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Additional Information

Post-extraction mesio-distal gap reduction assessment by CLSM – a clinical 3-month follow up study

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Running title: Mesio-distal distance changes by CLSM

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Clinical Relevance

Scientific rational for the study: A better understanding of the mesio-distal gap dimension (MDGD) changes after tooth extraction is essential in dental treatment planning.

Principal findings: MDGD changes after tooth extraction are greater during the first month following the extraction and continue for the second and the third month, but to a lesser extent. There are some situations related to a further reduction in MDGD.

Practical implications: Mesio-distal gap reduction could alter the patient's occlusion. For this reason, it is important to perform a retention of the adjacent teeth of the edentulous space to maintain the MDGD until the definitive treatment is achieved.

ABSTRACT

Aim: The aim of this three-month follow up study is to quantify the reduction in the mesio-distal gap dimension (MDGD) that occurs after tooth extraction through image analysis of three-dimensional images obtained with the CLSM technique.

Materials and Methods: Following tooth extraction, impressions of 79 patients one-month and 72 patients three-month after tooth extraction were obtained. Cast models were processed by CLSM and MDGD changes between time points were measured.

Results: The mean mesio-distal gap reduction one-month after tooth extraction was 343.4 μm and three-month after tooth extraction was 672.3 μm . The daily mean gap reduction rate during the first term (between baseline and one month post-extraction measurements) was 10.3 $\mu\text{m}/\text{day}$ and during the second term (between one- and three-month) was 5.4 $\mu\text{m}/\text{day}$.

Conclusions: The mesio-distal gap reduction is higher during the first month following the extraction and continues in time, but to a lesser extent. When the interdental contacts were absent the mesio-distal gap reduction is lower. When a molar tooth is extracted or the distal tooth to the edentulous space does not occlude with an antagonist the mesio-distal gap reduction is larger. The consideration of mesio-distal gap dimension changes can help improve dental treatment planning.

INTRODUCTION

Tooth extraction is one of the most common therapeutic procedures in dentistry. The periodontium is a tooth-dependent tissue that supports and develops with the eruption of the teeth. With tooth extraction, the alveolar process undergoes atrophy due to the loss of its function (Van der Weijden et al. 2009). The hard and soft tissues experience a remodelling process during healing after extraction, which continues even after the socket is refilled with new bone (Schropp et al. 2003). In addition, when a tooth is lost, the structural integrity of the dental arch is interrupted, leading to a realignment of the teeth to a new equilibrium state (Weinstein et al. 1963). Often the teeth adjacent to the edentulous space experience a displacement toward it, especially those teeth located distal to the edentulous space can move in mass, although a tipping movement is more common (Proffit 1978; Weinstein 1967). This realignment to the new equilibrium state is important when replacing the missing tooth, either through dental or implant-supported restoration. The mesial-distal gap dimension (MDGD) changes with time affect the aesthetics and functionality of the restoration process. Results reported in the literature about the mesio-distal gap reduction of an edentulous space are diverse (Christou and Kiliaridis 2007; Craddock et al. 2007; Gragg et al. 2001; Kiliaridis et al. 2000; Lindskog-Stokland et al. 2012; Lindskog-Stokland et al. 2013; Love and Adams 1971; Petridis et al. 2010; Shugars et al. 2000).

The Confocal Laser Scanning Microscopy (CLSM) technique consists of an optical microscope that includes a laser light as the light source and an electronic system for image collection. The main advantages of CLSM are: (1) removal of the veil that produces regions outside the in-focus plane, obtaining a greater contrast, (2) a greater resolution due to the use of a laser light as the light source, (3) the system can take images at different depths by varying the in-focus plane, (4) the images can be represented using three-dimensional.

Confocal microscopy is being widely used in the field of dentistry (García-Herraiz et al. 2012b). Some examples include the study of the surface of enamel and dentin (Belli et al. 2009; Belli et al. 2011; Brauchli et al. 2011; Yamada and Watari 2003), the analysis of the osseointegration (Nishikawa et al. 2006) and even the visualization of cementocytes (Scivetti et al. 2007).

CLSM can be used to study MDGD changes of the post-extraction area in study casts (García-Herraiz et al. 2012a). This technique allows the visualization of the soft tissues of post-extraction sockets. The radiation emitted by the Computed Tomography (CT) does not justify its use for prospective studies at different times. By contrast, CLSM is a non-invasive and non-damaging technique and absolutely harmless for the patient.

The aim of this three-month descriptive study is to quantify the changes in the mesio-distal gap dimension (MDGD) that occur after tooth extraction through image analysis of three-dimensional images obtained with the CLSM technique, as a new technique for the dimensional study of the post-extraction socket.

MATERIALS & METHODS

This study was approved by the ethical committee of Doctor Peset University Hospital of Valencia, Spain (Approval Protocol number 30/11).

The following article has been prepared following the guidelines of the STROBE statement.

Study population

Patients who visited Doctor Peset University Hospital Dentistry Service for a tooth extraction were selected sequentially for participating in this prospective study. All selected patients signed informed consent. The patients were enrolled and treated in a period of time between April 2011 and July 2013. The following exclusion criteria were applied:

- (1) Age under 18 years.
- (2) Systemic diseases preventing dental outpatient treatment.
- (3) Presence of relevant medical conditions (e.g. patients with diabetes mellitus, patients with medications altering bone metabolism or requiring antibiotic prophylaxis).
- (4) Pregnant women.
- (5) History of malignancy, radiotherapy or chemotherapy.
- (6) History of autoimmune disease.
- (7) Acute periodontal or periapical pathology in the moment of tooth extraction.

The extracted tooth must be flanked by a mesial and a distal tooth to allow stable

references during measurements.

Clinical procedure

Following the administration of local anaesthesia with articaine and a vasoconstrictor (Ultracain, Normon®, epinephrine 40/0.01 mg/ml), tooth extraction was performed in the most atraumatic form as possible to avoid damaging the gingiva or the alveolar bone support, especially at the vestibular level. The teeth were extracted without elevation of a mucoperiosteal flap and, if necessary, the teeth were sectioned to avoid traumatic extraction and to preserve bone walls. The post-extraction socket was curettaged to remove the granulation tissue. The post-extraction socket healed by first intention and it was not sutured.

After tooth extraction, a silicone print of the post-extraction site was taken (fluid silicone Aquasil Ultra LV on putty silicone Aquasil Soft Putty, Dentsply®). After that, the silicone print was filled with plaster (Vel-Mix Stone, ISO type IV, Kerr®) to obtain the study cast. When the plaster set down, the study of the post-extraction site was carried out by the CLSM technique. New silicone prints were taken to assess MDGD changes in the extraction area one and three months after tooth extraction.

The data and characteristics of the extraction collected during the study were:

- (1) Diseases.
- (2) Treatments.
- (3) Smoking status and cigarettes/day.
- (4) Periodontal status: To assess periodontal status, a complete full-mouth periodontal chart was performed. The exploration was carried out with a millimetre Williams periodontal probe (Hu-Friedy®). Subsequently, clinical attachment loss (CAL) of the adjacent teeth to the edentulous space was extracted. CAL average of 12 measurement locations of adjacent teeth, 6 at the mesial tooth and 6 at the distal, was obtained. Patients were classified as periodontal disease if CAL assessment was ≥ 3 mm (Armitage 1999).
- (5) Interdental contacts between the extracted tooth and the adjacent teeth: present or absent.
- (6) Dental type: anterior (incisors and canines), premolar or molar.
- (7) Location: maxillary or mandibular.

(8) Cause of extraction.

(9) Antagonist: the distal tooth to the extraction site occludes or not with another tooth placed in the opposite dental arch.

Evaluation of MDGD changes with CLSM

The analysis of the MDGD changes was conducted at the Chemical and Nuclear Engineering Department, Polytechnic University of Valencia.

Baseline, one- and three-month post-extraction study casts of each patient were processed by Confocal Laser Scanning Microscopy. The system used was the OLS3100-USS, LEXT model (Olympus®). This microscope allows the observation and the analysis of surfaces and microstructures of materials with a maximum resolution of 120 nm in X/Y and 10 nm in Z. The study casts were observed using a 2.5x lens, with a total magnification of 25X. The observations were made with a 4x6 grid (4 horizontal sections and 6 vertical sections). The sections were recorded using a size of 4.8x6.4 mm² and a lateral resolution of 6.25x6.25 μm². Each section had a maximum depth of 10 mm.

The samples were placed parallel to the line connecting the buccal surface of the adjacent teeth to the post-extraction site.

The measurements were taken on the section coinciding with the centre of the mesial tooth of the post-extraction site. Two parallel sections to the buccal and lingual surface of the mesial tooth were designed. These sections were placed tangent to the respective convexities of the tooth. Then, a third section (the green line in Figure 1A) placed in the centre of the other two was traced. Figure 1A shows the sections. Figure 1B shows the MDGD between the teeth adjacent to the post-extraction site in section number 3. The measurements were performed at the points of greatest protrusion of the mesial or distal surface of the teeth adjacent to edentulous space.

Examiner and technique accuracy

Surgical procedures were performed by one operator in the same clinic to ensure that the clinical procedure was performed similarly in all cases.

In the same way, the analysis of plaster models at the time of extraction, one- and three-month after tooth extraction were processed and measured by a single person, to keep the test as similar as possible.

The authors wanted to test the reliability of sample collection with silicone and subsequent plaster casting. For this aim, 12 extracted molars and plaster replicas were analysed by CLSM. No significant differences between matched pairs were found ($p > 0.05$; Wilcoxon test).

Statistical analysis

Sample size calculation was based on the data obtained in a previous study (García-Herrera et al. 2012a) and resulted in 67 subjects to estimate the MDGD with a 90% level of confidence, assuming a SD of 2500 microns and an accuracy of ± 500 microns (about 5% of the MDGD mean obtained in that previous work). Type I error probability was set at 0.05.

The statistical analysis was performed using two statistical software programs: SPSS (SPSS for Windows 17.0, SPSS Inc., Chicago, IL) and R program (R Core Team 2013).

A repeated measures design was carried out in which the response variable was MDGD. MDGD measurements taken at baseline, one- and three-month after tooth extraction allowed us to calculate one- and three-month post-extraction gap reduction. To minimize variations resulting from the fact that the measures were not taken at post-extraction day 30 and 90 in all patients, it was decided to consider the daily gap reduction rates. The daily gap reduction rates were analysed between baseline and one month post-extraction measurements (first term) and between one and three months post-extraction measurements (second term). The outcome variables were:

- (1) One-month gap reduction: $gr_{1m} = \text{baseline MDGD} - \text{one month MDGD}$
- (2) Three-month gap reduction: $gr_{3m} = \text{baseline MDGD} - \text{three months MDGD}$
- (3) Gap reduction rate in the first term: $rate_{1t} = (\text{One-month gap reduction}) / \text{days between baseline and one-month measurements}$
- (4) Gap reduction rate in the second term: $rate_{2t} = (\text{Three-month gap reduction} - \text{One-month gap reduction}) / \text{days between one- and three-month measurements}$

Kolmogorov-Smirnov tests were used to check the normality assumption. Differences in outcome variables and the influence of the subject or tooth extraction characteristics were tested using univariate analysis, specifically ANOVA and post hoc Tukey tests. Pearson test was used to compare the outcome variables with quantitative variables. The level of significance was set at $p < 0.05$.

RESULTS

The study population consisted of 79 adult patients at baseline and one month after tooth extraction (mean age 39, range 18-73, 33 females). The study population three months after tooth extraction consisted of 72 patients (mean age 39.6, range 18-73, 27 females). The renouncing rate was 9.8%. These withdrawals were not due to serious illness or death, but participants refused to follow the dental checking process.

The reasons for extraction were advanced caries lesions, endodontic treatment failures, fractures and orthodontic indication.

Table 1 displays the characteristics of the study population in the measurements at one- and three-month after tooth extraction. Diseases of the study population included hypertension, dyslipidaemia, dyspepsia, anaemia, depression and migraine. Other treatments included contraceptives, iron, aspirin and sumatriptan. Cigarette daily mean value among the smoking population was 15.19 cigarettes/day. In all instances in which the distal tooth to the extraction site occluded with an antagonist, it was a natural tooth, not a prosthetic restoration.

The results of the MDGD changes one- and three-month after tooth extraction are shown in Tables 2 and 3.

The mean mesio-distal gap reduction one-month after tooth extraction was 343.4 μm and three-month after tooth extraction was 672.3 μm . The comparison of the MDGD by paired t-test revealed significant differences between baseline data and one- and three-month after-tooth-extraction data.

The daily mean gap reduction rate in the first term was 10.3 $\mu\text{m}/\text{day}$. In the second term the daily gap reduction rate was 5.4 $\mu\text{m}/\text{day}$. Significant differences in daily gap reduction rates between the first and the second term were observed by paired t-tests. Figure 2 shows a reduction in MDGD over time depending on the gap reduction rates

during the first and the second term. The gradient of the line in the first term is more pronounced than in the second term, because the mesio-distal gap reduction rate is greater in the first term.

ANOVA tests showed a significant influence of the interdental contacts leading to a lower gap reduction one- and three-month post-extraction when they were absent. Dental type had significant influence on the MDGD. When a molar tooth was extracted the gap reduction was larger. Similarly, when the distal tooth to the extraction site did not occlude with an antagonist, the gap reduction one- and three-month after tooth extraction was greater. Table 4 shows the results of the mesio-distal gap reduction one- and three-month after tooth extraction based on each variable.

Age, gender, diseases and treatments did not affect MDGD one- and three-month after tooth extraction. No differences in MDGD were found in the smoking population nor Pearson correlation with the cigarettes/day. Additionally, periodontal status, measured by the CAL parameter, showed no Pearson correlation with mesio-distal gap reduction. Although it was observed a greater tendency in the mesio-distal gap reduction one- and three-month post-extraction and in the gap reduction rates in patients with greater CAL. Location and cause of extraction did not show any influence on MDGD one- and three-month after tooth extraction.

DISCUSSION

When a tooth is lost, the structural integrity of the dental arch is interrupted, and there is a realignment of the teeth until a new equilibrium state is reached (Weinstein et al. 1963). This realignment of the teeth, especially of the tooth placed distal to the edentulous space (Proffit 1978), causes a decrease in MDGD. This study quantified the mesio-distal gap reduction one- and three-month after tooth extraction with the purpose of optimizing functional and aesthetic outcomes of dental restorations and improving dental treatment planning.

The mean gap reduction was 343.4 μm one month post-extraction, and 672.3 μm three months after tooth extraction. Therefore, there is a marked reduction of the edentulous space one month after tooth extraction that continues for three months.

The mean daily gap reduction rate was 10.3 $\mu\text{m}/\text{day}$ in the first term, and 5.4 $\mu\text{m}/\text{day}$ in the second term. These results demonstrate that although MDGD changes continue throughout the evaluation period, they are higher during the first month after tooth extraction. Subsequently, the daily gap reduction rate decreases during the second and third months. This pattern of movement toward the edentulous space may be due to the new equilibrium state of the dental arch that is gained over time. As this balance is achieved, the daily gap reduction rate decreases. Similar results were reported in Lindskog-Storkland study (Lindskog-Stokland et al. 2013). In this study the authors found that the tipping movement is more pronounced immediately after extractions. Gragg et al. (Gragg et al. 2001) studied the changes in the distance between the teeth adjacent to the edentulous space pre-extraction and in follow-up radiographs. The distance was less than 1 mm during the first year post-extraction, and the distance continued to decrease at a successively slower rate each following year. Like in our results, these findings suggest that mesio-distal gap reduction is gradual and minor with time.

The original injury somehow accelerated the normal regional healing processes. This acceleration is the regional acceleratory phenomenon (RAP) (Frost 1983). RAP usually occurs after a fracture, arthrodesis, osteotomy, or bone-grafting procedure, and may involve recruitment and activation of precursor cells necessary for wound healing concentrated at the site of injury (Amit et al. 2012). Due to the injury, there is more cellular activity, leukocytes, signalling molecules, etc. The two main features of RAP in bone healing include decreased regional bone density and accelerated bone turnover, which are believed to accelerate tooth movement (Schilling et al. 1998; Verna 2016). It has been suggested that the RAP begins within a few days after injury, typically peaks at 1-2 months, usually lasts 4 months in bone and may take 6 to more than 24 months to subside (Schilling et al. 1998; Wilcko et al. 2001). RAP would explain that the mesio-distal gap reduction rate found in the study was higher one month after tooth extraction and decreased over time.

Lindskog-Storkland et al. (Lindskog-Stokland et al. 2012) reported a mean decrease of the edentulous space mesial to the molar during 12-year observation period of 0.2 mm. Since the majority of their cases were presented with the edentulous space already in the

baseline examination, the equilibrium state might have been reached before the start of the observation period, and no further tipping of the molar may take place.

The association between the mesio-distal gap reduction and interdental contacts between the extracted tooth and the adjacent teeth could be explained by the fact that when the interdental contacts were absent, like in a residual root, it is generally extracted after the loss of the crown. After the loss of the crown, the adjacent teeth migrate to the edentulous space even before the residual root is removed. Consequently, as the adjacent teeth have already experienced some realignment in order to reach the new equilibrium position, the mesio-distal gap reduction generated after removal of the residual root (base value of the present study) is lower than the gap reduction generated when a tooth with crown is extracted.

The statistical analysis revealed much more substantial mesio-distal gap reduction in molar tooth extractions. Probably molar teeth are subjected to higher forces in the dental arch, consequently their removal involves a greater gap reduction to reach the equilibrium state.

Greater mesio-distal gap reduction was found when the distal tooth of the post-extraction site did not occlude with an antagonist, which could be explained by the realignment of teeth after extraction. The new equilibrium state is more easily achieved if the distal tooth can occlude with an antagonist. These results agree with the positive correlation between overeruption and change in tooth inclination found by Lindskog-Storkland et al. (Lindskog-Storkland et al. 2012), which indicates that a change in occlusal loading might cause tipping of the tooth into the edentulous space. Analogous findings were reported in previous studies (Christou and Kiliaridis 2007; Kiliaridis et al. 2000).

Age, gender and location did not affect MDGD one- and three-month after tooth extraction. Same results were found in Petridis study (Petridis et al. 2010). The MDGD changes have been reported to be larger in the maxilla than in the mandible (Craddock et al. 2007). In the present study the results were not statistically significant, but a tendency of greater reduction of the MDGD in the maxilla was observed. This trend was also reported by Lindskog-Storkland et al. (Lindskog-Storkland et al. 2012).

In this study, periodontal status, measured by the CAL parameter, showed no Pearson correlation with the gap reduction, although a greater tendency was observed. The

periodontal status is related to hard and soft tissues of the teeth adjacent to the edentulous space support. Therefore, the worse the periodontal status of the teeth adjacent to the edentulous space, the greater the reduction in the mesial-distal gap, due to a higher attachment loss.

The technique used is based on an optical three-dimensional system (Thalmair et al. 2013; Windisch et al. 2007), however in this study the CLSM technique was used (García-Herraiz et al. 2012a) because it allows a great accuracy for measuring post-extraction changes and is an easily reproducible, non-invasive and non-radiation method. MDGD changes have been measured using different methods and heterogeneous results have been obtained. One of the techniques used to assess post-extraction tooth movements is 2D radiography (Gragg et al. 2001; Lindskog-Stokland et al. 2012; Lindskog-Stokland et al. 2013; Petridis et al. 2010; Shugars et al. 2000). This method does not allow measurements as accurate as CLSM. In addition, both radiographs as well as CT emit ionizing radiation, which is harmful to the patient. For this reason, this methodology would not be appropriate to carry out studies at different times. The technique used in this work allows the study of changes in the MDGD prospectively and at different times, without being detrimental to the patient. This new methodology allows the development of volumetric studies of the post-extraction socket that would help to predict the future outcomes of dental treatment.

Dental cast measuring is another method used to value post-extraction MDGD changes (Craddock et al. 2007; Petridis et al. 2010). Craddock et al. (Craddock et al. 2007) made alginate impressions on study casts of patients with an unopposed posterior tooth. These models were 3D scanned and, among other measures, the degree of tipping of the distal tooth towards the edentulous space was obtained. The results are not comparable, because they were measured in degrees instead of linear distance. In addition, in this study the patient should have opposed teeth missing for over 5 years. According to our results, as much time passes without interdental contacts, mesio-distal gap reduction will be greater. Otherwise, the use of alginate, instead of silicone for impressions, decreases the accuracy of plaster casts.

In our work, the optical scans were performed on study casts. The accuracy of silicone impressions and plaster casts could influence the method. The authors did not find

statistical differences when the extracted teeth were compared to their plaster replicas by CLSM, as has been previously mentioned. Also, there are many studies that support the accuracy of silicone impressions and plaster casts (Aguilar et al. 2010; Chen et al. 2004; Faria et al. 2008; Pereira et al. 2010; Thongthammachat et al. 2002).

In this study, the mesio-distal gap reduction between the adjacent teeth to the edentulous space has been evaluated, which is the most important parameter, from a clinical point of view, to plan and achieve an optimal dental reconstruction. However, individual movement of each adjacent tooth to the edentulous space has not been calculated. Therefore, it cannot be concluded that the decrease in MDGD is mainly caused by the distal tooth, as Proffit suggested (Proffit 1978). Future research can investigate the individual movement of each tooth adjacent to the edentulous space. Another line of research would be the rotations or tipping movements of the teeth adjacent to the edentulous space, as in the present study the mesio-distal gap reduction has been valued altogether.

In conclusion, the present prospective study demonstrates that mesio-distal gap dimension decreases after tooth extraction. The mesio-distal gap reduction is higher during the first month following tooth extraction and continues for the second and the third month, but to a lesser extent. When the interdental contacts were absent the mesio-distal gap reduction is lower. When a molar tooth is extracted or the distal tooth to the edentulous space does not occlude with an antagonist the mesio-distal gap reduction is larger. The consideration of mesio-distal gap dimension changes can help improve dental treatment planning.

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