Hard and soft tissue healing around teeth prepared with the biologically oriented preparation technique: an experimental in vivo preclinical investigation.

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ABSTRACT

Objectives: To evaluate the healing of the hard and soft tissues surrounding teeth prepared with the Biologically Oriented Preparation technique (BOPT), compared to teeth prepared with the Chamfer technique and to non prepared teeth.

Material and methods: 16 test (BOPT) and 16 control (Chamfer) teeth where prepared and provisionalised with PMMA crowns in 8 beagle dogs, following a random allocation sequence. 16 negative controls (non prepared teeth) were also selected for comparative analysis. The following histological outcomes at 4 and 12 weeks were evaluated: the descriptive histological composition and the histomorphometrical dimensions of the periodontal tissues, including the soft tissue height and thickness, as well as the horizontal and vertical bone remodelling.

Results: Test and control teeth exhibited a more apical location of the free gingival margin (1.1mm for both groups at 4 weeks ($p<0.05$) and 0.99mm for the test group at 12 weeks (p 0.043) with respect to the CEJ as compared to negative controls. 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 supra-crestal soft tissue complex, when compared to non prepared teeth.

1. INTRODUCTION

The biologically oriented preparation technique (BOPT, Loi & Di Felice 2013) is a vertical tooth preparation protocol for restorative purposes which is based on the following rationale:

a) a complete elimination of the cement-enamel junction landmark;

b) a controlled invasion of the gingival sulcus with the preparation protocol;

c) the creation of a new prosthetic emergence profile once the tooth has been prepared, through a

provisional crown that seals the space created within gingival sulcus.

In the last years, this preparation protocol has been advocated based on data from prospective case series (Agustín-Panadero et al. 2018, Serra-Pastor et al. 2019) and clinical trials comparing it to alternative techniques, as the Chamfer preparation (Paniz et al. 2016, Agustín-Panadero et al. 2020) where stable gingival margins and the maintenance of a natural gingival architecture were reported. However, these clinical studies cannot assess the healing characteristics elicited by the BOPT protocol and its impact on the morphology and structural organization of the periodontium.

It was therefore the objective of this *in vivo* preclinical investigation to compare the healing, the morphology and dimensions of the periodontal tissues surrounding teeth prepared with the BOPT, when compared to teeth prepared with an alternative tooth preparation protocol (Chamfer technique) or to un-prepared teeth.

2. MATERIAL AND METHODS

2.1 Experimental design

The present study was design as an experimental in vivo investigation, where each animal provided the test and the corresponding control in two different healing timepoints (early 4 weeks and delayed 12 weeks healing). This study was designed following the modified ARRIVE guidelines for reporting experimental preclinical investigations (Vignoletti and 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 Caceres, Spain, once the study protocol had been approved by the local Ethical Committee (REGA code: ES 100370001499). Test and control teeth were prepared in both hemimandibles using a randomized block group distribution.

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 and 22-21 Cº and were fed on a soft pellet diet. Every animal received an identification code printed in a sub- cutaneous RFID chip. Experienced veterinary doctors monitored the experimental animals during the entire course of this investigation.

2.3 Surgical Procedures.

2.3.1 Intervention 1. Root extractions

Using a computer-generated random allocation sequence, 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. Once the remaining roots were endodontically treated, 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 implant placement

Eight weeks after the first intervention, the contralateral hemi-mandible received the same extraction and endodontic treatment protocols, while in the other hemimandible the residual mesial

root of 4P4 and the distal one of 3P3 where randomly prepared with a Chamfer or a BOPT technique, following a randomized sequence, and received an immediate provisional crown.

In the test group, BOPT preparations where performed using a series of flame shaped diamond burs specifically designed for the BOPT protocol, with a decreasing grit of 125 to 20µm (BOPT preparation drills; Sweden & Martina, Italy), to eliminate the natural emergence profile of the tooth and create a vertically shaped abutment. After tooth preparation, 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), with the aim of developing a new prosthetic emergence profile, placing the restorative margin 0.5 mm below the gingival margin.

In the control group, Chamfer diamond burs with a decreasing grit of 151 to 25µm (856 series Chamfer burs, Komet - Gebr. Brasseler GmbH & Co. KG, Germany) were used to create a Chamfer preparation with a 1mm axial reduction and a finishing line 0,5mm below the gingival margin. The PMMA provisional was then delivered placing the restorative margin at the finishing line.

All preparations were performed under ×4.5 magnification using a 40.000-rpm hand piece. In both groups, a 1,5mm incisal reduction was performed during the preparation and provisional crowns were cemented with a temporary cement (Temp Bond Clear, Kerr Dental, Orange, CA, USA).

At the same surgical session, Prama® or Premium One® (Sweden & Martina, Italy) implants were placed, according to a randomization sequence, in the healed edentulous areas using a non-submerged standard implant placement protocol.

2.4.3 Intervention 3.

Eight weeks after the second intervention, the same tooth preparation and implant placement protocols where replicated in the contralateral hemi-mandibles.

2.5 Post-surgical care

After each surgery, 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 two 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 four weeks after the third intervention with an overdose of sodium pentothal (40-60 mg/kg/i.v., Dolethal, Vetoquinol, France). Each animai provided two hemi-mandibles with 4- and 12-weeks healing periods, respectively, which were freed from their attached tissues and sectioned in two halves between the centrai incisors. Each hemi-mandible was placed into a sealable container containing 4% formalin solution and stored in a secure area at constant temperature (5°C) from the time of collection unti! the shipment for histological processing. From each hemi-mandible, 5 tissue blocks where obtained containing 1 Prama® and 1 Premium One® implants, 1 BOPT and 1 Chamfer abutments, and 1 non prepared tooth, with their surrounding tissues.

2.7 Histological Processing

The tissue blocks from one randomly selected animai (#7) were processed by decalcification following a modification of the "fracture technique" (Berglundh, Lindhe et al. 1994), while the remaining blocks were processed by ground sectioning following the methodology described by Donath & Breuner (Donath and Breuner 1982).

Specimens allocated to the ground section technique were dehydrated in a graded series of ethanol and embedded in methyl methacrylate. Each block was cut in a bucco-lingual piane 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. (Figure 3).

This manuscript focuses on the descriptive histology and histometric measurements performed on the teeth sections from the calcified blocks. The results from the implants and from the decalcified sections of animal #7 will be reported in independent publications.

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, Munich, Germany) and assessed by histomorphometry by a calibrated examiner (DP) using a dedicated image analysis software (Zen lite Blue software, Carl Zeiss Microscopy). Intraclass correlation coefficients were generated to estimate the intra-examiner reproducibility.

2.9 *Histological outcomes*

2.9.1 Histomorphometry evaluation 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 junctional epithelium (aJE);*
- *Apical border of the provisional restoration (Pv).*
- *Apical border of the preparation (Prep).*
- *Bone crest (Bc).*

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

a) Hard tissues

- *Bone crest relative to the CEJ (CEJ-Bc);*
- *Bone crest relative to the apical border of the provisional (CEJ-Pv);*
- *Bone crest relative to the apical border of the preparation (CEJ-Prep);*
- *Width of the bone crest 1, 2, and 3mm apically to the peak of the crest (Bcw 1, 2, 3).*

b) Soft tissues

- *Height of the supra-crestal soft tissues (FGM-B);*
- *Height of the barrier epithelium (FGm-aJE);*
- *Height of the connective tissue attachment (aJE-B);*
- *Gingival margin relative to the CEJ (FGM-CEJ);*
- *Gingival margin relative to the apical border of the provisional (FGM-Pv);*
- *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-Pv);*
- *Width of the gingiva at the level of the apical border of the preparation (Gth-Prep);*
- *Width of the gingiva 1, 2, and 3mm apically to the FGM (Gth 1, 2, 3);*

All vertical linear measurements were replicated as continuous lines measurements in the three groups.

2.9 Statistical analysis

Outcome measurements were expressed as means and standard deviations (± SD), 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 implants. 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 <0.05. 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 7 out of 8 animals, however, in one animal (#5) the advent of 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.

3.2 Descriptive histology

At 4 weeks, healing occurred uneventfully around both test and control prepared teeth, which both presented a healthy periodontium. An inflammatory infiltrate was frequently observed within the supra-cresta l soft tissues in test abutments, in the areas in close vicinity with the provisional restorations. Signs of inflammation where also detected around control abutments, but to a minor extent and not in all sections.

At 12 weeks, in the area neighbouring the provisional restoration, a small inflammatory infiltrate was consistently present at both test and control abutments.

The organization of the supra-crestal soft tissues at both test and control abutments followed the emergence profile of the provisional restorations. In the test group, since the provisional restorations were horizontally over-contoured with respect to the abutment profile, the resulting supra-crestal tissue complex developed a more horizontal orientation. Conversely, in the control group, where the chamfer preparation did not completely erase the natural tooth emergence profile, the spacial organisation of the supracrestal tissues developed a less horizontal orientation, more similar to the one present in non prepared teeth.

3.3 Histometric measurements

The results from the histometric measurements are presented in Table 1-4. The intra-examiner

intra-class correlation coefficient was 0.995 (95% confidence intervals: 0.974 – 0.999).

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

At 4 weeks, the mean linear measurement of the soft tissue height at test abutments was 2.22 (SD 0.44) mm and 1.66 (SD 0.23) mm at the buccal and lingual aspects, respectively.

No statistically significant difference was observed when compared with the soft tissue height in control abutments (Δ = -0.37 and -0.29 mm; p=1.000). When compared with non prepared teeth, both test and control abutments demonstrated a significantly smaller buccal soft tissue height (test: Δ = -0.97mm, p 0.002; control: Δ = -0.97mm, p 0.005).
Interestingly, when a continuous, instead of a linear measurement was done, these differences with non prepared teeth were smaller and not statistically significant, suggesting that at 4 weeks of healing, the overall dimension of the soft tissue height was maintained fairly constant among the 3 groups, although a more horizontal development of the supra-cresta! soft tissues was present at test and contro! teeth.

A similar pattern was observed at 12 weeks, albeit differences between test and contro! groups as compared to non prepared teeth where smaller and did not reach statistica! significance neither with linear nor continuous line measurements. (Table 3-4)

3.3.2 Height of the junctional epithelium (FGm-aJE) and connective tissue (aJE-B):

No statistically significant nor clinically relevant differences could be observed at 4 weeks of healing between test and contro! abutments regarding the mean linear height of the junctional epithelium. However, both test and contro! abutments presented a significantly shorter linear height of the junctional epithelium at their buccal aspect, as compared to non prepared teeth (test: Δ = -0.98mm, p 0.001; control Δ = - l,06mm, p 0.001). Such difference was smaller and non-significant when comparing continuous line instead of linear measurements (test: Δ = -0.49mm, p 1.000; control Δ = -0.68mm, p 1.000).

At 12 weeks of healing, a significantly shorter linear measurement of the junctional epithelium was present at test as compared to contro! abutments (il. - l.04mm, p 0.004) and to non prepared teeth (il. - l.26mm, p 0.001). However, such differences where smaller and non-significant when comparing continuous versus linear measurements.

No significant nor relevant differences could be observed among the 3 groups regarding the height of the connective tissue attachment, at both 4 and 12 weeks of healing, in either linear or continuous line measurements. (Table 3-4)

3.3.3 Position of the free gingival margin (FGM-CEJ, FGM-Pv, FGM-Prep)

At 4 weeks, no significant differences were observed between test and control groups regarding the linear distance between the free gingival margin and the cement-enamel junction (CEJ) (Δ = -0.07mm, p 1.003). In both groups a significantly shorter FGM-CEJ distance was present when compared to non prepared teeth (test: Δ = -1,10mm, p 0.002; control: Δ = -1.18mm, p 0.003), thus showing that in non prepared teeth the gingival margin was placed in a more coronal position.

At 12 weeks, a significantly shorter linear distance between free gingival margin and CEJ was still present when comparing test abutments with non prepared teeth (Δ = -0.99mm, p 0.043),

while this difference was smaller and non-significant comparing non prepared teeth and control abutments (Δ = -0.42mm, p 1.000). Although the FGM-CEJ distance was shorter in test versus control abutments, these differences were not statistically significant (Δ = -0.80mm, p 0.651).

As described for the soft tissues height and junctional epithelium length, at both healing times, when the FGM-CEJ distance was calculated using continuous line measurements differences between groups and comparisons with non prepared teeth did not reach statistical significance. (Table 3-4).

Regarding the position of the free gingival margin relative to the provisional margin (FGM-Pv) or the preparation finishing line (FGM-Pr), no significant differences were observed between test and control abutments at both healing times, using both linear and continuous line measurements. (Table 3-4).

3.3.4 Soft tissues thickness at the level of the CEJ (Gth-CEJ), provisional margin (Gth-Pv) and preparation line (Gth-Pv):

At 4 weeks, the mean buccal and lingual soft tissue thicknesses at the level of the CEJ at test abutments were 1.06 (SD 0.29) and 1.08 (SD 0.29) mm, respectively. These differences were not statistically significant, nor when compared with those from non prepared teeth.

At 12 weeks, the soft tissue thickness was kept almost unchanged at the lingual aspect of test abutments (1.11mm, SD 0.23mm) while a reduction was observed at the buccal one (0.78mm SD 0.08mm), leading to a statistically significant difference in buccal tissue thickness between test abutments and non prepared teeth (Δ = -0.50mm, p 0.018). Similar results were obtained when comparing the soft tissue thickness of test and control abutments at the level of the provisional margin or the preparation finishing line. (Table 3-4).

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

At 4 weeks, the soft tissue thickness 1mm apical to the gingival margin (Gth1) was slightly higher at the buccal aspect of control, compared to test abutments, although these differences were not statistically different (Δ = -0.16mm, p 1.000). Both test and controls presented a thicker Gth1 when compared to non prepared teeth, but only control abutments showed a statistically significant difference (test: Δ = 0.37mm, p 0.159; control: Δ= 0.54mm, p 0.017).

In Gth 2, the buccal soft tissues thickness was similar in test and control abutments. In Gth 3 buccal tissues were thinner compared to non prepared teeth (test: Δ = -0.42mm, p 0.072; control: Δ = -0.40mm, p 0.173).

At 12 weeks, the buccal soft tissue thickness at test abutments remained almost unchanged, while control abutments presented thinner tissues at Gth1, compared to one at 4 weeks (Δ = -0,46mm, p 0.329). A similar tendency was observed when comparing test abutments with non prepared teeth, with tests presenting a thicker Gth1 (test: Δ = 0,41mm, p 0.259) and a thinner Gth3 (Δ = -0.32mm, p 1.000), albeit without reaching statistical significance. On the other hand, control abutments at 12 weeks presented a thinner Gth1 as compared to test ones (Δ=-0.40mm, p 0.861), which was almost equal to non prepared teeth (Δ = 0.08mm, p 1.000) (Table 2).

3.3.6 Bone crest position relative to the CEJ (BC-CEJ), provisional margin (BC-Pv) 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-Pv) or the preparation line (BC- Pr), using both linear and continuous line measurements. (Table 3-4)

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

Both at 4 and 12 weeks of healing, no significant differences could be found in the thickness of the buccal bone 1, 2 and 3mm apical to the BC, among test, controls and non prepared teeth. (Table 3-4)

4. CONCLUSIONS

Results from this study suggest that both the BOPT and Chamfer tooth preparation protocols induced the following qualitative and structural changes in the supra-crestal soft tissues complex: 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; c) establishment of a more apical free gingival margin with respect to the CEJ as compared to non prepared teeth, at both 4 and 12 weeks.

No significant differences where observed between test and control groups in both vertical and horizontal histometric measurements.

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FIGURES

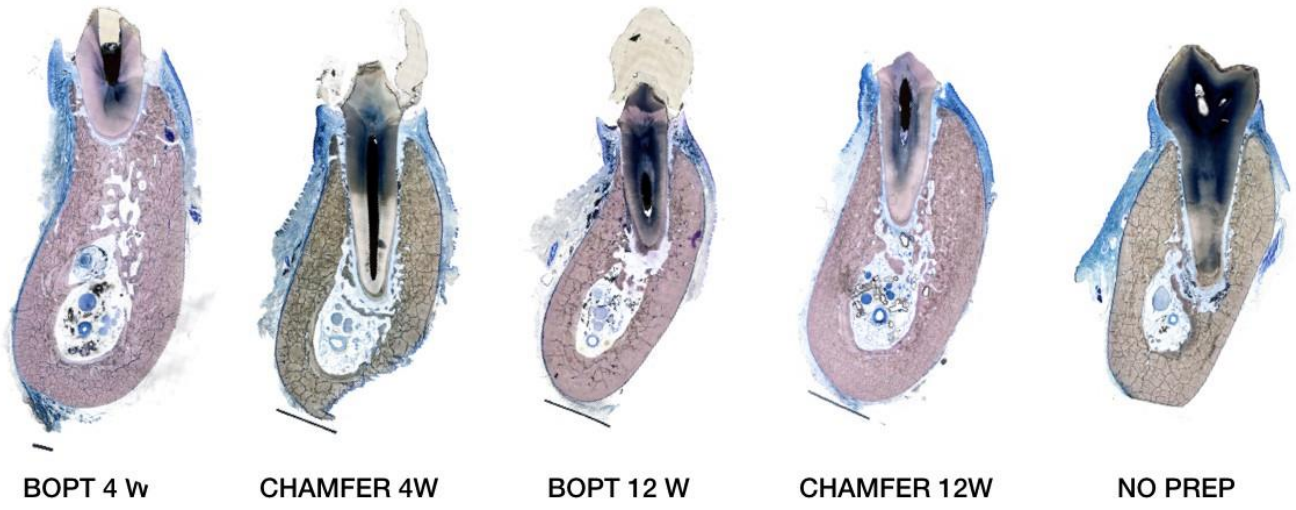

Figure 1 - Bucco-lingual histologic ground sections representing: a) Test abutments at 4 weeks; b) Control abutments at 4 weeks; c) Test abutments at 12 weeks; d) Control abutments at 12 weeks; e) Non prepared teeth.

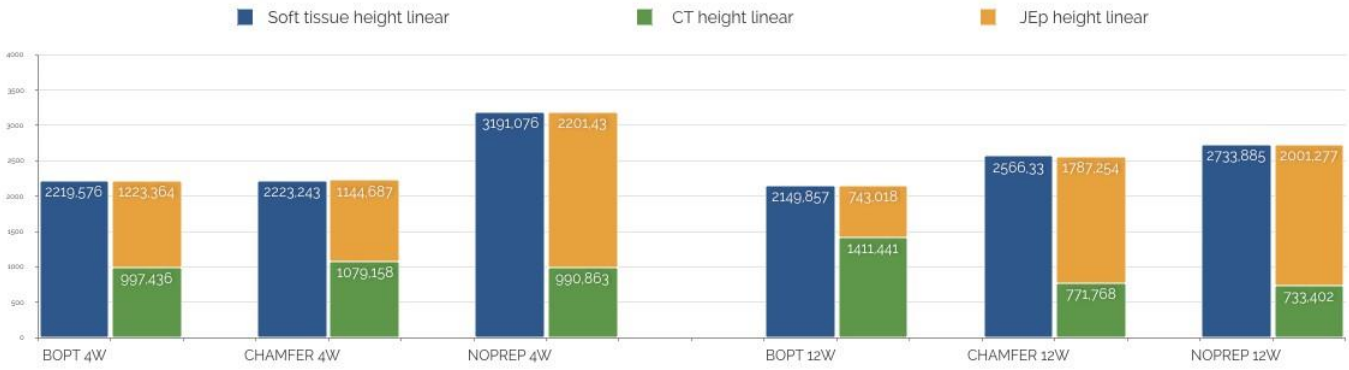

Figure 2 - Buccal linear measurements of the soft tissue height, connective tissue height, and junctional epithelium height at test abutments, control abutments, and non prepared teeth, at 4 and 12 weeks.

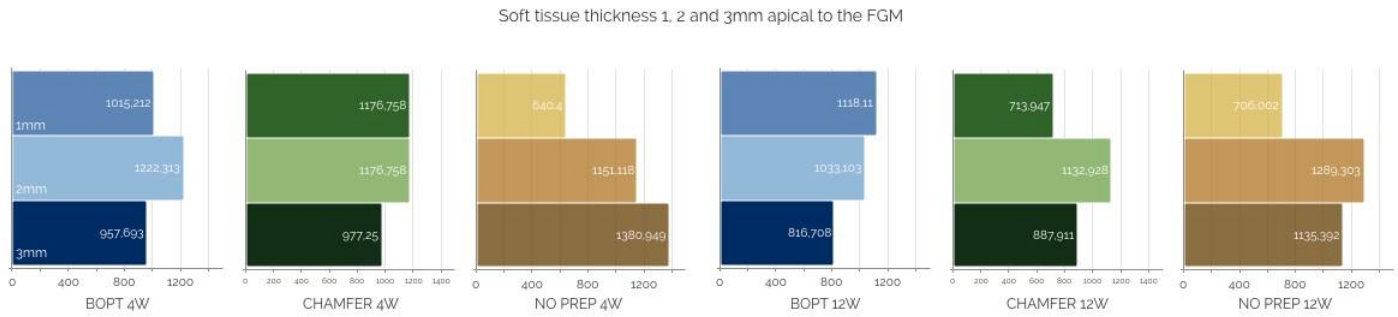

Figure 3 - Buccal measurements of the soft tissue thickness 1, 2 and 3mm apical to the gingival margin at test abutments, control abutments, and non prepared teeth, at 4 and 12 weeks.

Table 1 - Descriptive statistics of the buccal and lingual measurements at 4 weeks

	4W											
	BUCCAL						LINGUAL					
VARIABLE	BOPT 4w		CHAMFER 4w		NOPREP 4w		BOPT 4w		CHAMFER 4w		NOPREP 4w	
	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 *So# %ssue height LINEAR*	**2219,576**	**441,256**	**2223,243**	**328,315**	**3191,076**	**383,568**	**1662,439**	**226,965**	**1954,233**	**316,030**	**2304,341**	**416,838**
2 *Barrier Epithelium LINEAR*	**1223,364**	**222,352**	**1144,687**	**365,437**	**2201,430**	**404,787**	**956,506**	**196,437**	**1302,776**	**275,560**	**1483,021**	**400,423**
3 *Connec%ve %ssue a=achment LINEAR*	**997,436**	**389,837**	**1079,158**	**343,188**	**990,863**	**596,157**	**719,565**	**130,608**	**653,227**	**158,510**	**819,811**	**493,735**
4 *So# %ssue margin rela%ve to CEJ LINEAR*	**1073,034**	**313,243**	**997,252**	**370,165**	**2176,166**	**420,698**	**751,162**	**249,000**	**1293,042**	**276,607**	**1417,815**	**445,242**
5 *Bone Crest rela%ve to CEJ LINEAR*	**1148,856**	**473,952**	**1226,906**	**358,439**	**1014,653**	**606,626**	**922,568**	**277,506**	**665,054**	**163,786**	**886,007**	**485,275**
6 *So# %ssue margin rela%ve to provisional LINEAR*	**759,673**	**366,877**	**509,687**	**467,667**			**471,897**	**385,056**	**581,224**	**283,919**		
7 *Bone Crest rela%ve to provisional LINEAR*	**1466,816**	**472,414**	**1719,565**	**395,425**			**1168,659**	**464,958**	**1376,625**	**226,705**		
8 *So# %ssue margin rela%ve to prep line LINEAR*	**888,130**	**384,724**	**569,335**	**444,468**			**781,299**	**303,524**	**660,731**	**327,188**		
9 *Bone Crest rela%ve to prep line LINEAR*	**1332,792**	**521,448**	**1663,620**	**343,879**			**873,102**	**233,760**	**1295,282**	**301,445**		
1B *So# %ssue height CONTINUOUS LINE*	**3076,765**	**566,365**	**2976,125**	**428,886**	**3643,798**	**657,765**	**2341,175**	**318,529**	**2976,079**	**533,742**	**2792,640**	**423,850**
2B *Barrier Epithelium CONTINUOUS LINE*	**2041,565**	**281,137**	**1860,681**	**313,985**	**2536,186**	**715,527**	**1596,418**	**365,842**	**2298,030**	**519,744**	**1983,094**	**424,680**
3B *Connec%ve %ssue a=achment CONTINUOUS LINE*	**1035,199**	**397,681**	**1115,444**	**362,184**	**1107,612**	**663,872**	**744,757**	**139,343**	**678,048**	**160,633**	**809,546**	**526,579**
4B *So# %ssue margin rela%ve to CEJ CONTINUOUS LINE*	**1838,745**	**389,971**	**1705,770**	**429,329**	**2478,684**	**753,159**	**1305,595**	**500,238**	**2283,547**	**522,886**	**1854,885**	**526,333**
5B *Bone crest rela%ve to CEJ CONTINUOUS LINE*	**1238,020**	**554,743**	**1270,408**	**384,785**	**1165,114**	**650,750**	**1035,580**	**323,232**	**692,531**	**170,369**	**937,754**	**532,459**
6B *So# %ssue margin rela%ve to provisional CONTINUOUS*	**1578,982**	**184,904**	**679,835**	**660,608**			**681,683**	**684,478**	**1044,486**	**515,834**		

7B *Bone crest rela%ve to provisional CONTINUOUS LINE*	**1497,783**	**424,894**	**2296,344**	**543,577**			**1659,492**	**663,942**	**1931,593**	**695,620**		
8B *So# %ssue margin rela%ve to prep line CONTINUOUS LINE*	**1036,841**	**736,549**	**759,371**	**630,741**			**1230,107**	**500,886**	**1170,466**	**591,722**		
9B *Bone crest rela%ve to prep line CONTINUOUS LINE*	**2039,923**	**919,564**	**2216,807**	**546,866**			**1111,068**	**374,100**	**1805,613**	**731,998**		
10 *So# %ssue thickness 1mm apical to Gingival Margin*	**1015,212**	**275,128**	**1176,758**	**213,127**	**640,400**	**125,533**	**1565,330**	**257,855**	**1211,713**	**446,938**	**823,782**	**292,559**
11 *So# %ssue thickness 2mm apical to Gingival Margin*	**1222,313**	**324,169**	**1355,324**	**249,405**	**1151,118**	**201,091**	**1020,693**	**99,816**	**1367,901**	**385,841**	**1598,493**	**525,747**
12 *So# %ssue thickness 3mm apical to Gingival Margin*	**957,693**	**164,152**	**977,250**	**83,524**	**1380,949**	**348,914**	**807,794**	**33,262**	**899,138**	**64,158**	**1255,028**	**440,324**
13 *So# %ssue thickness at CEJ*	**1062,508**	**290,236**	**1301,450**	**320,348**	**1221,387**	**198,707**	**1084,874**	**294,708**	**1520,544**	**244,032**	**1321,684**	**267,192**
14 *So# %ssue thickness at provisional margin*	**717,900**	**369,455**	**527,812**	**345,574**			**723,448**	**472,555**	**721,797**	**209,810**		
15 *So# %ssue thickness at prepara%on line*	**834,430**	**397,270**	**607,220**	**324,743**			**1255,160**	**644,427**	**766,300**	**282,305**		
16 *Bone width 1mm apical to Bone Crest*	**479,984**	**134,729**	**639,781**	**149,865**	**837,751**	**184,236**	**1557,097**	**328,320**	**1237,506**	**136,343**	**1363,368**	**435,684**
17 *Bone width 2mm apical to Bone Crest*	**606,582**	**307,977**	**1069,422**	**543,979**	**1333,763**	**484,356**	**2206,696**	**472,880**	**1778,876**	**170,796**	**1810,498**	**531,552**
18 *Bone width 3mm apical to Bone Crest*	**1008,784**	**371,609**	**1207,707**	**478,289**	**1879,685**	**756,057**	**2952,386**	**756,949**	**2236,030**	**254,242**	**2041,722**	**346,391**

Table 2 - Descriptive statistics of the buccal and lingual measurements at 12 weeks

	12W											
	BUCCAL						LINGUAL					
VARIABLE	BOPT 12W		CHAMFER 12W		NOPREP 12W		BOPT 12W		CHAMFER 12W		NOPREP 12W	
	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 *So# %ssue height LINEAR*	2149,857	626,642	2566,330	148,812	2733,885	392,060	2000,514	202,227	1793,501	279,246	2181,955	727,779
2 *Barrier Epithelium LINEAR*	743,018	214,535	1787,254	405,882	2001,277	469,808	1093,620	250,524	1148,361	226,326	1506,738	731,503
3 *Connec%ve %ssue a=achment LINEAR*	1411,441	487,124	771,768	319,491	733,402	191,517	908,717	95,161	643,620	58,353	676,295	125,334
4 *So# %ssue margin rela%ve to CEJ LINEAR*	948,94604	797,814	1752,102	407,584	1948,394	503,996	879,400	534,987	1076,030	179,984	1461,113	719,839
5 *Bone Crest rela%ve to CEJ LINEAR*	1205,721	166,996	804,167	314,554	786,895	299,257	1111,557	318,186	712,499	124,786	720,829	139,995
6 *So# %ssue margin rela%ve to provisional LINEAR*	245,332	290,468	756,428	413,811			641,282	342,552	243,028	202,856		
7 *Bone Crest rela%ve to provisional LINEAR*	1900,336	449,867	1810,027	319,922			1356,404	141,136	1558,698	213,602		
8 *So# %ssue margin rela%ve to prep line LINEAR*	539,228	465,332	765,653	481,378			795,551	253,336	349,026	308,731		
9 *Bone Crest rela%ve to prep line LINEAR*	1611,894	162,496	1802,900	364,921			1207,161	53,708	1452,178	217,515		
1B *So# %ssue height CONTINUOUS LINE*	3319,07567	524,040	3149,483	342,294	3126,160	343,013	3083,417	514,743	2524,285	270,724	2560,865	806,262
2B *Barrier Epithelium CONTINUOUS LINE*	2234,868	666,970	2354,104	394,700	2351,678	419,905	2153,319	588,882	1856,872	227,629	1827,308	764,237
3B *Connec%ve %ssue a=achment CONTINUOUS LINE*	1084,207	160,031	795,379	327,853	774,482	191,859	930,099	84,843	667,414	68,436	733,557	158,044
4B *So# %ssue margin rela%ve to CEJ CONTINUOUS LINE*	1737,468	928,684	2312,547	412,986	2315,699	432,125	1919,323	632,531	1778,209	197,029	1761,077	756,777
5B *Bone crest rela%ve to CEJ CONTINUOUS LINE*	1581,607	626,281	836,936	325,094	810,461	211,710	1164,094	297,031	746,076	132,619	799,787	188,046
6B *So# %ssue margin rela%ve to provisional CONTINUOUS*	411,370	264,541	1084,911	637,219			1476,355	570,145	347,131	226,453		

7B *Bone crest rela%ve to provisional CONTINUOUS LINE*	**2983,477**	**330,001**	**2064,572**	**303,485**			**1607,063**	**315,912**	**2177,154**	**158,186**		
8B *So# %ssue margin rela%ve to prep line CONTINUOUS LINE*	**1061,073**	**739,796**	**1110,439**	**727,655**			**1803,588**	**569,725**	**663,404**	**517,145**		
9B *Bone crest rela%ve to prep line CONTINUOUS LINE*	**2258,003**	**353,576**	**2039,044**	**387,334**			**1279,829**	**60,216**	**1860,881**	**265,299**		
10 *So# %ssue thickness 1mm apical to Gingival Margin*	**1118,110**	**417,622**	**713,947**	**211,200**	**706,002**	**199,630**	**1201,975**	**397,641**	**1226,045**	**340,404**	**938,519**	**257,145**
11 *So# %ssue thickness 2mm apical to Gingival Margin*	**1033,103**	**179,623**	**1132,928**	**84,225**	**1289,303**	**345,476**	**1430,442**	**444,568**	**1270,036**	**394,963**	**1362,272**	**415,552**
12 *So# %ssue thickness 3mm apical to Gingival Margin*	**816,708**	**216,129**	**887,911**	**175,166**	**1135,392**	**224,960**	**774,102**	**125,424**	**849,554**	**87,331**	**1010,197**	**409,558**
13 *So# %ssue thickness at CEJ*	**781,290**	**81,071**	**1125,682**	**153,431**	**1282,614**	**141,303**	**1109,900**	**230,489**	**1336,172**	**281,872**	**1288,752**	**386,940**
14 *So# %ssue thickness at provisional margin*	**375,727**	**351,998**	**647,157**	**195,033**			**723,914**	**412,395**	**290,087**	**201,348**		
15 *So# %ssue thickness at prepara%on line*	**500,160**	**105,676**	**599,450**	**117,671**			**1103,020**	**289,515**	**481,320**	**390,504**		
16 *Bone width 1mm apical to Bone Crest*	**496,592**	**287,566**	**301,748**	**125,447**	**748,989**	**345,719**	**1391,426**	**226,772**	**1306,775**	**148,772**	**1279,402**	**260,938**
17 *Bone width 2mm apical to Bone Crest*	**852,141**	**303,165**	**620,111**	**240,751**	**1332,758**	**443,382**	**1988,557**	**180,763**	**1964,172**	**353,139**	**1773,378**	**332,768**
18 *Bone width 3mm apical to Bone Crest*	**1388,612**	**318,557**	**1317,439**	**673,518**	**2014,599**	**446,654**	**2774,545**	**593,145**	**593,145**	**441,607**	**2123,389**	**495,029**

Table 3 - Comparative statistics of the buccal and lingual measurements at 4 weeks

	ANOVA 4W											
	BUCCAL						LINGUAL					
VARIABLE	BOPT - CHAMFER		BOPT - NOPREP		CHAMFER-NOPREP		BOPT - CHAMFER		BOPT - NOPREP		CHAMFER-NOPREP	
	I-J	*P*	I-J	*P*	I-J	*P*	I-J	*P*	I-J	*P*	I-J	*P*
1 *So# %ssue height LINEAR*	**-3,667**	**1,000**	**-971,500**	**0,002***	**-967,833**	**0,005***	**-291,794**	**1,000**	**-641,901**	**0,307**	**-350,108**	**1,000**
2 *Barrier Epithelium LINEAR*	**78,677**	**1,000**	**-978,066**	**0,001***	**-1056,743**	**0,001***	**-346,270**	**1,000**	**-526,515**	**0,745**	**-180,245**	**1,000**
3 *Connec%ve %ssue a=achment LINEAR*	**-81,722**	**1,000**	**6,572**	**1,000**	**88,295**	**1,000**	**66,338**	**1,000**	**-100,246**	**1,000**	**-166,584**	**1,000**
4 *So# %ssue margin rela%ve to CEJ LINEAR*	**75,782**	**1,000**	**-1103,132**	**0,002***	**-1178,913**	**0,003***	**-541,880**	**1,000**	**-666,653**	**0,299**	**-124,773**	**1,000**
5 *Bone Crest rela%ve to CEJ LINEAR*	**-78,049**	**1,000**	**134,204**	**1,000**	**212,253**	**1,000**	**257,514**	**1,000**	**36,561**	**1,000**	**-220,953**	**1,000**
6 *So# %ssue margin rela%ve to provisional LINEAR*	**249,987**	**1,000**					**-109,327**	**1,000**				
7 *Bone Crest rela%ve to provisional LINEAR*	**-252,749**	**1,000**					**-207,966**	**1,000**				
8 *So# %ssue margin rela%ve to prep line LINEAR*	**318,795**	**1,000**					**120,568**	**1,000**				
9 *Bone Crest rela%ve to prep line LINEAR*	**-330,828**	**1,000**					**-422,181**	**1,000**				
1B *So# %ssue height CONTINUOUS LINE*	**100,640**	**1,000**	**-567,034**	**0,781**	**-667,674**	**0,529**	**-634,90370**	**1,000**	**-451,465**	**1,000**	**183,439**	**1,000**
2B *Barrier Epithelium CONTINUOUS LINE*	**180,885**	**1,000**	**-494,621**	**1,000**	**-675,506**	**0,572**	**-701,613**	**0,946**	**-386,676**	**1,000**	**314,936**	**1,000**
3B *Connec%ve %ssue a=achment CONTINUOUS LINE*	**-80,245**	**1,000**	**-72,413**	**1,000**	**7,832**	**1,000**	**66,709**	**1,000**	**-64,789**	**1,000**	**-131,497**	**1,000**
4B *So# %ssue margin rela%ve to CEJ CONTINUOUS LINE*	**132,975**	**1,000**	**-639,940**	**0,844**	**-772,914**	**0,512**	**-977,953**	**0,271**	**-549,291**	**1,000**	**428,662**	**1,000**
5B *Bone crest rela%ve to CEJ CONTINUOUS LINE*	**-32,388**	**1,000**	**72,906**	**1,000**	**105,294**	**1,000**	**343,049**	**1,000**	**97,826**	**1,000**	**-245,223**	**1,000**
6B *So# %ssue margin rela%ve to provisional CONTINUOUS*	**899,147**	**1,000**					**-362,803**	**1,000**				

7B *Bone crest rela%ve to provisional CONTINUOUS LINE*	**-798,561**	**1,000**						**-272,101**	**1,000**				
8B *So# %ssue margin rela%ve to prep line CONTINUOUS LINE*	**277,470**	**1,000**						**59,641**	**1,000**				
9B *Bone crest rela%ve to prep line CONTINUOUS LINE*	**-176,884**	**1,000**						**-694,545**	**1,000**				
10 *So# %ssue thickness 1mm apical to Gingival Margin*	**-161,547**	**1,000**	**374,812**	**0,159**	**536,359**	**0,017***		**353,616**	**1,000**	**741,548**	**0,003***	**387,931**	**0,691**
11 *So# %ssue thickness 2mm apical to Gingival Margin*	**-133,011**	**1,000**	**71,195**	**1,000**	**204,206**	**1,000**		**-347,208**	**1,000**	**-577,800**	**0,283**	**-230,592**	**1,000**
12 *So# %ssue thickness 3mm apical to Gingival Margin*	**-19,557**	**1,000**	**-423,256**	**0,072**	**-403,699**	**0,173**		**-91,344**	**1,000**	**-447,234**	**0,279**	**-355,890**	**1,000**
13 *So# %ssue thickness at CEJ*	**-238,94194**	**1,000**	**-158,878**	**1,000**	**80,064**	**1,000**		**-435,670**	**0,635**	**-236,811**	**1,000**	**198,859**	**1,000**
14 *So# %ssue thickness at provisional margin*	**190,088**	**1,000**						**1,650**	**1,000**				
15 *So# %ssue thickness at prepara%on line*	**227,210**	**1,000**						**488,860**	**1,000**				
16 *Bone width 1mm apical to Bone Crest*	**-159,797**	**1,000**	**-357,767**	**0,143**	**-197,970**	**1,000**		**319,591**	**1,000**	**193,729**	**1,000**	**-125,863**	**1,000**
17 *Bone width 2mm apical to Bone Crest*	**-462,839**	**1,000**	**-727,180**	**0,095**	**-264,341**	**1,000**		**427,819**	**1,000**	**396,197**	**1,000**	**-31,622**	**1,000**
18 *Bone width 3mm apical to Bone Crest*	**-198,923**	**1,000**	**-870,901**	**0,175**	**-671,978**	**1,000**		**716,356**	**0,920**	**910,664**	**0,057**	**194,308**	**1,000**

* Differences were deemed statistically significant when p was <0.05.

Table 4 - Comparative statistics of the buccal and lingual measurements at 12 weeks

	ANOVA 12W											
	BUCCAL						LINGUAL					
VARIABLE	BOPT - CHAMFER		BOPT - NOPREP		CHAMFER-NOPREP		BOPT - CHAMFER		BOPT - NOPREP		CHAMFER - NOPREP	
	I-J	*P*	I-J	*P*	I-J	*P*	I-J	*P*	I-J	*P*	I-J	*P*
1 *So# %ssue height LINEAR*	**-416,473**	**1,000**	**-584,028**	**0,518**	**-167,555**	**1,000**	**207,012**	**1,000**	**-181,441**	**1,000**	**-388,453**	**1,000**
2 *Barrier Epithelium LINEAR*	**-1044,236**	**0,043***	**-1258,259**	**0,001***	**-214,023**	**1,000**	**-54,741**	**1,000**	**-413,118**	**1,000**	**-358,377**	**1,000**
3 *Connec%ve %ssue a=achment LINEAR*	**639,673**	**1,000**	**678,039**	**1,000**	**38,366**	**1,000**	**265,097**	**1,000**	**232,423**	**1,000**	**-32,675**	**1,000**
4 *So# %ssue margin rela%ve to CEJ LINEAR*	**-803,156**	**0,651**	**-999,448**	**0,043***	**-196,293**	**1,000**	**-196,630**	**1,000**	**-581,714**	**1,000**	**-385,083**	**1,000**
5 *Bone Crest rela%ve to CEJ LINEAR*	**401,554**	**1,000**	**418,826**	**1,000**	**17,272**	**1,000**	**399,058**	**1,000**	**390,728**	**1,000**	**-8,330**	**1,000**
6 *So# %ssue margin rela%ve to provisional LINEAR*	**-511,096**	**1,000**					**398,254**	**1,000**				
7 *Bone Crest rela%ve to provisional LINEAR*	**90,309**	**1,000**					**-202,294**	**1,000**				
8 *So# %ssue margin rela%ve to prep line LINEAR*	**-226,425**	**1,000**					**446,524**	**1,000**				
9 *Bone Crest rela%ve to prep line LINEAR*	**-191,005**	**1,000**					**-245,018**	**1,000**				
1B *So# %ssue height CONTINUOUS LINE*	**169,593**	**1,000**	**192,915**	**1,000**	**23,323**	**1,000**	**559,132**	**1,000**	**522,553**	**1,000**	**-36,580**	**1,000**
2B *Barrier Epithelium CONTINUOUS LINE*	**-119,235**	**1,000**	**-116,810**	**1,000**	**2,425**	**1,000**	**296,447**	**1,000**	**326,011**	**1,000**	**29,564**	**1,000**
3B *Connec%ve %ssue a=achment CONTINUOUS LINE*	**288,828**	**1,000**	**309,725**	**1,000**	**20,897**	**1,000**	**262,685**	**1,000**	**196,542**	**1,000**	**-66,143**	**1,000**
4B *So# %ssue margin rela%ve to CEJ CONTINUOUS LINE*	**-575,078**	**1,000**	**-578,231**	**1,000**	**-3,152**	**1,000**	**141,114**	**1,000**	**158,246**	**1,000**	**17,132**	**1,000**
5B *Bone crest rela%ve to CEJ CONTINUOUS LINE*	**744,671**	**1,000**	**771,146**	**0,342**	**26,475**	**1,000**	**418,019**	**1,000**	**364,307**	**1,000**	**-53,711**	**1,000**
6B *So# %ssue margin rela%ve to provisional CONTINUOUS*	**-673,541**	**1,000**					**1129,224**	**1,000**				

7B *Bone crest rela%ve to provisional CONTINUOUS LINE*	**918,905**	**1,000**						**-570,091**	**1,000**				
8B *So# %ssue margin rela%ve to prep line CONTINUOUS LINE*	**-49,366**	**1,000**						**1140,184**	**1,000**				
9B *Bone crest rela%ve to prep line CONTINUOUS LINE*	**218,958**	**1,000**						**-581,052**	**1,000**				
10 *So# %ssue thickness 1mm apical to Gingival Margin*	**404,163**	**0,861**	**412,108**	**0,295**	**7,945**	**1,000**		**-24,070**	**1,000**	**263,456**	**1,000**	**287,526**	**1,000**
11 *So# %ssue thickness 2mm apical to Gingival Margin*	**-99,825**	**1,000**	**-256,200**	**1,000**	**-156,375**	**1,000**		**160,406**	**1,000**	**68,169**	**1,000**	**-92,237**	**1,000**
12 *So# %ssue thickness 3mm apical to Gingival Margin*	**-71,202**	**1,000**	**-318,684**	**1,000**	**-247,482**	**1,000**		**-75,452**	**1,000**	**-236,095**	**1,000**	**-160,644**	**1,000**
13 *So# %ssue thickness at CEJ*	**-344,391**	**0,795**	**-501,323**	**0,018***	**-156,932**	**1,000**		**-226,271**	**1,000**	**-178,852**	**1,000**	**47,420**	**1,000**
14 *So# %ssue thickness at provisional margin*	**-271,430**	**1,000**						**433,827**	**1,000**				
15 *So# %ssue thickness at prepara%on line*	**-99,290**	**1,000**						**621,700**	**1,000**				
16 *Bone width 1mm apical to Bone Crest*	**194,845**	**1,000**	**-252,397**	**1,000**	**-447,242**	**0,133**		**84,651**	**1,000**	**112,024**	**1,000**	**27,373**	**1,000**
17 *Bone width 2mm apical to Bone Crest*	**232,031**	**1,000**	**-480,616**	**1,000**	**-712,647**	**0,319**		**24,384**	**1,000**	**215,179**	**1,000**	**190,795**	**1,000**
18 *Bone width 3mm apical to Bone Crest*	**71,172**	**1,000**	**-625,987**	**1,000**	**-697,159**	**1,000**		**294,605**	**1,000**	**651,156**	**0,992**	**356,551**	**1,000**

* Differences were deemed statistically significant when p was <0.05.