
1. Soft tissue height LINEAR	−291.79	−1317.04	733.46	641.13	1.00	−641.90	−1479.01	195.21	585.27	.31	−350.11	−1254.29	554.08	565.43	1.00
2. Barrier Epithelium LINEAR	−346.27	−1352.98	660.44	629.54	1.00	−526.52	−1348.49	295.46	574.69	.75	−180.25	−1068.08	707.59	555.20	1.00
3. Connective tissue attachment LINEAR	66.34	−562.52	695.20	393.25	1.00	−100.25	−613.71	413.22	358.99	1.00	−166.58	−721.19	388.02	346.82	1.00
4. Soft tissue margin relative to CEJ LINEAR	−541.88	−1601.70	517.94	662.75	1.00	−666.65	−1531.99	198.69	605.01	.30	−124.77	−1059.45	809.90	584.49	1.00
5. Bone Crest relative to CEJ LINEAR	257.51	−518.72	591.84	425.28	1.00	36.56	−518.72	591.84	388.23	1.00	−220.95	−820.73	378.82	375.06	1.00
6. Soft tissue margin relative to provisional LINEAR	−158.44	−643.01	326.14	469.88	.47										
7. Bone Crest relative to provisional LINEAR	−185.16	−708.31	337.99	507.28	.44										
8. Soft tissue margin relative to prep line LINEAR	72.71	−369.50	514.92	428.80	.71										
9. Bone Crest relative to prep line LINEAR	−401.14	−765.76	−36.52	353.57	.04										
1B. Soft tissue height CONTINUOUS LINE	−634.90	−1818.60	548.79	740.22	1.00	−451.47	−1417.95	515.02	675.72	1.00	183.44	−860.48	1227.36	652.81	1.00
2B. Barrier Epithelium CONTINUOUS LINE	−701.61	−1862.48	459.26	725.94	.95	−386.68	−1334.52	561.17	662.69	1.00	314.94	−708.86	1338.73	640.22	1.00
3B. Connective tissue attachment CONTINUOUS LINE	66.71	−609.27	742.69	345.15	1.00	−64.79	−616.72	487.15	385.89	1.00	−131.50	−727.66	464.66	372.80	1.00
4B. Soft tissue margin relative to CEJ CONTINUOUS LINE	−977.95	−2225.95	270.05	780.43	.27	−549.29	−1568.28	469.70	712.43	1.00	428.66	−671.97	1529.30	688.27	1.00
5B. Bone crest relative to CEJ CONTINUOUS LINE	343.05	−409.57	1095.67	470.64	1.00	97.83	−516.68	712.34	429.64	1.00	−245.22	−908.97	418.52	415.07	1.00
6B. Soft tissue margin relative to provisional CONTINUOUS LINE	−358.58	−1201.56	484.39	817.41	.36										
7B. Bone crest relative to provisional CONTINUOUS LINE	−182.04	−1129.63	765.56	918.85	.67										
8B. Soft tissue margin relative to prep line CONTINUOUS LINE	59.64	−679.35	798.64	716.58	.86										
9B. Bone crest relative to prep line CONTINUOUS LINE	−600.26	−1389.89	189.36	765.68	.12										
10. Soft tissue thickness 1 mm apical to gingival margin	353.62	−322.39	1029.62	421.40	1.00	741.55	189.59	1293.50	384.68	.00	387.93	−208.25	984.11	371.64	.69
11. Soft tissue thickness 2 mm apical to gingival margin	−347.21	−1257.35	562.93	569.15	1.00	−577.80	−1320.93	165.33	519.56	.28	−230.59	−1033.26	572.08	501.94	1.00

TABLE 1 (Continued)

Variable	Lingual													
	BOPT−chamfer					BOPT−noprep				Chamfer−noprep				
	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
12. Soft tissue thickness 3 mm apical to gingival margin	−91.34	−794.69	612.01	438.44	1.00	−447.23	−1021.52	127.05	400.24	.28	−355.89	−976.19	264.41	462.16	1.00
13. Soft tissue thickness at CEJ	−435.67	−1091.64	220.30	410.20	.64	−236.81	−772.41	298.78	374.46	1.00	198.86	−379.65	777.37	361.77	1.00
14. Soft tissue thickness at provisional margin	10.39	−512.12	532.90	506.66	.97										
15. Soft tissue thickness at preparation line	485.78	−225.06	1196.61	689.28	.15										
16. Bone width 1 mm apical to bone crest	319.59	−366.01	1005.19	427.37	1.00	193.73	−366.06	753.52	390.14	1.00	−125.86	−730.50	478.78	376.91	1.00
17. Bone width 2 mm apical to bone crest	427.82	−449.62	1305.26	545.10	1.00	396.20	−333.38	1125.77	506.73	1.00	−31.62	−817.64	754.40	488.30	1.00
18. Bone width 3 mm apical to bone crest	716.36	−469.71	1902.42	633.22	.92	910.66	−15.21	1836.54	638.15	.06	194.31	−556.33	1432.77	587.01	1.00

Abbreviations: BOPT, biologically oriented preparation technique; CI, confidence interval.

[a]Differences were assessed with Kruskal–Wallis test and deemed statistically significant when *p* was <.05.

unprepared tooth sites, in both test and control teeth, the buccal soft tissue height was significantly smaller (Test: $\Delta = -0.97$ mm; SD = 0.49 mm; 95% CI (−1.67; −0.27 mm); $p = .00$. Control: $\Delta = -0.97$ mm; SD = 0.47 mm; 95% CI (−1.72; −0.21 mm); $p = .00$; Figure 4). However, if assessed by continuous line measurements, these differences were smaller and not statistically significant (Figure A4).

At 12 weeks, differences between test and control groups were not significant, neither with linear nor continuous line measurements (Tables 1 and 2).

3.3.2 | Height of the barrier epithelium and connective tissue

No statistically significant differences were observed at 4 weeks between test and control sites regarding the mean linear height of the barrier epithelium. However, both test and control sites had a significantly shorter linear height of the barrier epithelium at their buccal aspect, when compared with non-prepared teeth (Test: $\Delta = -0.98$ mm; SD = 0.48 mm; 95% CI (−1.67; −0.29 mm); $p = .00$. Control: $\Delta = -1.06$ mm; SD = 0.47 mm; 95% CI (−1.80; −0.31 mm); $p = .00$) (Figure A3). Such difference was smaller and non-significant when using continuous line measurements (Test: $\Delta = -0.49$ mm; SD = 0.64 mm; 95% CI (−1.41; 0.43 mm); $p = 1.00$. Control: $\Delta = -0.68$ mm; SD = 0.62 mm; 95% CI (−1.67; 0.32 mm); $p = 1.00$) (Figure A4).

At 12 weeks of healing, a significantly shorter linear measurement of the barrier epithelium was present at test as compared with control abutments ($\Delta = -1.04$ mm; SD = 0.56; 95% CI (−2.07; 0.18 mm); $p = .00$) and to non-prepared teeth ($\Delta = -1.26$ mm; SD = 0.45 mm; 95% CI (−2.09; 0.43 mm); $p = .00$). However, these differences disappeared when comparing continuous line measurements.

In regard to the height of the connective tissue attachment, there were no significant nor relevant differences among the three groups at both 4 and 12 weeks of healing, in neither linear nor continuous line measurements (Tables 1 and 2).

3.3.3 | Position of the FGM (FGM-CEJ, FGM-Prov and FGM-Prep)

At 4 weeks, there were no significant differences between test and control sites regarding the linear distance between the FGM and the CEJ ($\Delta = -0.07$ mm; SD = 0.62 mm; 95% CI (−0.92; 1.07 mm); $p = 1.003$). In both groups, this distance (FGM-CEJ) was significantly shorter when compared with non-prepared teeth (Test: $\Delta = -1.10$ mm; SD = 0.57 mm; 95% CI (−1.92; −0.29 mm); $p = .00$. Control: $\Delta = -1.18$ mm; SD = 0.55 mm; 95% CI (−2.06; 0.29 mm); $p = .00$), hence being the gingival margin in non-prepared teeth located in a more coronal position.

At 12 weeks, a significantly shorter linear distance between FGM and CEJ was still present when comparing test sites with non-prepared teeth ($\Delta = -0.99$ mm; SD = 0.53 mm; 95% CI (−1.98; −0.19 mm); $p = .04$); however, this difference was smaller and non-significant when

TABLE 2 Comparative statistics of the buccal and lingual measurements at 12 weeks.

	Buccal														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
Variable[a]	I-J (μm)	95% CI INF	95% CI SUP	SD (μm)	p[a]	I-J (μm)	95% CI INF	95% CI SUP	SD	p	I-J (μm)	95% CI INF	95% CI SUP	SD	p
1. Soft tissue height LINEAR	−416.47	−1460.95	628.00	565.65	1.00	−584.03	−1426.11	258.05	456.04	.52	−167.56	−1009.64	674.53	456.04	1.00
2. Barrier Epithelium LINEAR	−1044.24	−2070.73	−17.74	555.91	.04	−1258.26	−2085.85	−430.67	448.19	.00	−214.02	−1041.61	613.57	448.19	1.00
3. Connective tissue attachment LINEAR	639.67	−462.77	1742.12	597.04	1.00	678.04	−210.78	1566.86	481.35	1.00	38.37	−850.46	927.19	481.35	1.00
4. Soft tissue margin relative to CEJ LINEAR	−803.16	−2019.11	412.80	658.52	.65	−999.45	−1979.78	−19.12	530.91	.04	−196.29	−1176.63	784.04	530.91	1.00
5. Bone Crest relative to CEJ LINEAR	401.55	−751.65	1554.76	624.53	1.00	418.83	−510.91	1348.57	503.51	1.00	17.27	−912.47	947.01	503.51	1.00
6. Soft tissue margin relative to provisional LINEAR	−590.31	−1744.57	563.95	811.01	.20										
7. Bone Crest relative to provisional LINEAR	−167.72	−936.34	600.90	540.05	.54										
8. Soft tissue margin relative to prep line LINEAR	−474.86	−1691.57	741.85	854.89	.30										
9. Bone Crest relative to prep line LINEAR	−283.83	−1151.25	583.59	609.47	.37										
1B. Soft tissue height CONTINUOUS LINE	169.59	−1164.70	1503.89	722.60	1.00	192.92	−882.83	1268.66	582.58	1.00	23.32	−1052.42	1099.07	582.58	1.00
2B. Barrier Epithelium CONTINUOUS LINE	−119.24	−1491.46	1252.99	743.15	1.00	−116.81	−1223.13	989.51	599.14	1.00	2.43	−1103.90	1108.75	599.14	1.00
3B. Connective tissue attachment CONTINUOUS LINE	288.83	−857.69	1435.34	620.91	1.00	309.73	−614.62	1234.07	500.59	1.00	20.90	−903.45	945.25	500.59	1.00
4B. Soft tissue margin relative to CEJ CONTINUOUS LINE	−575.08	−2109.53	959.37	831.00	1.00	−578.23	−1815.34	658.88	669.97	1.00	−3.15	−1240.26	1233.96	669.97	1.00
5B. Bone crest relative to CEJ CONTINUOUS LINE	744.67	−527.77	2017.12	689.11	1.00	771.15	−254.73	1797.02	555.58	.34	26.48	−999.40	1052.35	555.58	1.00
6B. Soft tissue margin relative to provisional CONTINUOUS LINE	−718.36	−2344.56	907.85	1142.61	.25										
7B. Bone crest relative to provisional CONTINUOUS LINE	738.71	−24.75	1502.17	536.43	.05										
8B. Soft tissue margin relative to prep line CONTINUOUS LINE	−475.61	1215.32	−2205.29	1254.07	.45										
9B. Bone crest relative to prep line CONTINUOUS LINE	382.31	−665.15	1429.77	735.97	.33										
10. Soft tissue thickness 1 mm apical to gingival margin	404.16	−250.10	1058.43	353.20	.86	412.11	−122.10	946.31	288.39	.30	7.95	−526.26	542.15	288.39	1.00
11. Soft tissue thickness 2 mm apical to gingival margin	−99.83	−800.73	601.09	378.38	1.00	−256.20	−828.49	316.09	308.95	1.00	−156.38	−728.67	415.92	308.95	1.00

TABLE 2 (Continued)

	Buccal														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
Variable[a]	I-J (μm)	95% CI INF	95% CI SUP	SD (μm)	*p*[a]	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
12. Soft tissue thickness 3 mm apical to gingival margin	−71.20	−732.45	590.05	356.97	1.00	−318.68	−858.59	221.23	291.47	1.00	−247.48	−787.39	292.43	291.47	1.00
13. Soft tissue thickness at CEJ	−344.39	−891.24	202.46	295.22	.80	−501.32	−947.83	−54.82	241.04	.02	−156.93	−603.44	289.57	241.04	1.00
14. Soft tissue thickness at provisional margin	−432.49	−1120.47	255.49	483.39	.14										
15. Soft tissue thickness at preparation line	−67.22	−418.48	284.04	246.80	.59										
16. Bone width 1 mm apical to bone crest	194.85	−420.37	810.06	330.99	1.00	−252.40	−762.51	257.72	274.44	1.00	−447.24	−957.35	62.87	274.44	.13
17. Bone width 2 mm apical to bone crest	232.03	−902.40	1366.46	605.65	1.00	−480.62	−1421.24	460.00	502.18	1.00	−712.65	−1653.27	227.97	502.18	.32
18. Bone width 3 mm apical to bone crest	71.17	−1417.85	1560.19	791.52	1.00	−625.99	−1860.62	608.64	656.29	1.00	−697.16	−1931.79	537.47	656.29	1.00

	Lingual														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
Variable	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
1. Soft tissue height LINEAR	207.01	−960.29	1374.31	632.17	1.00	−181.44	−1200.34	837.46	551.80	1.00	−388.45	−1306.88	529.97	574.33	1.00
2. Barrier Epithelium LINEAR	−54.74	−1200.93	1091.45	620.74	1.00	−413.12	−1413.60	587.36	541.82	1.00	−358.38	−1260.20	543.44	563.95	1.00
3. Connective tissue attachment LINEAR	265.10	−450.89	981.09	387.75	1.00	232.42	−392.55	857.39	338.46	1.00	−32.68	−596.01	530.66	352.28	1.00
4. Soft tissue margin relative to CEJ LINEAR	−196.63	−1403.29	1010.03	653.48	1.00	−581.71	−1634.97	471.55	570.41	1.00	−385.08	−1334.48	564.31	593.70	1.00
5. Bone Crest relative to CEJ LINEAR	399.06	−375.24	1173.36	419.33	1.00	390.73	−285.14	1066.60	366.02	1.00	−8.33	−617.55	600.89	380.97	1.00
6. Soft tissue margin relative to provisional LINEAR	305.50	−360.10	971.11	339.03	.27										
7. Bone Crest relative to provisional LINEAR	−155.39	−641.54	330.76	247.63	.43										
8. Soft tissue margin relative to prep line LINEAR	394.29	−364.10	1152.69	386.30	.22										
9. Bone Crest relative to prep line LINEAR	−236.48	−697.84	224.89	235.00	.23										
1B. Soft tissue height CONTINUOUS LINE	559.13	−788.56	1906.83	729.86	1.00	522.55	−653.81	1698.92	637.08	1.00	−36.58	−1096.94	1023.78	663.09	1.00
2B. Barrier Epithelium CONTINUOUS LINE	296.45	−1025.26	1618.16	715.79	1.00	326.01	−827.67	1479.69	624.79	1.00	29.56	−1010.35	1069.48	650.30	1.00
3B. Connective tissue attachment CONTINUOUS LINE	262.69	−506.95	1032.32	416.81	1.00	196.54	−475.25	868.34	363.82	1.00	−66.14	−671.69	539.40	378.67	1.00

(Continues)

TABLE 2 (Continued)

Variable	Lingual														
	BOPT—chamfer					BOPT—noprep					Chamfer—noprep				
	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*	I-J (μm)	95% CI INF	95% CI SUP	SD	*p*
4B. Soft tissue margin relative to CEJ CONTINUOUS LINE	141.11	−1279.80	1562.03	769.51	1.00	158.25	−1082.03	1398.52	671.69	1.00	17.13	−1100.84	1135.10	699.11	1.00
5B. Bone crest relative to CEJ CONTINUOUS LINE	418.02	−438.88	1274.91	464.06	1.00	364.31	−383.65	1112.27	405.07	1.00	−53.71	−727.91	620.49	421.61	1.00
6B. Soft tissue margin relative to provisional CONTINUOUS LINE	1355.71	508.83	2202.58	431.37	.01										
7B. Bone crest relative to provisional CONTINUOUS LINE	−507.19	−1109.43	95.05	306.76	.08										
8B. Soft tissue margin relative to prep line CONTINUOUS LINE	1456.75	348.21	2565.29	564.65	.02										
9B. Bone crest relative to prep line CONTINUOUS LINE	−608.24	−1164.35	−52.12	200.30	.04										
10. Soft tissue thickness 1 mm apical to gingival margin	−24.07	−793.74	745.60	415.50	1.00	263.46	−418.78	945.69	368.30	1.00	287.53	−329.58	904.63	384.68	1.00
11. Soft tissue thickness 2 mm apical to gingival margin	160.41	−875.83	1196.65	561.19	1.00	68.17	−836.34	972.67	489.85	1.00	−92.24	−907.55	723.07	509.85	1.00
12. Soft tissue thickness 3 mm apical to gingival margin	−75.45	−876.25	725.35	432.31	1.00	−236.10	−945.93	473.74	383.20	1.00	−160.64	−802.71	481.42	400.24	1.00
13. Soft tissue thickness at CEJ	−226.27	−973.12	520.58	404.47	1.00	−178.85	−830.76	473.05	353.05	1.00	47.42	−540.20	635.04	367.46	1.00
14. Soft tissue thickness at provisional margin	456.73	−357.44	1270.90	414.71	.19										
15. Soft tissue thickness at preparation line	782.34	−42.12	1606.79	419.95	.06										
16. Bone width 1 mm apical to bone crest	84.65	−695.94	865.24	421.40	1.00	112.02	−579.89	803.94	373.53	1.00	27.37	−598.49	653.23	390.14	1.00
17. Bone width 2 mm apical to bone crest	24.38	−974.63	1023.40	537.47	1.00	215.18	−670.35	1100.71	476.42	1.00	190.80	−610.20	991.79	497.60	1.00
18. Bone width 3 mm apical to bone crest	294.61	−945.81	1535.03	662.24	1.00	651.16	−448.36	1750.67	587.01	.99	356.55	−638.00	1351.10	613.11	1.00

Abbreviations: BOPT, biologically oriented preparation technique; CI, confidence interval.

[a]Differences were assessed with Kruskal–Wallis test and deemed statistically significant when *p* was <.05.

FIGURE 4 Clinical healing of test and control abutments at 4 and 12 weeks. (a) Buccal soft tissues vertical linear measurements (point-to-point): overall soft tissue height, connective tissue attachment and barrier epithelium height at test, positive control and negative control teeth at 4 and 12 weeks of healing. (b) Buccal soft tissues vertical continuous line measurements (true tissue profile): overall soft tissue height, connective tissue attachment and barrier epithelium height at test, positive control and negative control teeth at 4 and 12 weeks of healing. (c) Buccal hard and soft tissues vertical linear measurements (point-to-point): position of the bone crest and soft tissue margin relative to the cement-enamel junction (CEJ). (d) Buccal hard and soft tissues vertical continuous line measurements (true tissue profile): position of the bone crest and soft tissue margin relative to the CEJ.

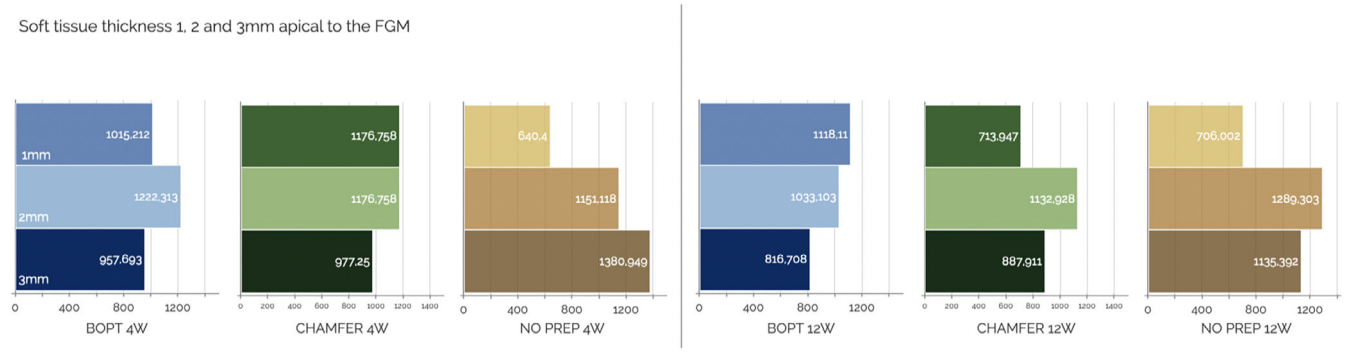

FIGURE 5 Buccal soft tissues thickness 1, 2 and 3 mm apical to the free gingival margin.

comparing non-prepared teeth and control sites ($\Delta = -0.19$ mm; SD = 0.53 mm; 95% CI (−1.17; 0.78 mm); $p = 1.00$). Although the FGM-CEJ distance was shorter in test versus control sites, these differences were not statistically significant ($\Delta = -0.80$ mm; SD = 0.66 mm; 95% CI (−2.02; 0.41 mm); $p = .65$) (Figure A3).

At both healing times, when the FGM-CEJ distance was calculated using continuous line measurements, differences between groups and comparisons with non-prepared teeth were not statistically significant (Tables 1 and 2; Figure A4).

Regarding the position of the FGM relative to the margin of the provisional restoration (FGM-Prov) or the preparation finishing line (FGM-Pr), no significant differences were observed between test and control sites at both healing times, using both linear and continuous line measurements (Tables 1 and 2).

3.3.4 | Thickness of the soft tissues at the level of the CEJ (Gth-CEJ), margin of the provisional restoration (Gth-Prov) and preparation line (Gth-Prov)

At 4 weeks, the mean buccal and lingual soft tissue thicknesses at the level of the CEJ at test sites were 1.06 (SD = 0.29) and 1.08 (SD = 0.29) mm, respectively. When compared with control sites and unprepared teeth, differences were not significant. At 12 weeks, compared with test sites, non-prepared teeth presented statistically significant thicker buccal soft tissues at the level of the CEJ ($\Delta = 0.50$ mm; SD = 0.24 mm; 95% CI (−0.95; −0.05 mm); $p = .02$). At the level of the provisional margin or the preparation finishing line ($\Delta = 0.1$–0.27 mm, $p = 1.00$), the soft tissue thickness was similar when comparing test and control sites (Tables 1 and 2; Figure A5).

3.3.5 | Soft tissues thickness 1, 2 and 3 mm apical to the gingival margin (Gth 1, 2 and 3)

At 4 weeks, the soft tissue thickness of 1 mm apical to the gingival margin (Gth1) was similar between test and control sites (Figure 5). When compared with non-prepared teeth, only control sites showed a statistically significant thicker Gth1 (Test: $\Delta = 0.37$ mm; SD = 0.31 mm; 95% CI (−0.06; 0.81 mm); $p = .16$. Control: $\Delta = 0.54$ mm; SD = 0.29 mm; 95% CI (0.06; 1.01 mm); $p = .02$). In Gth2, the buccal soft tissues thickness was similar in test and control abutments. In Gth3, buccal tissues were thinner compared with non-prepared teeth (Test: $\Delta = -0.42$ mm; SD = 0.31 mm; 95% CI (−0.87; 0.02 mm); $p = .07$. Control: $\Delta = -0.40$ mm; SD = 0.30 mm; 95% CI (−0.88; 0.08 mm); $p = .17$).

At 12 weeks, the buccal Gth1 soft tissue thickness at test sites was higher compared with control and negative control sites, although differences were not statistically significant. Similar dimensions were observed at Gth2, whereas a thinner tissue was observed at Gth3 at test sites as compared with control and negative control sites, although differences were not significant (Figure A5).

3.3.6 | Bone crest position relative to the CEJ (BC-CEJ), provisional margin (BC-Prov) and preparation line (BC-Pr)

Both at 4 and 12 weeks of healing, the position of the bone crest relative to the CEJ (BC-CEJ) did not show significant differences comparing test, controls and non-prepared teeth, using both linear and continuous line measurements.

Similarly, no significant differences could be found between tests and controls when assessing the position of the bone crest relative to the provisional margin (BC-Prov) or the preparation line (BC-Pr), using both linear and continuous line measurements (Tables 1 and 2).

3.3.7 | Bone crest width 1, 2 and 3 mm apically to the bone crest

Both at 4 and 12 weeks of healing, no significant differences were present in the thickness of the buccal bone 1, 2 and 3 mm apical to the BC, when comparing test, controls and non-prepared teeth (Tables 1 and 2).

4 | DISCUSSION

The aim of the present experimental in vivo investigation was to compare the short-term morphological and dimensional changes that occur within the periodontium of teeth rehabilitated with the BOPT (test) or the chamfer technique (positive control), as compared with unprepared teeth (negative control).

Test and positive control did not present significant differences in vertical and horizontal histometric measurements. Both groups, however, demonstrated a more apical location of the FGM relative to the CEJ when compared with the negative control ($\Delta = 1.1$ mm for both groups at 4 weeks ($p < .05$), 0.99 mm for the test group ($p = .043$) and 0.20 mm for control group ($p = 1.000$) at 12 weeks).

The early (4 weeks) and late (12 weeks) healing in both preparation techniques occurred uneventfully, albeit a mild inflammatory infiltrate was consistently present at the most coronal part of the soft tissues in contact with the provisional restoration. Such findings are in line with previous histomorphometry studies assessing the placement of restorative margins in the subgingival space (Karlsen, 1970; Newcomb, 1974; Tarnow et al., 1986) and with clinical studies describing gingival inflammation at teeth treated with either the BOPT or chamfer techniques. One year after treatment of teeth not presenting BOP at baseline, Paniz et al. (2016) reported the presence of BOP at 52.2% of teeth prepared with a vertical finishing line and 36.5% with a horizontal one. Similarly, Schatzle et al. (2001) reported significantly greater gingival inflammation around restorations with subgingival margins compared with those with supra-gingival margins, without significant differences in supra-gingival plaque levels (Schatzle et al., 2001).

When evaluating the soft tissue height at early healing (4 weeks), its overall dimension was approximately 1 mm longer at non-prepared teeth as compared with BOPT and chamfer abutments ($p < .05$). This difference was due to a longer barrier epithelium and a higher distance between the FGM and the CEJ ($p < .05$). However, there were no differences among the three groups in the linear height of the connective tissue attachment ($p = 1.000$).

At 12 weeks, the soft tissue height at BOPT sites had an overall dimension approximately 0.5 mm shorter than at positive and negative controls, albeit without statistical significance ($p > .05$). Furthermore, a significantly shorter barrier epithelium was observed at BOPT sites, by approximately 1 mm ($p < .05$). This finding is partially in contrast with previous studies comparing the supra-crestal soft tissue height of natural versus restored teeth. Vacek et al. (1994) reported similar connective tissue height between natural and restored teeth, although with a longer barrier epithelium in restored versus natural teeth. However, Tarnow et al. (1986) reported shorter barrier epithelium lengths at restored teeth, combined with a mean apical recession of the gingival margin of 1.2 mm (0.4–2.2 mm). These differences may be related to the type of tooth preparation, the morphology of the restorations and the position of the restoration margin relative to the gingival margin.

In the present study, the margin of the provisional restoration was placed 0.5 mm below the gingival margin at the time of crown delivery and was used as a reference point to assess the occurrence of gingival recession. Both at 4 and 12 weeks, no relevant differences were observed between BOPT and chamfer abutments, and in both groups, the margin of the provisional restoration was kept subgingivally without being exposed.

Nevertheless, this lack of gingival recession should be interpreted with caution due to the short follow-up. In the study by Agustin-

Panadero et al. (2021), there was no gingival recession (absent or <1 mm) either in BOPT or in chamfer preparations at 1 year. However, at 5 years, the BOPT group still presented no gingival recessions while the first chamfer presented a 31.6% incidence of 1–2 mm recessions (Agustin-Panadero et al., 2021).

These differences in the overall soft tissue height and barrier epithelium length, however, were smaller and non-significant ($p > .05$), when these vertical measurements were done using a continuous line following the soft tissue profile (true profile dimension; Tomasi et al., 2014), instead of a point-to-point linear measurement. This finding points out that the true dimension of the supra-crestal soft tissue profile was kept fairly unchanged in spite of the tooth preparation, although in the teeth prepared with the BOPT technique the area pertaining to the barrier epithelium had been apically and buccally displaced by the emergence profile of the provisional crowns; because in this protocol, the provisional restorations are horizontally overcontoured with respect to the abutment profile.

Since BOPT and chamfer abutments resulted in a free gingiva following the emergence profile of the provisional crown, their buccal soft tissues were thicker 1 mm apical to the gingival margin (Gth1) as compared with unprepared teeth, both at 4 and 12 weeks of healing.

When comparing BOPT and chamfer-prepared teeth, while at 4 weeks the thickness of the buccal soft tissues 1 mm apical to the gingival margin (Gth1) was similar (0.16 mm thicker in chamfer group; SD = 0.34 mm; 95% CI (−0.70; 0.44 mm); $p = 1.000$), at 12 weeks, BOPT abutments had a thicker Gth1, although these differences were not statistically significant ($\Delta = 0.40$ mm; SD = 0.35 mm; 95% CI (−0.25; 1.06 mm); $p = .861$). Such finding suggests the potential role of the BOPT protocol in promoting a phenotype increase in the submarginal portion of the supra-crestal soft tissues surrounding the provisional restoration, as a consequence of providing more horizontal space for the supra-crestal soft tissue complex, in the area between the bottom of the preparation and the margin of the provisional crown.

In previous reports, gingival inflammation and attachment loss have been reported when the restorative margins of prosthetic restorations are placed invading the junctional epithelium within the crevicular space (Nevins & Skurow, 1984; Reeves, 1991), and similarly, evidence of bone loss has been reported in histological studies evaluating restorative margins invading the connective tissue attachment space (Carnevale et al., 1983; Tarnow et al., 1986). In this investigation, despite the extension of the preparation within the sulcus, there were no differences among the two preparation techniques in the position of the bone crest relative to the CEJ, nor in the position of the bone crest relative to the provisional crown margin. This finding suggests that albeit the BOPT protocol implies an invasion of the sulcus during tooth preparation, the placement of the restorative margins coronally to the bottom of the preparation (0.5 mm subgingival) does not result in an apical migration of the soft tissue margin or the bone crest.

A variable degree of inflammation was noted in sections from both treatment groups. In some specimens, the infiltrated connective tissue extended over the entire dimension of the pocket epithelium, whereas in others it was limited to the most coronal sulcular epithelium or it was almost undetected. However, there was no inflammation in the control sections, and the extent of this inflammatory infiltrate was independent from the treatment group, which is suggestive that its presence was related to the provisional restorations, rather than the type of tooth preparation.

This in vivo investigation presents clear limitations inherent to all preclinical models with a reduced sample size, and therefore, these results should be interpreted with caution. The fact that no statistically significant differences were found between the two tooth preparation techniques should not imply equal histological and clinical behaviours. Furthermore, the external validity of this study is limited by: (a) the short follow-up, which could mask the onset of gingival recessions during a longitudinal observation; (b) the need to splint adjacent crowns to increase their retention and to avoid their decementation during the study period; (c) the absence of a standardized reference point (notch) on the root surface; (d) the difference in morphology with human teeth that may have influenced the fabrication of the provisional crowns; (e) the frequent observation of altered passive eruption in the adopted experimental animal, which may have influenced the magnitude of the dimensional changes that were observed; (f) the provision of acrylic temporary restorations fabricated with a direct technique, with a lesser precision in the marginal fit compared with an indirect fabrication, what resulted in the presence of marginal gaps filled by temporary cement and (g) the possible presence of fractures and/or dislodgement of the temporary crowns during the sawing and grinding procedure during the histological processing. Thus, albeit the provision of an immediate temporary restoration represents an essential, integral part of the biologic rationale on which the BOPT protocol stands, since the emergence profile of the direct provisional crown provides immediate sealing and mechanical support to the horizontal space created within the sulcus during the vertical preparation, the clear limitations associated with such kind of interim restorations may represent a confounding factor in the assessment of the differences between the two preparation techniques. At the same time, these limitations may magnify the differences between the two groups with provisional restorations with the negative control group with unprepared teeth, since the presence of the referred gap may potentially promote plaque accumulation and localized inflammation.

In conclusion, despite the adoption of an animal model, the limited follow-up, the absence of standardized reference points and the provision of splinted acrylic temporary restorations, the results from this study suggest that both the BOPT and chamfer tooth preparation protocols induced similar qualitative and structural changes in the supra-crestal soft tissues complex including: (a) presence of a mild inflammatory infiltrate in the most coronal part of the soft tissues in contact with the provisional restoration; (b) development of a supra-crestal soft tissues profile, which follows the emergence profile of the provisional restoration and (c) establishment of a more apical FGM with respect to the CEJ as compared with non-prepared teeth, at both 4 and 12 weeks. There were no significant differences between the test and control tooth preparation techniques in both vertical and horizontal histometric measurements.

AUTHOR CONTRIBUTIONS

David Palombo: Histometric analysis, literature review, data analysis and interpretation and manuscript drafting. **Maryam Rahmati:** Histological processing, histometric analysis, data analysis and manuscript revision. **Fabio Vignoletti:** Study concept/design, execution of the experimental phase, data analysis and interpretation and critical review of the manuscript. **Javier Sanz-Esporrin:** Execution of the experimental phase, statistical analysis, data analysis and interpretation and critical review of the manuscript. **Mari Paz Salido:** Execution of the experimental phase, statistical analysis, data analysis and interpretation and critical review of the manuscript. **Håvard Jostein Haugen:** Supervisor of the histological processing and critical review of the manuscript. **Mariano Sanz:** Project supervisor, study concept/design, execution of the experimental phase, data analysis and interpretation and critical review of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A

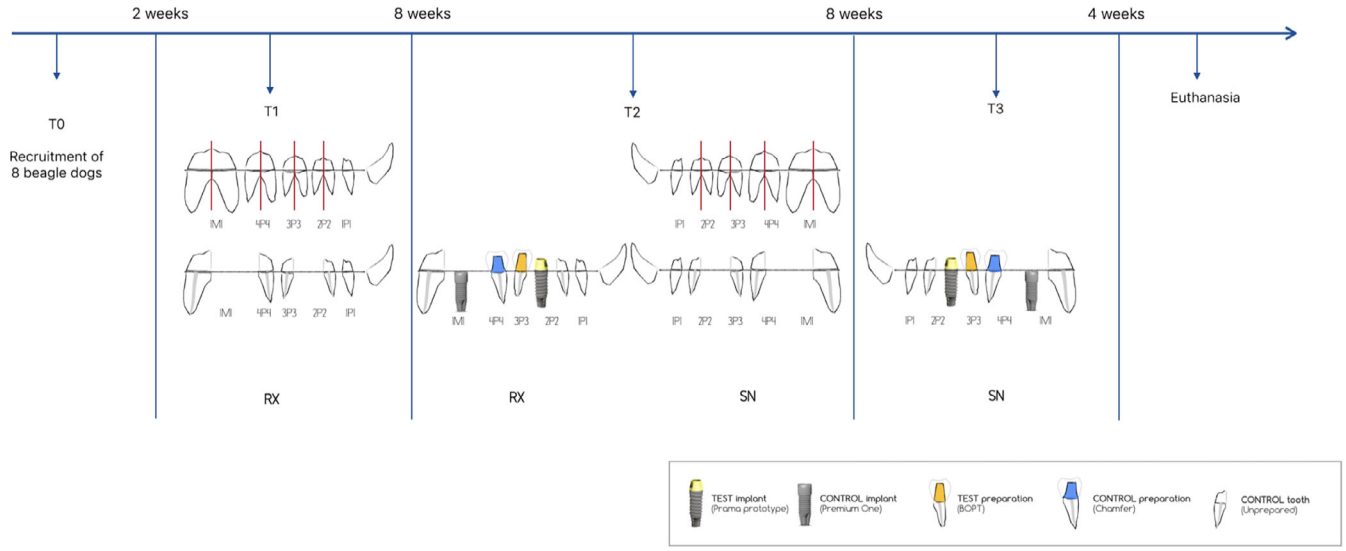

FIGURE A1 Graphical representation of the random allocation distribution followed for every experimental animal.

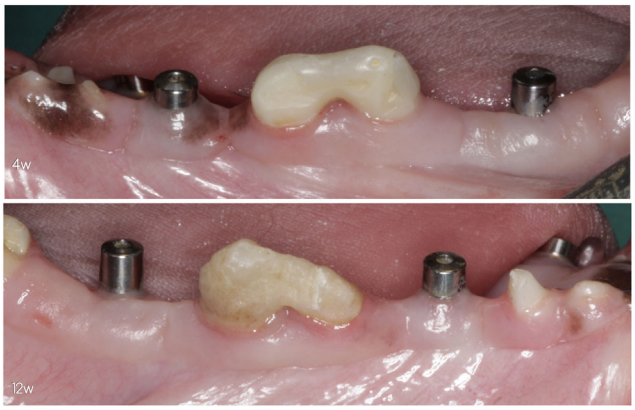

FIGURE A2 Clinical appearance 4 and 12 weeks after the study interventions.

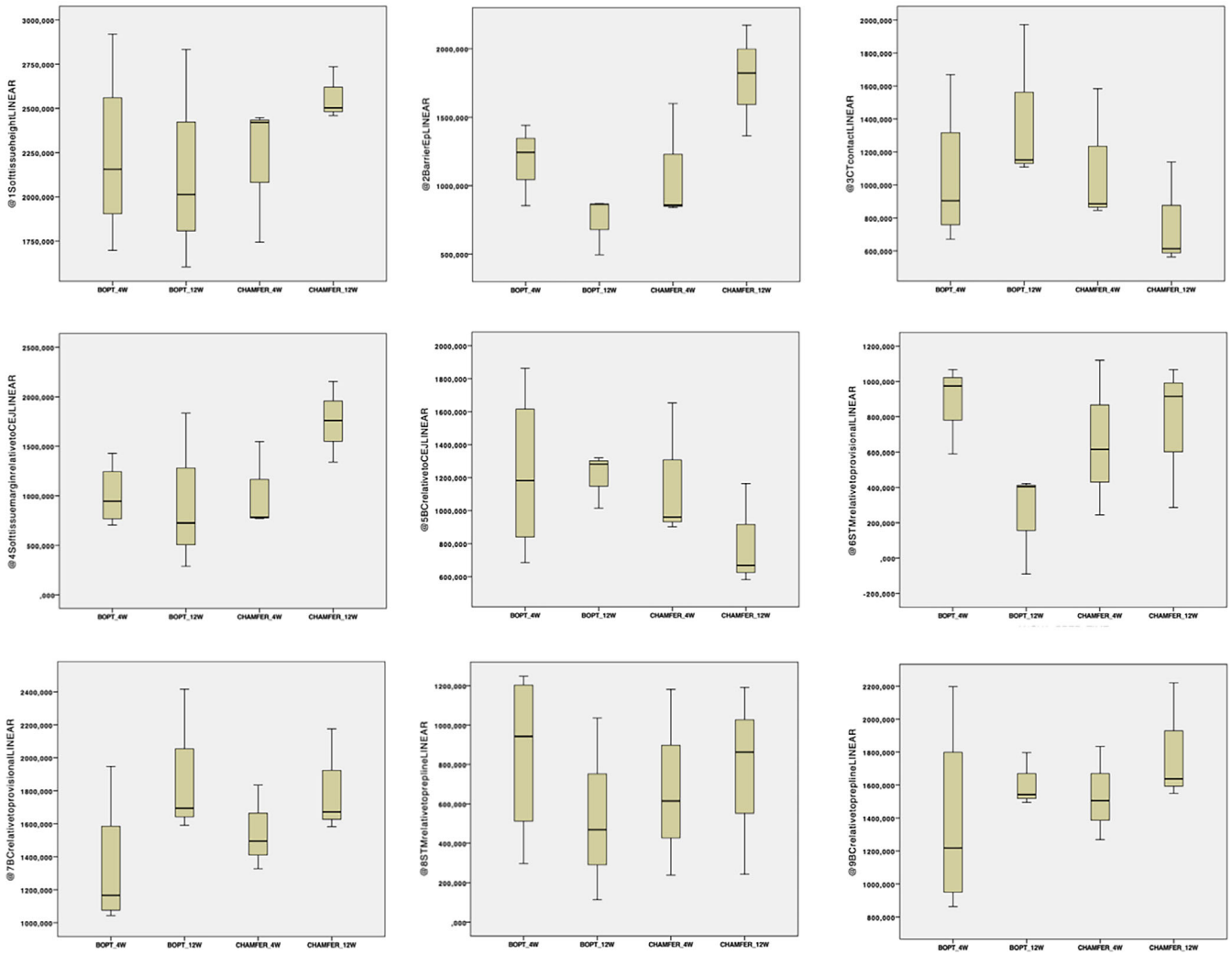

FIGURE A3 Boxplots of the vertical linear/continuous and horizontal measurements of hard and soft tissues at test: (1) height of the supra-crestal soft tissues (FGM-B)—linear measurement; (1b) height of the supra-crestal soft tissues (FGM-B)—continuous line; (2) height of the barrier epithelium (FGm-aJE)—linear measurement; (2b) height of the barrier epithelium (FGm-aJE)—continuous line; (3) height of the connective tissue attachment (aJE-B)—linear measurement; (3b) height of the connective tissue attachment (aJE-B)—continuous line; (4) gingival margin relative to the CEJ (FGMCEJ)—linear measurement; (4b) gingival margin relative to the CEJ (FGM-CEJ)—continuous line; (5) bone crest relative to the CEJ (CEJ-Bc)—linear measurement; (5b) bone crest relative to the CEJ (CEJ-Bc)—continuous line; (6) gingival margin relative to the apical border of the provisional (FGM-Pv)—linear measurement; (6b) gingival margin relative to the apical border of the provisional (FGM-Pv)—continuous line; (7) bone crest relative to the apical border of the provisional (B-Pv)—linear measurement; (7b) bone crest relative to the apical border of the provisional (B-Pv)—continuous line; (8) bone crest relative to the apical border of the preparation (B-Prep)—linear measurement; (8b) bone crest relative to the apical border of the preparation (B-Prep)—continuous line; (9) gingival margin relative to the apical border of the preparation (FGM-Prep)—linear measurement; (9b) gingival margin relative to the apical border of the preparation (FGM-Prep)—continuous line.

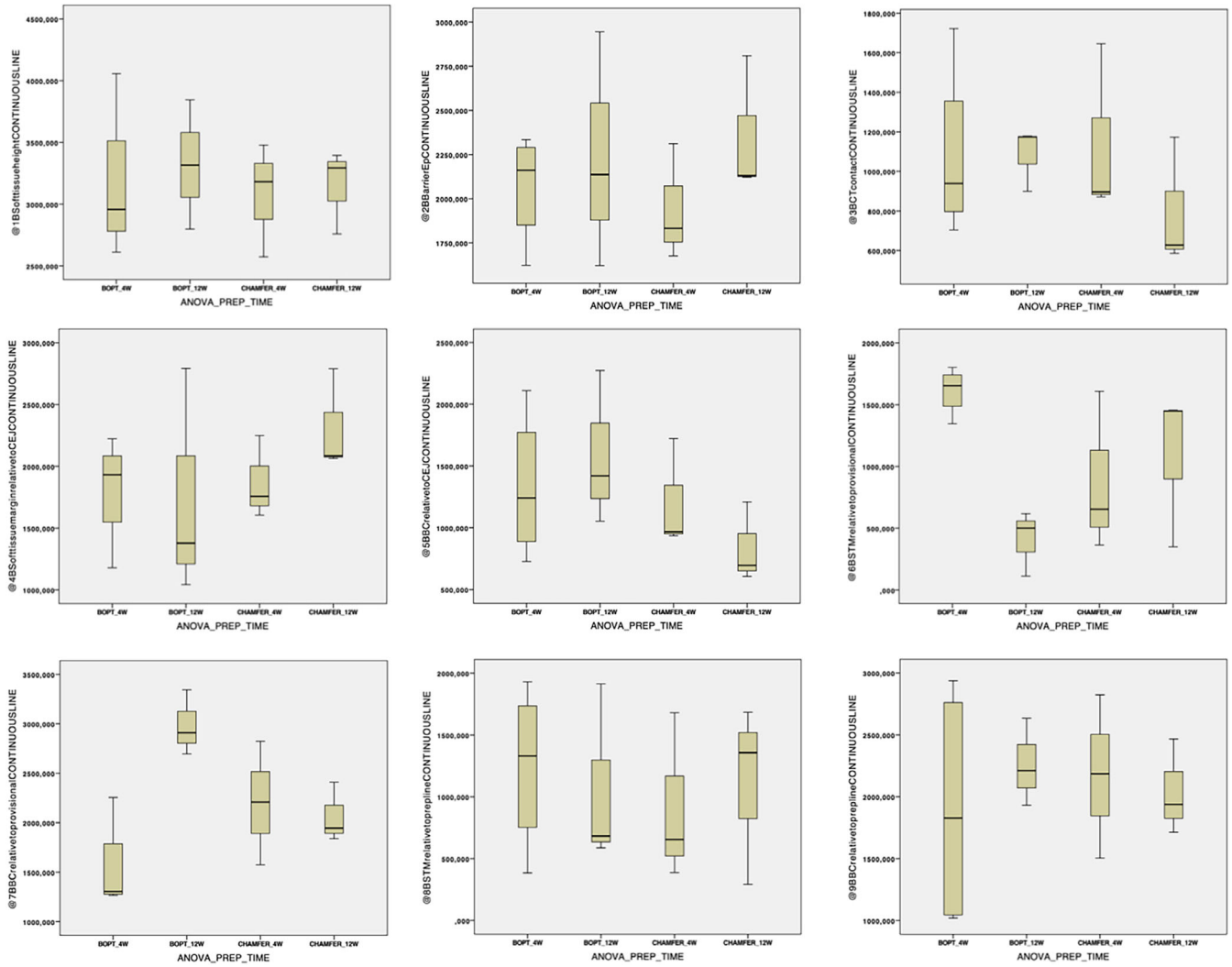

FIGURE A4 Boxplots of the vertical continuous line measurements of hard and soft tissues at test, positive control and negative control sites: (1b) Height of the supra-cresta soft tissues (FGM-B)—continuous line; (2b) height of the barrier epithelium (FGm-aJE)—continuous line; (3b) height of the connective tissue attachment (aJE-B)—continuous line; (4b) gingival margin relative to the cement-enamel junction (CEJ) (FGM-CEJ)—continuous line; (5b) bone crest relative to the CEJ (CEJ-Bc)—continuous line; (6b) gingival margin relative to the apical border of the provisional (FGM-Pv)—continuous line; (7b) bone crest relative to the apical border of the provisional (B-Pv)—continuous line; (8b) bone crest relative to the apical border of the preparation (B-Prep)—continuous line; (9b) gingival margin relative to the apical border of the preparation (FGM-Prep)—continuous line.

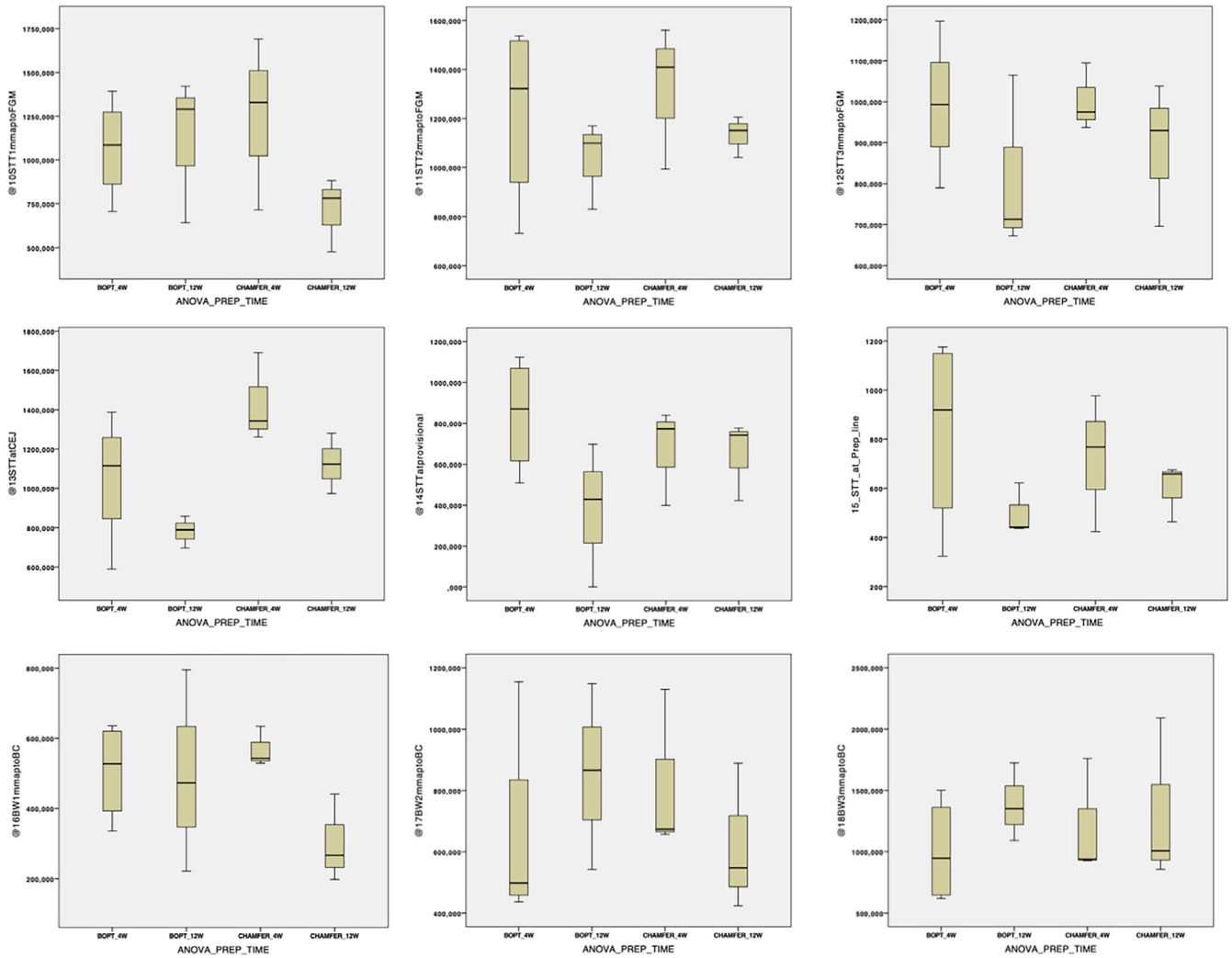

FIGURE A5 Boxplots of the horizontal measurements of hard and soft tissues at test, positive control and negative control sites: (10) Width of the gingiva at cement-enamel junction (CEJ) (Gth-CEJ); (11) width of the gingiva at provisional (Gth-Pv); (12) width of the gingiva at prep line (Gth-Prep); (13) width of the gingiva 1 mm apically to free gingival margin (FGM) (Gth 1); (14) width of the gingiva 2 mm apically to FGM (Gth 2); (15) width of the gingiva 3 mm apically to FGM (Gth 3); (16) bone thickness 1 mm apical to BC (Bcw1); (17) bone thickness 2 mm apical to BC (Bcw2); (18) bone thickness 3 mm apical to BC (Bcw3).

TABLE A1 Descriptive statistics of the buccal and lingual measurements at 4 weeks.

	4 weeks											
	Buccal						Lingual					
	BOPT 4 weeks		Chamfer 4 weeks		Noprep 4 weeks		BOPT 4 weeks		Chamfer 4 weeks		Noprep 4 weeks	
Variable	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)
1. Soft tissue height LINEAR	2219.58	441.26	2223.24	328.32	3191.08	383.57	1662.44	226.97	1954.23	316.03	2304.34	416.84
2. Barrier Epithelium LINEAR	1223.36	222.35	1144.69	365.44	2201.43	404.79	956.51	196.44	1302.78	275.56	1483.02	400.42
3. Connective tissue attachment LINEAR	997.44	389.84	1079.16	343.19	990.86	596.16	719.57	130.61	653.23	158.51	819.81	493.74
4. Soft tissue margin relative to CEJ LINEAR	1073.03	313.24	997.25	370.17	2176.17	420.70	751.16	249.00	1293.04	276.61	1417.82	445.24
5. Bone crest relative to CEJ LINEAR	1148.86	473.95	1226.91	358.44	1014.65	606.63	922.57	277.51	665.05	163.79	886.01	485.28
6. Soft tissue margin relative to provisional LINEAR	759.67	366.88	509.69	467.67			471.90	385.06	581.22	283.92		
7. Bone crest relative to provisional LINEAR	1466.82	472.41	1719.57	395.43			1168.66	464.96	1376.63	226.71		
8. Soft tissue margin relative to prep line LINEAR	888.13	384.72	569.34	444.47			781.30	303.52	660.73	327.19		
9. Bone crest relative to prep line LINEAR	1332.79	521.45	1663.62	343.88			873.10	233.76	1295.28	301.45		
1B. Soft tissue height CONTINUOUS LINE	3076.77	566.37	2976.13	428.89	3643.80	657.77	2341.18	318.53	2976.08	533.74	2792.64	423.85
2B. Barrier epithelium CONTINUOUS LINE	2041.57	281.14	1860.68	313.99	2536.19	715.53	1596.42	365.84	2298.03	519.74	1983.09	424.68
3B. Connective tissue attachment CONTINUOUS LINE	1035.20	397.68	1115.44	362.18	1107.61	663.87	744.76	139.34	678.05	160.63	809.55	526.58
4B. Soft tissue margin relative to CEJ CONTINUOUS LINE	1838.75	389.97	1705.77	429.33	2478.68	753.16	1305.60	500.24	2283.55	522.89	1854.89	526.33
5B. Bone crest relative to CEJ CONTINUOUS LINE	1238.02	554.74	1270.41	384.79	1165.11	650.75	1035.58	323.23	692.53	170.37	937.75	532.46
6B. Soft tissue margin relative to provisional CONTINUOUS LINE	1578.98	184.90	679.84	660.61			681.68	684.48	1044.49	515.83		
7B. Bone crest relative to provisional CONTINUOUS LINE	1497.78	424.89	2296.34	543.58			1659.49	663.94	1931.59	695.62		
8B. Soft tissue margin relative to prep line CONTINUOUS LINE	1036.84	736.55	759.37	630.74			1230.11	500.89	1170.47	591.72		
9B. Bone crest relative to prep line CONTINUOUS LINE	2039.92	919.56	2216.81	546.87			1111.07	374.10	1805.61	732.00		
10. Soft tissue thickness 1 mm apical to gingival margin	1015.21	275.13	1176.76	213.13	640.40	125.53	1565.33	257.86	1211.71	446.94	823.78	292.56
11. Soft tissue thickness 2 mm apical to gingival margin	1222.31	324.17	1355.32	249.41	1151.12	201.09	1020.69	99.82	1367.90	385.84	1598.49	525.75
12. Soft tissue thickness 3 mm apical to gingival margin	957.69	164.15	977.25	83.52	1380.95	348.91	807.79	33.26	899.14	64.16	1255.03	440.32
13. Soft tissue thickness at CEJ	1062.51	290.24	1301.45	320.35	1221.39	198.71	1084.87	294.71	1520.54	244.03	1321.68	267.19
14. Soft tissue thickness at provisional margin	717.90	369.46	527.81	345.57			723.45	472.56	721.80	209.81		
15. Soft tissue thickness at preparation line	834.43	397.27	607.22	324.74			1255.16	644.43	766.30	282.31		
16. Bone width 1 mm apical to bone crest	479.98	134.73	639.78	149.87	837.75	184.24	1557.10	328.32	1237.51	136.34	1363.37	435.68
17. Bone width 2 mm apical to bone crest	606.58	307.98	1069.42	543.98	1333.76	484.36	2206.70	472.88	1778.88	170.80	1810.50	531.55
18. Bone width 3 mm apical to bone crest	1008.78	371.61	1207.71	478.29	1879.69	756.06	2952.39	756.95	2236.03	254.24	2041.72	346.39

Abbreviation: BOPT, biologically oriented preparation technique.

TABLE A2 Descriptive statistics of the buccal and lingual measurements at 12 weeks.

	12 weeks											
	Buccal						Lingual					
	BOPT 12 weeks		Chamfer 12 weeks		NOPREP 12 weeks		BOPT 12 weeks		Chamfer 12 weeks		NOPREP 12 weeks	
Variable	Mean (μm)	SD (μm)	Mean (μm)	SD (μm)	Mean (μm)	SD (μm)	Mean (μm)	SD (μm)	Mean (μm)	SD (μm)	Mean (μm)	SD (μm)
1. Soft tissue height LINEAR	2149.86	626.64	2566.33	148.81	2733.89	392.06	2000.51	202.23	1793.50	279.25	2181.96	727.78
2. Barrier Epithelium LINEAR	743.02	214.54	1787.25	405.88	2001.28	469.81	1093.62	250.52	1148.36	226.33	1506.74	731.50
3. Connective tissue attachment LINEAR	1411.44	487.12	771.77	319.49	733.40	191.52	908.72	95.16	643.62	58.35	676.30	125.33
4. Soft tissue margin relative to CEJ LINEAR	948.95	797.81	1752.10	407.58	1948.39	504.00	879.40	534.99	1076.03	179.98	1461.11	719.84
5. Bone crest relative to CEJ LINEAR	1205.72	167.00	804.17	314.55	786.90	299.26	1111.56	318.19	712.50	124.79	720.83	140.00
6. Soft tissue margin relative to provisional LINEAR	245.33	290.47	756.43	413.81			641.28	342.55	243.03	202.86		
7. Bone crest relative to provisional LINEAR	1900.34	449.87	1810.03	319.92			1356.40	141.14	1558.70	213.60		
8. Soft tissue margin relative to prep line LINEAR	539.23	465.33	765.65	481.38			795.55	253.34	349.03	308.73		
9. Bone crest relative to prep line LINEAR	1611.89	162.50	1802.90	364.92			1207.16	53.71	1452.18	217.52		
1B. Soft tissue height CONTINUOUS LINE	3319.08	524.04	3149.48	342.29	3126.16	343.01	3083.42	514.74	2524.29	270.72	2560.87	806.26
2B. Barrier epithelium CONTINUOUS LINE	2234.87	666.97	2354.10	394.70	2351.68	419.91	2153.32	588.88	1856.87	227.63	1827.31	764.24
3B. Connective tissue attachment CONTINUOUS LINE	1084.21	160.03	795.38	327.85	774.48	191.86	930.10	84.84	667.41	68.44	733.56	158.04
4B. Soft tissue margin relative to CEJ CONTINUOUS LINE	1737.47	928.68	2312.55	412.99	2315.70	432.13	1919.32	632.53	1778.21	197.03	1761.08	756.78
5B. Bone crest relative to CEJ CONTINUOUS LINE	1581.61	626.28	836.94	325.09	810.46	211.71	1164.09	297.03	746.08	132.62	799.79	188.05
6B. Soft tissue margin relative to provisional CONTINUOUS LINE	411.37	264.54	1084.91	637.22			1476.36	570.15	347.13	226.45		
7B. Bone crest relative to provisional CONTINUOUS LINE	2983.48	330.00	2064.57	303.49			1607.06	315.91	2177.15	158.19		
8B. Soft tissue margin relative to prep line CONTINUOUS LINE	1061.07	739.80	1110.44	727.66			1803.59	569.73	663.40	517.15		
9B. Bone crest relative to prep line CONTINUOUS LINE	2258.00	353.58	2039.04	387.33			1279.83	60.22	1860.88	265.30		
10. Soft tissue thickness 1 mm apical to gingival margin	1118.11	417.62	713.95	211.20	706.00	199.63	1201.98	397.64	1226.05	340.40	938.52	257.15
11. Soft tissue thickness 2 mm apical to gingival margin	1033.10	179.62	1132.93	84.23	1289.30	345.48	1430.44	444.57	1270.04	394.96	1362.27	415.55
12. Soft tissue thickness 3 mm apical to gingival margin	816.71	216.13	887.91	175.17	1135.39	224.96	774.10	125.42	849.55	87.33	1010.20	409.56
13. Soft tissue thickness at CEJ	781.29	81.07	1125.68	153.43	1282.61	141.30	1109.90	230.49	1336.17	281.87	1288.75	386.94
14. Soft tissue thickness at provisional margin	375.73	352.00	647.16	195.03			723.91	412.40	290.09	201.35		
15. Soft tissue thickness at preparation line	500.16	105.68	599.45	117.67			1103.02	289.52	481.32	390.50		
16. Bone width 1 mm apical to bone crest	496.59	287.57	301.75	125.45	748.99	345.72	1391.43	226.77	1306.78	148.77	1279.40	260.94
17. Bone width 2 mm apical to bone crest	852.14	303.17	620.11	240.75	1332.76	443.38	1988.56	180.76	1964.17	353.14	1773.38	332.77
18. Bone width 3 mm apical to bone crest	1388.61	318.56	1317.44	673.52	2014.60	446.65	2774.55	593.15	593.15	441.61	2123.39	495.03

Abbreviation: BOPT, biologically oriented preparation technique.