Effectiveness of a personalized device in the evaluation of mandibular second molar periodontal healing after surgical extraction of adjacent third molar

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Summary

Aim. **The primary aim of the present study was to validate the effectiveness of a personalized device able to guide periodontal probing in evaluation of second molar periodontal healing after adjacent third molar surgical extraction. Secondarily, the study analyzed if any patient and tooth related factors affected the second molar periodontal healing as well as if they were able to affect the periodontal probing depth performed with or without the personalized device.**
Materials and methods. **Thirty-five lower second molars were evaluated after extraction of the adjacent third molar. Pre-operative as well as 3 and 12 month post-operative probing depths of the distal surface of the second molar were evaluated. All measurements were taken by two different methods: standard two-point and four-point probing using a personalized onlay-type guide. Periapical radiographs were also evaluated. The Pearson product moment and the general linear model with backward stepwise procedure were used for inferential statistics.**
Results. **The mean 12-month post-operative probing depth/mean pre-operative probing depth ratio obtained with the guided probing method showed a highly significant effect on the 12-month radiographic post-operative/pre-operative radiographic measure ratio. None of the examined patient- or tooth-related factors showed a significant effect on pre-operative/12-month post-operative radiographic measure ratio.**
Conclusions. **The use of the proposed personalized device seems to provide a more reliable estimate of second molar periodontal healing after adjacent third molar surgical extraction. No patient- or tooth-related factors seem to be able to affect either second molar periodontal healing or probing depth measures obtained with or without the personalized device in individuals younger than 25 years old. It can be therefore recommended that lower third molar surgical extraction be performed in young adults.**

Key words: periodontal healing, periodontal probing, radiographic measurements, smoking, statistics.

Introduction

Many studies have been performed over the years to verify periodontal modifications of the lower second molar distal surface after adjacent third molar surgical extraction (1-14) with regard to several risk factors, such as flap design, the infra-bony pre-operative pocket on the second molar distal surface, the patient's age, the width of the contact area between second and third molar and the mesial width of third molar follicular space. However, clinical evaluation of periodontal defects on the distal surface of the lower second molar can be affected by non-repeatability of the measurements due to unavoidable variation in the probing site, as well as of the insertion axis of the periodontal probe. The use of a device to guide periodontal probing theoretically minimizes inter- and intra-examiner variability, making the measurements obtained at different times comparable. The primary aim of the present study was to validate the effectiveness of a personalized device built to guide periodontal probing in the clinical evaluation of lower second molar periodontal healing after adjacent third molar surgical extraction, in comparison with the conventional periodontal probing technique, using the radiographic assessment of bone healing as an objective reference. Second, this study analyzed whether any patient- or tooth-related factors were able to affect second molar periodontal healing, as well as periodontal probing depth performed with or without the personalized device.

Materials and methods

Sampling

The examined sample included 35 second molars that were clinically and radiographically evaluated before and after extraction of the adjacent third molar, from 30

subjects among the patients treated with the same common protocol by the same operator over a 2-year period at the Oral Surgery Unit of the Department of Oral and Maxillo Facial Sciences at the "Sapienza" University of Rome. The inclusion criteria included the following: at least one lower extracted third molar; vertical, mesio-angular or horizontal third molar class B-depth impaction; the presence of the first two molars and the second premolar on the extraction side, to assure stent stability during measurements; and the patient's consent to use the clinical data for statistical evaluation. The exclusion criteria included the following: any systemic disease and local or generalized periodontitis; pregnancy or lactation; any medication in the last 6 months that could influence wound healing after surgery; any type of reconstruction on the distal surface of the second molar, as well as a second molar prosthetic crown on the extraction side; caries on the occlusal and inter-proximal surfaces of the first two molars, as well as on the second premolar; a history of root planing performed immediately after surgical extraction; a history of problems in stent seating at the 3- and 12-month probing follow-ups; missing or incomplete periodontal data or radiographs; any type of conservative or prosthetic rehabilitation performed on the molars or second premolar on the extraction side before the 12-month follow-up; and a history of professional calculus or plaque removal after surgery before the 12-month follow-up. All of the clinical and radiographic measurements were obtained under magnification using a Head-Worn Loupe KS (Carl Zeiss, Germany) with the following technical features: magnification ratio: 4x; working distance: 300 mm; free distance to object: 220 mm; field of view: 50 mm; and length of optical system: 51.5 mm.

All of the following examinations and treatments were performed by the same investigator.

Periodontal examinations and measurements

- WHO periodontal screening and recording (PSR), with a PCP 11 5B probe (Hu-Friedy, USA), and furcation probing of first and second molars, with a #2 Nybers probe (PQ2N6 - Hu-Friedy, USA), were performed to exclude generalized periodontitis or localized periodontitis on the extraction side.
- Periodontal probing depth (PD) of the distal surface of the lower second molar was always performed with the same straight, tapered, 15 mm probe (42-746-02 2A Martin, Germany) pre-operatively, as well as at 3 and 12 months post-operatively, on the extraction side by two different methods:
A - standard (stand) two-point probing, disto-buccal (DB) and disto-lingual (DL); and
B - four-point probing (dev), buccal (B), disto-buccal (DB), centro-distal (CD) and disto-lingual (DL), using a personalized onlay-type guide or stent (Fig. 1), directly made in the patient's mouth during a preliminary session using auto-polymerizing acrylic resin (Jet Kit, Lang Dental MFG Co. Inc. Wheeling, IL, USA) and then completed in the laboratory. During resin polymerization, 4 vertical grooves were made on the distal side of the
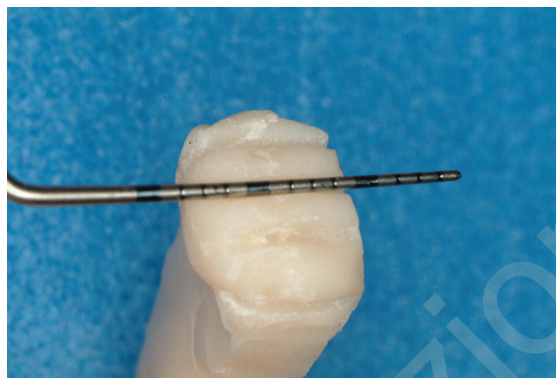

Figure 1. The personalized acrylic stent used for the study with the probe positioned in one of the four 6 mm high grooves.

stent at the level of the four points chosen for probing, using a probe similar to that used for the measurements. Each stent was approximately 2 mm thick, involved three teeth from the second molar to the second premolar and extended for 4-5 mm from the occlusal surface on the distal face of the second molar, ending approximately 4 mm coronally from the gingival margin, so the grooves were at least 6 mm in height. A slight overlap (approximately 1 mm) of the stent on the lateral surfaces of all of the teeth involved was allowed to assure retention and stability.

All of the probing measurements were obtained to the nearest millimeter from the free gingival margin to the bottom of the pocket, using the same probe. To improve visibility, the measurements were obtained in an indirect manner, using a #5 mouth mirror (Kerr Co. USA) positioned distally to the second molar.

Radiographic examinations and measurements

- Periapical radiographs were performed pre-operatively, as well as at 3 and 12 months post-operatively (Fig. 2), with a Castellini radiographic machine (70 KVp – 8 mA – 0.38 kW tube type Cox 70G8CEI), using the parallel technique, with a long cone and a precision device (Rinn® XCP Instruments Kit, Dentsply, Elgin, IL, USA) and a focal object distance of approximately 20 cm. Before the film-holder was positioned in the mouth, for each radiograph, a millimetric adhesive grid (Phil x-Ray Grid - Pat. Pending) was applied on the film side facing the teeth. The radiographs were examined on a diaphanoscope (230 V, ~ 50-60 Hz, 85 W - Titanox, Italy). Study measurements were obtained with a millimetric ruler, from the cement-enamel junction (CEJ) to the bottom of the radiographically detectable bone defect just near the distal surface of the second molar. For each radiograph, the definitive unknown measurement was calculated by applying a mathematical proportion, in which the known terms were the actual millimetric length of ten vertically contiguous squares of the grid and the millimetric value of the defect, both obtained from the radiographs. Each value obtained in

Figure 2. The complete three-radiograph set of one of the studied second molars. Pre-operative x-ray (left); 3-month post-operative x-ray (middle); 12-month post-operative x-ray (right).

this manner was then approximated to the nearest milli-metric unit. All of the radiographic measurements were performed by the examiner at the end of the study.

- The following data resulting from pre-operative panoramic radiographs of all of the patients were also considered: contact area width between the third and second molar (≤ 3 mm / ≥ 4 mm); and third molar incli-nation (0-29°/30-59°/60-90°).

Surgical technique

Third molar extraction was performed according to the following standard protocol: pre-operative oral antibiotic prophylaxis with amoxicillin plus clavulanic acid (2 g) 1 hour before the procedure; 3% mepivacaine anesthesia of the inferior alveolar nerve, plus 2% mepivacaine infil-trative anesthesia with adrenaline in the buccal mucosa of the third molar region; angular flap; tooth removal af-ter ostectomy and/or tooth sectioning, if needed; re-moval of follicular remnants and inflammatory tissue from the residual cavity; final cleaning with sterile saline irrigation; 3-0 silk sutures with a taper-cut needle; and granular nimesulide 100 mg immediately after surgery and further doses, if needed, every 12 hours following suture removal at 1 week post-operatively. No root planing of the distal surface of the second molar was performed before, during or after surgery. No specific instructions on oral hygiene were provided to the pa-tients after surgery. Surgical extractions in the same patient were performed at different times.

Other clinical features

The following data were also collected as binary vari-ables (yes/no): pre-operative inflammatory signs or symptoms; third molar communication with the oral en-vironment; visible plaque presence on the distal surface of the second molar at least in one of the follow-up ex-aminations; and the patient's smoking habits.

Statistical methods

Each lower second molar was considered an indepen-dent statistical unit. The mean value, as the central

trend index, the standard error (SE), as the variability index, and the Kolmogorov-Smirnov test (K-S), as the distribution normality test, were used for descriptive sta-tistics of quantitative variables. Absolute and percent-age frequencies were used for qualitative variables. Pearson's product moment (r) and the general linear model (GLM) with the backward stepwise procedure (BSP) were used for inferential statistics.

The study was conducted with the approval of the local Ethical Committee, with protocol number 725/12. Ethi-cal principles for medical research, as stated by the Helsinki Declaration, were followed. Informed consent/assent was obtained from all of the study par-ticipants.

Study design

For each of the proposed aims, a different study design was planned.

The first aim was to verify the degree of correspon-dence between the periodontal healing evaluation per-formed by radiographic measurements and the probing depths measured with and without the personalized de-vice as a guide.

As a first step, to evaluate the possible improve-ment/worsening over time of the second molar peri-odontal status, the mean pre-operative PD/post-oper-ative mean PD at 3 and 12 months, as a ratio mea-sured with (dev3/pre and dev12/pre) and without (stand3/pre and stand12/pre) the personalized device, were calculated, in which 1 indicated stableness, val-ues < 1 indicated improvement, and values > 1 indi-cated worsening. Moreover, the ratios of measure-ments obtained from pre-operative radiographs (xrpre) to those obtained from 3- (xr3) and 12- (xr12) month post-operative radiographs were calculated (xr3/pre and xr12/pre, respectively) as an objective control.

The postulate was that if the probing depth measure-ments obtained with the personalized device provided an effective measurement of the second molar peri-odontal improvement/worsening, then a statistically sig-nificant correlation would exist between those measure-ments and the radiographic measurements. In other words, it should be possible to predict the radiographic outcome from the probing depth measurements ob-tained using the personalized device. Moreover, if the

latter were more reliable than the results obtained with the standard probing method, one might expect that the correlation between the measurements obtained with the personalized device and the radiographic measurements would be stronger than the correlation between measurements obtained with the classic probing method and the radiographic measurements. Because a very strong correlation was found between dev12/pre and dev3/pre ($r = 0.91$), as well as between stand12/pre and stand3/pre ($r = 0.81$), meaning that the second molars, the periodontal status of which improved after 12 months, were basically the same as those found to have improved periodontally after 3 months, only the ratios calculated at 12 months were used (dev12/pre; stand12/pre; xr12/pre) to shorten and simplify the subsequent analysis.

Two GLMs were therefore developed: the first one used xr12/pre as a dependent variable and dev12/pre as an explanatory variable; the second one, in which xr12/pre was used as a dependent variable and stand12/pre as an explanatory variable, was then developed.

To investigate more closely the comparison between the standard and guided probing methods, the previous analysis was repeated using the PD measurements obtained in the only comparable point of the two methods, named "B" in the guided method and "DB" in the standard method, instead of the mean measurements obtained with each of the two methods. Hence, the ratios between the 12-month post-operative PD and the pre-operative PD, respectively obtained with and without the stent (B12/pre and DB12/pre), were used. Two further GLMs were therefore developed, using xr12/pre, as a dependent variable and, respectively, B12/pre and DB12/pre as explanatory variables.

The second aim was to verify whether and which patient-related and third molar-related variables influenced periodontal second molar healing.

Radiographic measurements after 12 months (xr12/pre) have been used as objective measurements of second molar periodontal healing. A GLM with the BSP ($p > 0.05$) has been used to analyze the effects of the following explanatory variables on xr12/pre: patient's age, sex, and smoking habits (yes/no); visible plaque presence at follow-up examinations (yes/no); contact area between the third and second molars (≤ 3 mm/≥ 4 mm); pre-operative local signs and/or symptoms of infection (yes/no); pre-operative third molar communication with oral environments (yes/no); and third molar inclination relative to the second molar axis ($\leq 30°/>30°$). For convenience, third molar inclination was also considered a binary variable because only 5 third molars were found to have inclinations between 30° and 60°.

The third aim was to verify whether and which patient-related and third molar-related factors influenced the correlation between guided probing depths (dev12/pre) and radiographic measurements (xr12/pre) and to determine whether any correlation existed between standard probing depths and radiographic measurements.

For this type of analysis, the residuals of the two general linear models, developed to accomplish the first aim, were used. Residuals are the deviations between the observed values of the dependent variable (xr12/pre) and the predicted values on the basis of the explanatory variables (dev12/pre or stand12/pre). The postulate was therefore that, if a variable positively or negatively influenced the correlation between xr12/pre and dev12/pre or stand12/pre, that variable should also positively or negatively influence the deviation values of the two xr12/pre models, respectively developed with dev12/pre (xrdev12/preRES) and stand12/pre (xrstand12/preRES) as explanatory variables. Two different GLMs with BSP were used to test, respectively the effects on xrdev12RES and xrstand12RES of the above-mentioned variables: patient's age, sex, and smoking habits (yes/no); visible plaque presence at follow-up examinations (yes/no); contact area between third and second molars (≤ 3 mm/≥ 4 mm); third molar inclination relative to the second molar axis ($\leq 30°/>30°$); pre-operative local signs and/or symptoms of infection (yes/no); and pre-operative third molar communication with oral environments (yes/no).

Results

Descriptive results

All of the surgical procedures were uneventful. The age of the patients at the time of surgery ranged from 16 to 46 years old, with a mean age of 24.37 ± 0.93 years old. Nineteen of the 35 third molar extractions were performed in female patients (54.29%) and 16 in male patients (45.71%). Pre-operative symptoms, as well as communication with the oral environment, were present in 19 of 35 cases (54.29%). In 18 of 35 cases (51.43%), third molar inclination was less than 30°, and in 23 cases (65.71%), the contact area between the second and third molar was ≥ 4 mm. Twenty-three extractions were performed in smokers (65.71%), and in 24 of 35 cases (80%), visible plaque was detectable on the distal surface of the second molar in at least one of the follow-up examinations.

The mean probing depths detected pre-operatively (pre), as well as at three (3) and twelve (12) months post-operatively with (dev) and without (stand) the personalized device, were the following: devpre=3.10 ± 0.16; dev3=3.08 ± 0.10; dev12=2.75 ± 0.11; standpre=5.50 ± 0.27; stand3=4.94 ± 0.25; and stand12=4.00 ± 0.16.

The mean radiographic measurements of bone defects were the following: pre-operative (xrpre)=6 ± 0.38; 3 months post-operatively (xr3)=4.94 ± 0.40; and 12 months post-operatively (xr12)=3.68 ± 0.32.

Inferential analysis results

First aim

The results obtained with the first GLM (Fig. 3) (coefficient $\pm$ SE=0.52 ± 0.15; t=3.56; p=0.0011) showed that dev12/pre had a highly significant effect on xr12/pr ($F_{1, 33}$=12.68; p=0.0011), although the correlation between
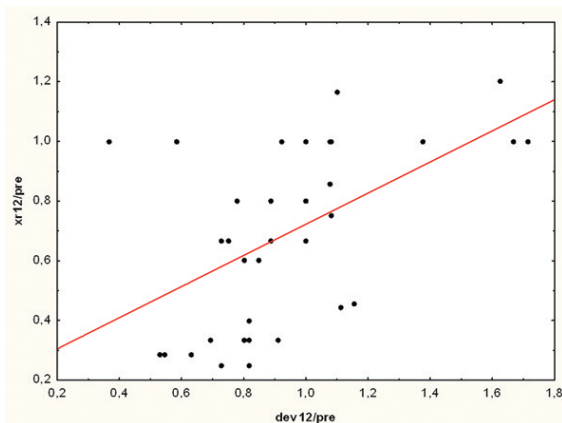
Figure 3. Ratio of the 12-month post-operative radiographic measures to the pre-operative radiographic measures versus ratio of the mean 12-month guided probing depths to the mean pre-operative guided probing depths.

Figure 4. Ratio of the 12-month post-operative radiographic measures to the pre-operative radiographic measures versus ratio of the mean 12-month standard probing depths to the mean pre-operative standard probing depths.

the observed xr12/pre values and the predicted values, on the basis of dev12/pre, was moderate (R=0.53), indicating that dev12/pre variability explained a moderate portion of xr12/pre variability. Finally, the residuals (the differences between the observed xr12/pre values and the predicted values on the basis of dev12/pre) showed an almost normal distribution (K-S: d=0.11; p>0.20). This finding confirms the estimate of the standard error regression coefficient. In other words, a statistically significant, although moderate, correspondence existed between radiographic measurements and periodontal probing depth obtained with the personalized device.

Results from the second GLM (Fig. 4) (coefficient $\pm$ SE=0.65 $\pm$ 0.25; t=2.63; p=0.01) were generally similar to those obtained with the previous GLM ($F_{1, 33}$=6.92; p=0.0128), although stand12/pre had a less significant effect on xr12/pre than dev12/pre (higher p value), and it explained a smaller portion of xr12/pre than dev12/pre (lower R value). In other words, this correspondence was lower than that between radiographic measurements and measurements obtained with the guided probing technique. The use of the personalized device therefore seemed to provide a more reliable estimate of second molar periodontal healing.

The results from the last two GLMs (Fig. 5: $F_{1, 33}$=12,79; p=0.0011; Fig. 6: $F_{1, 33}$=7.72; p=0.0089) confirmed those obtained with the two previous GLMs (Figs. 3, 4), in that the guided method produced findings were slightly more correlated with the radiographic findings (coefficient $\pm$ SE=0.42 $\pm$ 0.19; t=3.58; p=0.001) than those obtained with the standard method (coefficient $\pm$ SE=0.50 $\pm$ 0.18; t=2.79; p=0.009).

Figure 5. Ratio of the 12-month post-operative radiographic measures to the pre-operative radiographic measures versus ratio of the 12-month guided probing depths in the B point to the pre-operative guided probing depths at the same point.

Figure 6. Ratio of the 12-month post-operative radiographic measures to the pre-operative radiographic measures versus ratio of the 12-month guided probing depths in the DB point to the pre-operative guided probing depths at the same point.

Second aim

Because no models were developed by the BSP, none of the examined patient-and tooth-related factors had significant effects (Tab. 1) (p-values < 0.05 with the T-test) on the ratio between pre-operative and 12-month post-operative radiographic measurements (xr12/pre).
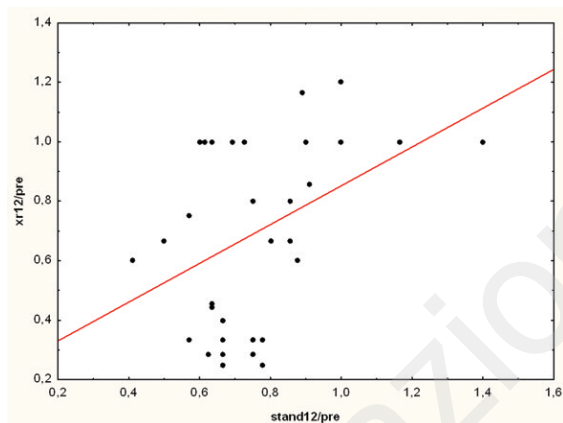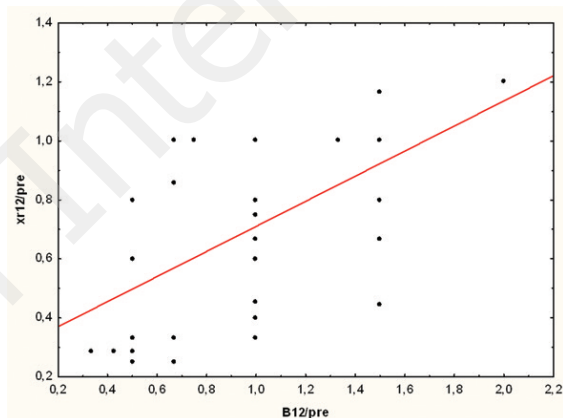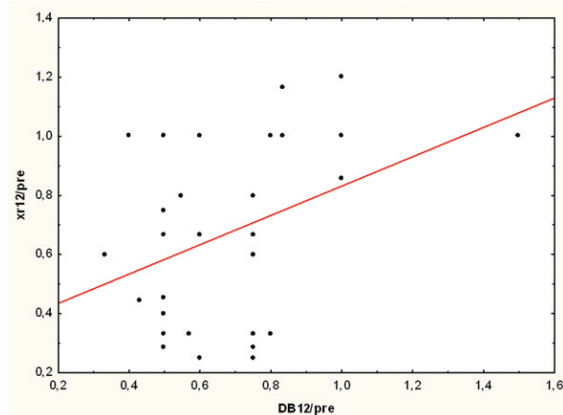
Table 1. Partial correlations (PC) between the explanatory variables not included in the general linear model and xr12/pre (dependent variable).

Explanatory variables	PC	T	p
Gender	-0.084	-0.486	0.630
Smoking habits	-0.022	-0.129	0.898
Plaque	0.109	0.630	0.533
Contact area	-0.194	-1.135	0.264
Symptoms	0.085	0.490	0.627
Inclination	-0.192	-1.125	0.269
Communication with oral cavity	0.000	0.002	0.998
Age	0.137	0.797	0.431

Table 2. Partial correlations (PC) between the explanatory variables not included in the general linear model and xrdev12/preRES (dependent variable).

Explanatory variables	PC	T	p
Gender	0.155	0.902	0.373
Smoking habits	0.093	0.540	0.592
Plaque	0.057	0.326	0.747
Contact area	-0.077	-0.446	0.658
Symptoms	0.012	0.069	0.945
Inclination	-0.225	-1.326	0.194
Communication with oral cavity	0.000	0.002	0.998
Age	0.191	1.118	0.272

Table 3. Partial correlations (PC) between the explanatory variables not included in the general linear model and xr stand12/pre RES (dependent variable).

Explanatory variables	PC	T	p
Gender	-0.236	-1.397	0.171
Smoking habits	-0.075	-0.432	0.668
Plaque	0.120	0.694	0.492
Contact area	-0.212	-1.246	0.222
Symptoms	0.119	0.688	0.496
Inclination	-0.178	-1.039	0.306
Communication with oral cavity	0.057	0.331	0.743
Age	0.103	0.597	0.554

Third aim

Because no models were developed with the BSP, none of the examined patient- or tooth-related factors had significant effects (Tab. 2) (p-values < 0.05 with the t-test) on the ratio between pre-operative and 12-month post-operative radiographic measurements (xr12/pre) or on the deviations between the observed value of xr12/pre and the values predicted on the basis of each considered factor (Tab. 3) (dev12/pre or stand12/pre).

Discussion

In the present study, 35 second molars were examined after adjacent third molar extraction in 30 patients. In 5 patients, both lower third molars were extracted at dif-

ferent times. The use of each lower second molar of the same patient as a single statistical unit has already been reported in previous studies (5-7) because, from the analysis of variance concerning probing depths and intra-bony defects, it seemed there was no dependence between two operations performed in the same patient, even if performed at different times.

The mean age of the patients in the present study (24,30 ± 0,98) was younger than that in previous studies (27,2 ± 6,35 (3); 27 ± 7 (10); 30, 37 ± 2,32) (14). If the only patient older than 30 years old (46 years old) was excluded, the mean age of the patients (23,63 ± 0,73) was similar to that in Montero and Mazzaglia's study (13). In the studies of Cetinkaya et al. (11) and Faria et al. (14) the mean age (18,53 ± 1,60 (11); 21,03 ± 4,38) (14) was younger than in the present study.

Only two previous studies (5, 10) have evaluated the periodontal condition of any other tooth, to assess whether second molar periodontal involvement was a local problem due to third molar impaction or periodontitis. Specifically, Kuegelberg (5) examined the first mandibular molar, while Kan et al. (10) examined the entire dentition. To avoid any influence that local or generalized periodontitis might have on post-surgical second molar periodontal healing, the present study analyzed patients in whom the WHO PSR, which was previously applied, excluded any type of periodontal involvement (not including the second molar distal surface).

With the same purpose, to avoid any positive or negative influence on the healing process, in the present study, patients with systemic conditions that might have an effect on bone growth and periodontal healing, such as uncontrolled diabetes mellitus or immunosuppressive treatments, and participants who were subjected to professional plaque and calculus removal after surgery, before the 12-month follow-up, or to root planing immediately after surgical extraction were excluded from this study. As a routine measure, calculus removal was gently performed only intra-operatively, if present. Actually, root planing of the second molar distal surface has been found to improve healing in the presence of pre-existing periodontal impairment (PD > 4 mm); however, it can determine periodontal deterioration if performed for any indication, for example, in subjects with healthy preoperative second molar periodontal status (16). Osborne et al. (17) previously found minimal benefits by root planing and curetting the second molar in individuals younger than 25 years old at the time of third molar removal.

Accuracy is especially needed in clinical studies aimed to verify differences in the effectiveness of different diagnostic or therapeutic procedures, to avoid any possible sources of error. All of the steps of each procedure are subject to the risk of error. Inter-examiner and intra-examiner variability is also a clear source of error in clinical studies.

Many attempts have been undertaken in the past to standardize clinical and radiographic methods in studies aimed to evaluate periodontal healing after different therapeutic procedures. Methodological standardization certainly limits the intra- and inter-ex-

aminer variability. Furthermore, inter-examiner variability is clearly avoided if the measurements are obtained by the same examiner.

In the present study, all of the procedures were performed by the same expert surgeon, and much effort was undertaken to apply reproducible methods of investigation and treatment to limit any possible causes of error. Details were provided for all of the steps of the research, although simple, partly self-made and easily available methods were used because they are commonly used in daily practice, they do not require extensive training for use, and they are not uncomfortable for the patients. The only disadvantage was the direct chair-side making of the stent, which was fairly time-consuming and which develops heat and an unpleasant taste. An indirect technique could otherwise be used, so the guide could be made on a plaster cast, obtained from a previous alginate impression, although in this case, direct adaptation in the patient's mouth was sometimes necessary nevertheless before its use.

Although both clinical and radiographic examinations have limitations in measurement accuracy, and many attempts have been undertaken in the past to eliminate every source of error and to reduce inter- and intra-examiner variability, they are commonly used in clinical practice, as well as in research studies.

In the present study, probing depth was the only probing parameter considered. Clinical attachment level (CAL) was not evaluated because the cemento-enamel junction (CEJ) was sometimes not visible on the distal aspect of the second molar due to its sub-gingival location, and therefore it could not be used as a clinical reference point. Moreover, the main aim of the present study was not to obtain a real measurement of the periodontal (bone and soft tissue) level but to verify the different ability of the two diagnostic clinical methods applied in evaluating periodontal healing under standardized conditions.

The landmarks for radiographic measures were the CEJ and the bottom of the bone defect on the distal surface of the second molar.

Probing measurements

There are three possible main sources of error related to probing measurements in the second molar region (18-20): visual, tactile and positional. The first is related to the difficulty in visual perception of the probing depth on the distal surface of the second molar with the stent in place. The second is mainly related to the presence of a third molar crown or calculus, which can somewhat affect the pre-operative probing depth, as well as probing force and probe-tip diameter and calibration. The third, which the use of the stent was intended to reduce, is related to the different probing point and inclination from one measurement to another.

A mirror and a 4x loupe were used in the present study to improve visibility during probing measurements. The mirror was used not only to look at the reflected image, because direct reading was obstructed by the stent, but also to reflect the light, thus increasing illumination,

whereas the loupe was used to magnify the image. Much better reading was therefore guaranteed.

As for probing force, it is usually thought that measurements obtained by the same expert investigator are comparable to each other or at least that the existing differences are diluted among all measurements. Hassel et al. (21) found poor correlation between PD measurements and the probing force applied, so they concluded that probing force had only a moderate influence on PD measurements and that the probing technique was more critical in PD measurement than the pressure applied to the probe. Inter and inter-examiner variability in PD measurements of a same defect is therefore more related to different areas of the defect being measured at different times than to the probing force applied in different measurements.

Because probe tip diameter and calibration have also been considered as further variables able to influence periodontal probing and because these characteristics have been found to change from one probe type to another, as well as in the same batch of instruments (22), in the present study, the same probe was always used for all of the probing measurements, to improve accuracy and reproducibility.

As for positional error, in the present study, a personalized device was used to guide the probe during measurements. The use of a personalized guide or stent for periodontal probing depth has already been described in the literature, (1, 2, 18-20, 23, 24) as well as to evaluate lower second molar periodontal healing after adjacent third molar removal (11). However, Watts (18) reported that, despite the use of the stent to guide periodontal probing, the greater source of error in his study was just the position at which the probe was placed. Nevertheless, in Watts's study (18), the guidelines were marked on the stent with a heated burnisher, but no information was provided about the diameter or the depth of the grooves or their height. Cetinkaya et al. (11) reported no information about the stent at all. In the present study, the grooves were furrowed during resin hardening, using a probe similar to that used for the measurements, and the groove height was at least 6 mm to guide the probe properly each time into the same position and with the same inclination, despite possibly aberrant pocket anatomy (25), scanty visibility and limited patient cooperation due to discomfort or pain caused by gingival inflammation. Moreover, to exclude any interference in the correct choice of the axis of probe insertion during groove furrowing, no cases with reconstructions or prosthetic crowns on second molars were included in the present study because imperfect distal surfaces might have altered the anatomic relationship between the distal tooth surface and the periodontium, giving rise to difficulties in probe insertion and therefore possibly altering probing depth measurements, which could indicate a false periodontal condition.

Despite the use of the personalized device, some degree of error is however to be expected. Watts (18) previously suggested that changes in probing depth over a period of time are merely due to localized examiner error. Because the variation in horizontal

probing position seems to have been an important error in all of the studies in which individual sites were analyzed over time (19), the depth and the height of the grooves seemed to be important features to minimize positional differences, making subsequent measures comparable. In the present study, the grooves were furrowed during resin hardening, using a probe similar to that used in periodontal probing, so they were almost perfectly aligned with the site that was to be probed. If they were furrowed afterward with a cylindrical burr, they could have incorrectly guided the probe into the gingival sulcus due to a wrong inclination. Moreover, the grooves ended approximately 4 mm coronally to the gingival margin, so the probe was clearly visible during insertion. Nevertheless, slight inclination of the probe is possible despite the height of the grooves, which was approximately 6 mm, as well as their depth, which was slightly greater than the probe diameter, especially in subsequent measurements, because the tapered morphology of the probe does not allow the probe diameter to fit the groove diameter in cases of PD less than the pre-surgical PD, so attention was always paid to assure that the probe was in the groove for the entire 6 mm tract during the measurement. A parallel-sided probe could therefore be more adequate in guided probing than a tapered one, although this type of probe is less frequently used in clinical practice because it is less able to penetrate periodontal tissues.

Moreover, because it was possible that the stent became unfit over time and could not be used either due to slight tooth movements caused by occlusal forces, orthodontics, tooth extraction or periodontal disease or due to conservative or prosthetic tooth rehabilitation possibly performed during the study period, patient exclusion from the present study was undertaken for these reasons. Furthermore, the same stent would most likely be unfit after many years in the case of further measurements to analyze second molar periodontal modification better after adjacent third molar extraction over a long period of time, as performed by some authors (5, 10).

Because bone regeneration is certainly greater where the infra-bony defect is deeper, which is more often in the middle of the second molar distal surface due to third molar crown impaction, the choice to consider four-point measurements with the guided probing method, instead of the two line-angle measurements with the standard method, could explain the greater reliability of the guided method because it might be able to evaluate the healing process, that is, a site-specific process, in greater detail than the usual method. However, because the statistical analysis was performed using the mean of the measurements obtained with each method, the possible greater sensitivity of the guided method, due to the more detailed evaluation of the infra-bony defects on the distal surface of the second molar, could have been diluted. Furthermore, slightly greater reliability of the guided method was demonstrated, although measurements performed with the two methods only at similar line-angle points were considered.

Another possible cause of probing error and low reproducibility of measurements was previously found to be the depth of sites (19, 25), most likely because the deeper the pocket was, the higher the risk of positional changes of the tip of the probe during its penetration was, although the use of the stent theoretically minimized this type of possible error as well.

Finally, the state of periodontal tissues can influence PD measurements as well due to different resistance to penetration between variously inflamed and healthy tissues, as well as the different responses of patients to probe penetration at various degrees of inflammation. The use of the stent also avoids that the probe insertion axis having to be modified relative to the lower compliance of the patient due to possible tissue inflammation.

Radiographic measurements

The choice to use the radiographic measurements of bone defects as objective references might be questionable; however, it is a safe and universally recognized method to obtain subsequent comparable measurements of the bone height in a standardized manner, without surgical re-entry or traumatic periodontal probing (4). However, many sources of technical radiographic errors exist, including vertical or/and horizontal changes in projection geometry due to different angulations between the central beam and the film or the teeth and different distances between the teeth and the film or the tube (4, 26-29). Because intra-oral radiographic exams provide two-dimensional representations of a three-dimensional condition, in which it can be difficult to distinguish between technical artifacts and real anatomic changes, a highly standardized method is necessary to limit inter-exam variability and to improve the interpretation of the radiographic anatomy. Actually, a horizontal, abnormal central beam angulation can determine superimposition between the tooth root and the bony defect, which can otherwise be obscured by the root (29). Similarly, abnormal vertical angulations can determine false double bone levels, with the lingual more or less apical than the buccal and the bottom of the defect more or less close to the alveolar crest (29).

In the present study, the reproducibility of radiographic images was always achieved using the long cone parallel technique, with a precision device and a constant focal object distance of approximately 20 cm. To make the subsequent measurements comparable, patients in whom prosthetic crowns, fillings or inlays/onlays were performed on the second molar before the 12-month follow-up, with possible alteration of the second molar crown distal surface, were excluded from the study. Moreover, a millimetric grid was used to measure radiographic bone defects and to verify the reproducibility of geometric projection. The use of the grid, applied on the film before the radiograph was obtained, is a simple and cost-effective method from a biological, as well as an economic, point of view, to minimize errors possibly caused by radiographic distortion and enlargement caused by slight differences in film position and/or focal

object distance. However, to the best of the author's knowledge, there have been no studies on periodontal healing of the lower second molar distal surface after adjacent third molar extraction but only a report on healing after periodontal treatment in which the grid has been used (30), and this report showed high accuracy in bone defect assessment. For radiation hygiene reasons, no second radiographs were obtained at the same time to test the image reproducibility of the applied method. In contrast, because the first radiographs were always of good quality, second radiographs were never necessary. The reproducibility of radiographic images has been verified in that the millimetric length of ten contiguous squares of the grid, measured on each radiograph of the same tooth, was never 0,5 mm greater or less than 10 mm, that is, within the approximation range of periodontal PD measurements. In fact, low levels of variability have already been shown in the past in the lower molar region, especially if the parallel technique with the long cone was used (4, 26-28, 31, 32), regardless of the use of a personalized film-holder, applied by means of acrylic resin or impression material (28, 32), to reproduce the same position of the film each time relative to the teeth and therefore to obtain super-imposable subsequent dental radiographs.

Another source of error in bone defect depth measurement is difficulty in radiographic CEJ location (31, 32) because of interference from anatomic structures due to horizontal and/or vertical angulation of the central X-ray beam, although the use of the long cone parallel technique seems to result in no significant differences in locating the correct CEJ position when applied in the lower molar region. To improve the examiner's ability to locate the radiographic reference points, in the present study, measurements were obtained under a 4x magnification view. Higher magnifications have already been reported to be useless (4).

The removal of decimals from the definitive radiographic measurements for statistical analysis, with approximation to the nearest unit, has already been applied by Kuegelberg et al., (4) and it can be justified by the limited influence that decimals have on final data. Moreover, if clinical measurements are related to radiographic measures, it is plausible that the two types of measurements fall in the same range of approximation.

The possible, although minimal, residual variability of radiographic measurements in the present study, caused by all of the above-mentioned sources of error, was not very important in the present investigation in that each radiographic measurements, used as objective reference of periodontal healing, was related to the mean PD found at the same time with the two different probing methods applied.

Descriptive results

The first aspect to be analyzed is the difference found between the mean PD measured with the stent and the mean standard PD. One possible explanation for this difference is that, while with the free probing method, the deepest defect point is sought, and this technique can also explain both the inter- and intra-examiner variability of the method, the guided method compels the probe to penetrate each time at the same point and with the same inclination. In fact, the probe is an investigation instrument, which should be used to freely search the contour of the bone defect and to detect and measure its deepest point. The personalized device used in the present study, as well as some other devices already proposed in the past, seems to be more reliable for comparative research studies, in which the definition of the real anatomy of the defect is not an aim, while the free probing method should be preferably used in clinical practice.

The second aspect is the difference between the mean radiographic measurements and both the guided and free probing mean measurements. The present results are difficult to explain and disagree with those of Kuegelberg et al., (4) who found a mean infra-bony increase of approximately 0.6 mm for every millimeter of increased PD, starting from 3 mm of PD and 1 mm of infra-bony defect and who suggested that the presence of pseudo-pockets on the distal surface of second molars explained the discrepancy.

Some considerations can help in understanding the present results. Probing depth is a linear measurement, which employs the free gingival margin as a superficial reference point and the deepest point of the bone defect as a deep reference point. Periodontal probes certainly never reach the latter point, except in intra-operative probing or probing at surgical re-entry, although probing to bone, under local anesthetics, has been proposed as a more precise method for obtaining more realistic measurements (33). Some anatomic variables can influence PD measurement: gingival tissue thickness; bone defect topography; the presence of obstacles to probe penetration, such as calculus; and different consistency and thickness of connective attachment, which are also relevant with regard to different degrees of inflammation.

The mean pre-surgical measurements found in the present study (xrpre: 6 ± 0.38; devpre 3.10 ± 0.16; standpre 5.50 ± 0.27) can be mainly explained by the interference with probe penetration, possibly due to the third molar crown and sometimes due to the calculus, especially in the guided method because it more extensively studied the distal surface of the second molar, and owing to the stent, it did not allow the probe to avoid obstacles during penetration. As for the 3-month measurements (xr3 = 4.94 ± 0.40; dev3 = 3.08 ± 0.10; stand3 = 4.94 ± 0.25), they can be explained by the resistance to probe penetration offered by the last 1-3 mm connective attachment fibers because healing had occurred by then, and the gingival margin was near final maturation. Finally, increased gingival tissue, as Kuegelberg et al. (4) already suggested, can explain the results found in the present study regarding the 12-month measurements (xr12: $3,68 \pm 0,32$; dev12: $2,75 \pm 0,11$; stand12: $4,00 \pm 0,16$).

The third aspect to be considered is the progression, over time, of periodontal healing on the distal surface of the lower second molar after adjacent third molar extraction. The present results seem to demonstrate pro-

gressive improvement of soft and hard tissue healing after surgery. This finding is supported by the results reported by the Critically Appraised Topic of Richardson and Dodson (16), based on 8 articles they reviewed, in which PD measurements were found, on average, to be unchanged or to have improved 6 to 12 months after third molar extraction, although the present results refer to 3- and 12-month post-operative measurements.

Inferential results

The results from the comparison between periodontal probing measurements with and without the personalized device with regard to radiographic measures should be considered with some caution because the differences between the two models are small, and they could also have been due to random fluctuations. Replication of this study will therefore be needed to confirm the apparent greater reliability of the guided probing depth.

Because a low correlation between dev 12/pre and stand 12/pre (r = 0.15) was found, it is also possible that the two methods provide complementary and not redundant information on second molar periodontal healing. Actually, because the healing process occurs in a progressive but quantitatively non-homogeneous manner, probing depth at different points could detect different quantitative aspects of the same process.

It is also important to emphasize that the results obtained by the two probing methods were only moderately correlated with the results obtained by radiographic measurements, as already found by Kuegelberg et al. (4). This finding is true either if the mean PD obtained from each of the two probing techniques was used for statistical analysis or if the measurements obtained with the two techniques at their only similar point (B and DB) were used. This low correlation can be explained by considering that probing depth and radiographic bone level are two aspects of the same periodontal picture, which are only partially related to each other. Hausmann et al. (34) reported that probing attachment level and radiographic bone level were different features of periodontitis and that the weak correlation between their respective changes could the result of a number of causes, which range from no relationship to a time lag, which could obscure any relationship.

A very weak correlation between probing attachment changes and radiographic changes over a 2-year period was reported by Pilgram et al., (35) who suggested that although there were some physio-pathologic reasons to believe that a relationship existed between attachment level and bone height, this relationship seemed to be weak, complex and/or not time-related.

Another likely reason for the low correlation between clinical and radiographic measurements is that the changes over the 1-year study period were within the range of errors with the measuring techniques used, as already suggested by others (36).

Other previous studies (33, 34, 36, 37) have investigated the correlation between clinical and radiographic assessments of periodontal healing, but they were under-

taken in patients with periodontal disease, in which healing could be impaired either by the older age of patients or by the presence of the disease.

The results of the present study, which did not find correlations between any study variable and the healing process on the distal surface of the second molar, are in line with those of Kuegelberg et al., (5) who showed that patient sex and age at the time of surgery, the presence of visible plaque, bleeding on probing, probing depths ≥ 7 mm on the distal surface of the second molar and a widened third molar follicle did not affect the healing process in individuals younger than 20 years of age, although the present results are not supported by those of other studies, which found correlations between one or more variables and the healing process. Montero and Mazzaglia (13) found that third molar depth was strongly correlated with both baseline periodontal PD and post-surgical changes. In the study by Kan et al., (10) detectable plaque at the distal surface of the second molar, mesio-angular third molar impaction and the presence of a crestal radiolucency apical to the third molar were found to be related to periodontal probing depth in the regression model in individuals just older than 25 years old (27 ± 7). Although it is plausible that the presence of plaque on the distal surface of the lower second molar could influence periodontal healing after adjacent third molar removal, and because the distal surface of the second molar has been previously found to show a higher plaque score than other surfaces, this surface could be a locus *minoris resistentiae* for the development of local periodontitis, as well as for deterioration of the healing process (3). However, despite only a few patients have previously been found to be plaque-free on this surface, plaque was not found to be related to second molar periodontal healing in patients younger than 20 years old (6). To verify whether the presence of plaque did or did not have an impact on second molar periodontal healing, no cases were included in the present study in which the patient's oral hygiene following extraction was supervised or enhanced, although it is possible that the two scheduled follow-up examinations nevertheless positively influenced patient oral health. However, Kuegelberg et al. (6) already showed that enhanced plaque control during the initial phase of healing did not affect the prevalence of intra-bony defects.

No other authors, with the exclusion of Kuegelberg (5), who found post-surgical healing impairment due only to smoking in individuals older than 30 years old, have investigated the correlation between smoking habits and lower second molar periodontal healing after adjacent third molar extraction. The present results seem to disagree with those of the systematic review by Patel et al., (38) who found that smoking had a negative effect on bone regeneration. However, these authors analyzed bone regeneration after regenerative therapies in periodontally compromised patients, while in the present study, bone regeneration was spontaneous, and the patients were younger and were free from periodontal disease.

Because the non-significant effect of each of the patient- or tooth-related examined variables could also

have been due to the qualitative nature of some of the variables (e.g., smoking, plaque, etc.), further studies are needed, in which only quantitative measurements, such as the number of cigarettes smoked, the plaque amount and the degree of pre-operative infection, and/or an increased sample size should be evaluated to investigate whether one or more of the study variables have significant effects on second molar periodontal healing.

Conclusions

In conclusion, within the limits of the present study, a personalized device seems to provide a more reliable estimate than the standard probing method of periodontal healing on the distal surface of the lower second molar after adjacent third molar extraction.
Because all of the examined patient- and tooth-related factors seemed to not be able to affect either second molar periodontal healing or probing depth measurements obtained with or without the proposed personalized device in individuals younger than 25 years old, it can be recommended that lower third molar surgical extraction be performed in young adults.

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