

Assessment of Changes in the Outcome of Autogenous Tooth Grafts Over Time: A Clinical Study Evaluating Periodontal Healing in Bone Defects After Lower Third Molar Removal



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Background: The removal of impacted lower third molars (ILTM) is associated with bone defects in the distal area of second molars. Different methods have been described to minimize these defects.

Purpose: The primary objective was to assess changes in probing depth (PD) over time (up to 36 months) between test (grafted) and control (ungrafted) groups; the graft was obtained from the extracted ILTM.

Study Design, Setting, Sample: This split-mouth randomized clinical trial was conducted at the Postgraduate Course in Oral Surgery of the Faculty of Dentistry of the Complutense University of Madrid. Adult patients requiring bilateral ILTM extraction with adjacent second molars were recruited, excluding pregnant/lactating women, patients in treatment with nonsteroidal anti-inflammatory drugs and patients with periodontal diseases.

Predictor/Exposure/Independent Variable: The predictor variable was the graft technique. The bone defect after ILTM removal was treated with autogenous tooth graft (ATG) in the test group, leaving the control group ungrafted.

Main Outcome Variable: PD on the distobuccal, distomedial, and distolingual surfaces was recorded in both groups and averaged at baseline (T0), 3 (T1), 6 (T2), and 36 months (T3) postoperatively.

Covariates: Sex, age, surgical time, ILTM situation and position between groups were assessed.

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Analyses: ANOVA repeated measures for comparisons between groups and the Friedman test for comparisons within the groups over time were applied. Statistical significance was established with a confidence interval of 95% ($P < .05$).

Results: The sample comprised 22 patients (6 males, 16 females) with a mean age of 21.68 ± 2.19 years; 44 ILTM extractions were performed. Statistically significant differences in PD average were found between groups ($P < .001$, 95% confidence interval) at 3 (1.63 ± 0.29), 6 (1.76 ± 0.3), and 36 months (1.74 ± 0.36). Reductions from T0 to T3 of 2.74 ± 0.28 ($P < .001$) and 0.54 ± 0.3 ($P = .43$) were observed in test and control groups, respectively.

Conclusion and Relevance: ATG placed on the distal surface of lower second molars and almost completely filling the extraction socket improved PD 3, 6 and 36 months after ILTM. Furthermore, no significant changes in PD were observed over time; no major complications occurred. ATG appears to be a viable alternative graft material for this procedure.

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Lower third molars (LTMs) are the last teeth to erupt into the human dental arch. They are also the most commonly impacted,¹ perhaps because there remains insufficient space for them to erupt normally.² Impacted LTM (ILTM) removal is often associated with complications and damage to the adjacent second molar, including periodontal pockets and gingival bleeding on its distal surface.³

After surgical removal of ILTM, there is some controversy regarding the gain or loss of periodontal attachment level on the distal surface of the lower second molar roots.^{2,4} To maintain periodontal health after ILTM removal, it is not clear which techniques—mechanical or ultrasonic debridement of the distal root of the lower second molar,^{2,5} or a specific flap design^{6,7}—are really useful.

Some authors claim that ILTM removal does not damage distal surface of lower second molars⁸⁻¹¹ but others recommend bone regeneration techniques such as coadjutant treatment.^{12,13} Some argue that unless bone regeneration techniques are performed, periodontal defects can remain pathological after surgery,^{14,15} especially in cases of mesioangular LTM impaction.^{15,16}

Various bone graft materials are available for regenerating defects after ILTM including autologous bone,¹⁷ xenografts,¹⁸ or synthetic bone.¹⁹ While autologous bone graft remains the gold standard, it also suffers drawbacks such as its limited availability and associated morbidity at the donor site.^{17,20}

So, in the last few years, autogenous tooth graft (ATG) has been investigated as an alternative material for regenerating defects after ILTM extraction.²¹⁻²³ However, as far as the authors are aware, no studies have assessed the medium/long-term outcomes of these ATG.

Therefore, this randomized clinical trial aimed to evaluate the efficacy of ATG in decreasing the risk of persistent periodontal defects among patients undergoing ILTM removal.

The hypothesis put forward in the present investigation was that the probing depth (PD) after ILTM extraction would be lower in the experimental group compared with the control group, and that in turn, no significant changes in PD will be observed in the experimental group after 36 months of follow-up.

The specific aim was to record the PD on the distal surface of the second molar before (T0) and after ILTM removal (T1, T2, and T3) by comparing variations in PD in test and control groups in a split-mouth model.

Material and Methods

STUDY DESIGN, SAMPLE, AND APPROVAL

The study included a total of 44 ILTM extractions in 22 patients. It was designed as a nonblinded split-mouth randomized clinical trial (RCT) and conducted at the Postgraduate Oral Surgery Clinic of the Faculty of Dentistry, Complutense University of Madrid (Spain). All patients were provided with full information about the purpose of the study and the procedures involved and gave their informed consent to take part.

Inclusion criteria were as follows: patients of either sex, aged between 18 and 25 years, presenting indications for bilateral extraction of LTMs; presence of second molars; and patients being able to understand and carry out the researchers' instructions. Exclusion criteria were as follows: pregnant or lactating women; patients in treatment with nonsteroidal anti-inflammatory drugs; and patients with periodontal diseases.

All procedures fulfilled ethical standards for research involving human subjects established by institutional and/or national research committees in accordance with the 1964 Helsinki declaration and subsequent amendments. The study protocol was approved by the Ethics Committee of the San Carlos Clinical Hospital, Madrid (Reg N^o 18/203-E) dated May 25, 2018. The study was conducted following the Consolidated Standards of Reporting Trials guidelines (<http://www.consort-statement.org/>).

VARIABLES

The independent variable was the graft technique. Extraction sites were grafted with ATG or left ungrafted.

The dependent or outcome variable was the PD evolution over time (from T0 to T3).

The covariates in this study were divided into preoperative, intraoperative, and postoperative.

Preoperative variables were age, sex, medical and dental history, clinical location and position of the ILTM, close or otherwise relationship to the inferior alveolar nerve, observed in a panoramic radiograph.

Intraoperative variables were surgical time, complications and correct ATG placement in the alveolus.

Postoperative variables were complications and ATG aspect.

DATA COLLECTION

For ATG preparation, the tooth (whether complete or divided by odontosection) was cleaned with a sterile gauze to remove soft tissues, and dried with compressed air. A Smart Dentin Grinder (Kometa Bio, Bioner, Barcelona, Spain), was used to grind the tooth for 3 seconds, and the resulting material was sieved for 20 seconds to ensure a particle size of 300-1,200 μm . Then the material was mixed with a liquid solution (0.5 molar sodium hydroxide and 20% ethanol for 12 minutes) followed by saline solution for 3 minutes.

PD was recorded with a millimeter periodontal probe by a calibrated examiner in both groups at T0 (preoperative), T1 (3 months postoperatively), T2 (6 months postoperatively), and T3 (36 months postoperatively). PD was measured preoperatively (T0) and postoperatively (T1, T2, and T3) on the distobuccal, distomedial, and distolingual surfaces, and averaged.

Age was measured in years, sex was classified as male or female, situation was measured as partial or total coverage, position was recorded as mesioangular, horizontal, vertical or distoangular, and surgical time was measured in minutes.

Intraoperative complications were registered and the correct ATG placement in the alveolus was assessed by means of a periapical radiograph (Fig 1).

Postoperative complications were registered and ATG aspect was analyzed with a panoramic radiograph and a periapical x-ray (Fig 2) 3 years after intervention.

SAMPLE SIZE, BLINDING, CALIBRATION, AND RANDOMIZATION

Determining sample size, randomization, and blinding processes have been described in detail in a previous study by the same authors.²¹

Sample size was decided according to the primary variable (PD), based on an earlier pilot study which enrolled 5 patients (10 alveoli), obtained a mean reduction of 2.4 ± 1.09 mm and 1 ± 0.66 mm on test and control side, and considering an alpha-type error of 5% and a beta-type error of 5%, sample size estimation performed with specialized software (G*Power 3.1.9.4), resulting in 10 patients per group.

Before conducting the study, intraexaminer reproducibility was established, calibrating the main variable (PD) with 15 patients on the distobuccal, distomedial, and distolingual surfaces. As this was a quantitative variable, the intraclass correlation

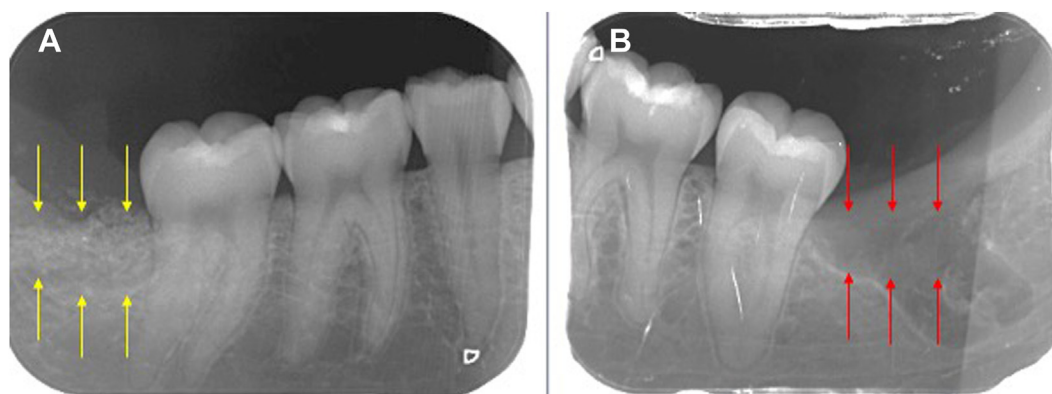


FIGURE 1. Periapical radiography (A) ATG placed on the test side (yellow arrows). (B) Ungrafted control side (red arrows).

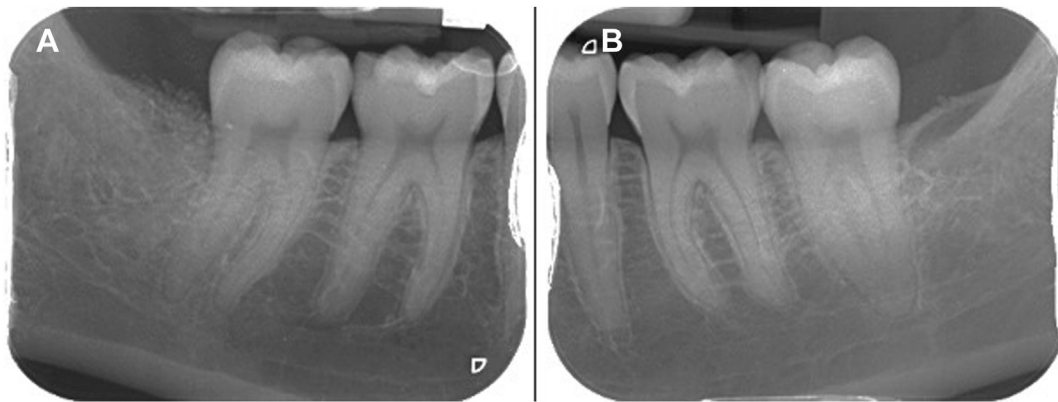


FIGURE 2. Periapical radiography showing bone formation 3 years after ILTM removal. (A) Test side. (B) Control side.

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coefficient was calculated: 0.994 (confidence interval 95%: 0.985 to 0.998) indicating excellent agreement.

Blinding of patients and the oral surgeon was not possible. However, a randomization process was performed by another researcher (J.M.M.-G.) using random number generator statistical software, to generate information about the side of the first surgery (right or left) and the type of treatment (ATG or blood clot). Stratified random sampling was performed by type of treatment and side of first surgery. The information was revealed from opaque envelopes minutes before the surgeries.

SURGICAL PROCEDURE

All ILTM extractions were performed by the same oral surgeon (L.S.L), using 4% articaine with 1:100,000 adrenaline (Ultracaine, Normon SL, Madrid, Spain) to block the inferior alveolar, lingual, and long buccal nerves. An envelope flap and a full-thickness mucoperiosteal flap were raised in all cases, performing bone removal and/or tooth sectioning with a tungsten carbide round bur and handpiece. After extractions, curettage of the distal aspect of the second molars was performed, thereafter placing an ATG made from the extracted wisdom tooth on the test side, and leaving the control side ungrafted. A Gelatamp fibrin sponge (Gelatamp, Coltene, Langenau, Germany) was placed on each alveolus before suturing with 4/0 silk (Aragó, Barcelona, Spain).

STATISTICAL ANALYSIS

Data were entered on an Excel spreadsheet (MS Excel 2019, Microsoft Inc, Redmond, WA, USA) and sent to an independent statistician for analysis using version 28.0.0 of SPSS statistical software (IBM SPSS, Chicago, IL, USA).

First, descriptive statistics were calculated for all variables (frequency, mean, standard deviation, me-

dian, minimum, and maximum), and normal distribution was checked using the Shapiro-Wilk test. It was found that the data did not display normal distribution. In turn, the variables "third molar position" and "third molar situation" were analyzed using the χ^2 test in order to analyze possible variations between the 2 sides that could affect the final outcome.

For the main variable, PD, ANOVA repeated measures for comparisons between groups and the Friedman test with Bonferroni correction for comparisons within the groups over time were applied.

For all results, a 95% confidence interval was recorded (significance level $P < .05$, 2-tailed).

Results

PATIENTS AND ILTM CHARACTERISTICS

A total of 26 patients were initially included in this RCT but 4 dropped out over the course of the 3-year follow-up. So finally, complete data were analyzed for 22 patients who underwent 44 ILTM surgeries. Of the 22 patients, 6 were males (27.3%) and 16 females (72.7%), with an average age of 21.68 ± 2.19 years (Table 1). Moreover, PD values in both groups were recorded preoperatively (T0), with no statistically significant differences between the 2 groups (Table 2).

Fourteen of these patients had already been assessed in a previously published study²¹ and 8 more patients were added to make up the sample size. In the previous study, patients were only monitored over 6 months after ILTM removal.

There were also no differences between the 2 groups in terms of ILTM characteristics. The frequencies in each group, as well as the overall frequencies regarding ILTM positions, ILTM situations, and the duration of the interventions ($11.16 + 15$ minutes for test and 12.79 minutes for control group) are shown in Table 1. No statistically significant

Table 1. BIVARIATE ANALYSES OF COVARIATES VS. TREATMENT GROUP.

Covariates	Test Group (n = 22)	Control Group (n = 22)	Total (n = 44)	P Value
Sex				
Male	6/22	6/22	12/44	0.99
Female	16/22	16/22	32/44	
Age (yrs old)	21.68 ± 2.19	21.68 ± 2.19	21.68	0.99
ILTM position				
Mesioangular	10/22 (45.5%)	13/22 (59.1%)	23/44 (52.3%)	.661
Horizontal	6/22 (27.3%)	5/22 (22.7%)	11/44 (25%)	
Vertical	5/22 (22.7%)	4/22 (18.2%)	9/44 (20.5%)	
Distoangular	1/22 (4.5%)	0/22 (0%)	1/44 (2.3%)	
ILTM situation				
Partial coverage	19/22 (86.4%)	18/22 (81.8%)	37/44 (84.1%)	.680
Total coverage (included)	3/22 (13.6%)	4/22 (18.2%)	7/44 (15.9%)	
Main intervention duration (minutes)	11.16 ± 5.46	12.79 ± 5.49	NA	NA
	+ 15 (ATG preparation)			

Abbreviations: ILTM, impacted lower third molar; NA: Not applicable.

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differences existed between test and control groups regarding sex, age, ILTM situation or position.

When comparing ILTM within the same patient, the initial position was not exactly the same in 40.91% of cases, the situation being similar in 95.45% of patients and the PD (outcome variable) in 100%.

ATG was placed in 22 alveoli, while the other 22 alveoli were ungrafted.

CHANGES IN PD

Comparing preoperative PD between test and control groups, no statistically significant differences were found at baseline ($P = .18$). When comparing PD evolution from baseline (T0) to 36 months postoperatively (T3) between groups, final PD in the test group was 3.74 ± 0.91 versus 5.48 ± 1.41 in the control group, with a mean difference of 1.74 ± 0.36 ($P < .001$), as shown in Table 2.

Differences over time within the test and the control group are shown in Table 3. When analyzing differences between T1 and T2 and T2 and T3 within each group over time, no statistically significant differences were obtained in either the test group or control group, as shown in Table 3.

COMPLICATIONS

Intraoperative and postoperative complications in the short term (6 months) have been described in a previous study,²¹ but did not exhibit differences between groups; no complications or adverse effects were recorded at the 3-year follow-up in any of the 22 patients (44 ILTMs).

Discussion

ILTM is the most frequent intervention performed by oral and maxillofacial surgeons^{13,20,24,25} and may

Table 2. DIFFERENCES BETWEEN TEST AND CONTROL GROUP AT T0 (BASELINE), T1 (3 MONTHS), T2 (6 MONTHS), AND T3 (36 MONTHS). AVERAGES ON DB, DM AND DL SURFACES. MEAN PD DIFFERENCES BETWEEN GROUPS AT T0, T1, T2, AND T3. DIFFERENCES WITHIN GROUPS TO T3

Time of Assessment	Test Group (n = 22)	Control Group (n = 22)	Mean Difference	P Value
T0 DB-DM-DL	6.48 ± 1.15	6.03 ± 1.07	0.45 ± 0.33	.18
T1 DB-DM-DL	4.18 ± 0.92	5.82 ± 1.01	1.63 ± 0.29	<.001
T2 DB-DM-DL	3.98 ± 0.79	5.74 ± 1.17	1.76 ± 0.30	<.001
T3 DB-DM-DL	3.74 ± 0.91	5.48 ± 1.41	1.74 ± 0.36	<.001
Within group T0 to T3 P value	<.001	.43	NA	NA

Abbreviations: DB, distobuccal; DL, distolingual; DM, distomedial.

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Table 3. ASSESMENT OF PD REDUCTION ON DISTAL SURFACE (AVERAGE OF DB, DM, AND DL SURFACES) OVER TIME WITHIN TEST AND CONTROL GROUP. NOTE THAT THERE ARE STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN T0 AND T1, T0 AND T2, AND T0 AND T3 WHEREAS THERE ARE NO DIFFERENCES BETWEEN T1 AND T2 OR T2 AND T3 IN THE TEST GROUP. IN THE CONTROL GROUP, NO DIFFERENCES ARE FOUND OVER TIME. DIFFERENCES BETWEEN GROUPS TO T0 TO T1, T0 TO T2, T0 TO T3, T1 TO T2, T1 TO T3, AND T2 TO T3

PD Differences on Distal Surface	Test Group (n = 22)	P Value	Control Group (n = 22)	P Value	Intergroup P value
PD differences on distal surface					
T0 to T1	2.30 ± 0.24	<.001	0.21 ± 0.18	.85	<.001
T0 to T2	2.5 ± 0.22	<.001	0.28 ± 0.22	.75	<.001
T0 to T3	2.74 ± 0.28	<.001	0.54 ± 0.30	.43	<.001
T1 to T2	0.2 ± 0.12	.53	0.076 ± 0.11	.98	.470
T1 to T3	0.44 ± 0.19	.16	0.33 ± 0.23	.66	.724
T2 to T3	0.24 ± 0.16	.59	0.26 ± 0.19	.73	.952

Abbreviations: DB, distobuccal; DL, distolingual; DM, distomedial; PD, probing depth.

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suffer a number of complications.^{16,24} It can also lead to periodontal problems characterized by bone loss on the distal aspect of the lower second molar.²¹

To reduce the risk of developing periodontal defects on the distal surface of the second molar, various treatment strategies are available.¹² Regenerative procedures and/or bone grafting techniques have been found superior to spontaneous healing.^{6,13}

The main objective of the present clinical trial was to assess the evolution of PD over 36 months when using ATG after 22 ILTM extraction surgeries and to compare the outcomes with PD in ungrafted alveoli. The results of the study showed statistically significant differences in PD at 3 months, 6 months, and 36 months between the test (ATG) and control (ungrafted) groups, showing that ATG may offer an effective bone substitute for regenerating defects after ILTM removal.

One of the advantages of this autologous graft material is that it does not require a second donor site and so is well accepted by patients and reduces morbidity considerably.

The chemical composition of ATG is similar to that of autologous bone, which is still considered the gold standard in regenerative processes due to its osteogenesis, osteoconductive, and osteoinductive properties.¹⁷ Both tissues share similar organic (25 vs 20%) and inorganic (65 vs 70 to 75%) content, as well as water (10%). ATG's inorganic content is made up of 4 types of calcium phosphate (hydroxyapatite, tricalcium phosphate, octacalcium phosphate, and amorphous calcium phosphate), and 90% of the organic content consists of type I collagen, 10% of non-collagenous proteins (osteopontin, osteocalcin, bone morphogenetic protein, insulin-like growth factor-II, and transforming growth factor-beta).²⁵ It is the organic compound that provides ATG with its osteoin-

ductive capacity, while inorganic content provides its osteoconductive property.^{21,26,27}

Guided bone regeneration with ATG has also been investigated, showing good preliminary results in both animal²⁸ and human studies.^{29,30} It has also obtained encouraging results in sinus lifting³¹ and alveolar ridge preservation procedures.³²

Regarding the purpose of the present RCT, various studies have evaluated PD on the distal surface of the second molar after ATG placement^{21,22,27} following ILTM removal. But these studies assessed only short-term results. All studies of the medium/long-term evolution of bone defects after ILTM removal have investigated other graft materials or conventional approaches without graft materials.

A classic landmark study by Kugelberg et al¹⁴ evaluated periodontal healing 2 years after ILTM surgery, finding that 43.3% of second molars showed PDs of over 7 mm, and 32.1% of the sample exhibited bone loss greater than 4 mm. These results can be compared (to some extent) with those of the present study in which PDs in the control group did not show significant improvement over time, with PDs of around 6 mm at the 3-year evaluation.

In contrast, statistically significant improvement was observed in bone defects regenerated with ATG, with PDs of less than 4 mm at all locations after 3 years of follow-up. As far as the authors are aware, no medium/long-term studies to date have evaluated the 36-month evolution of ATG after ILTM removal. So, according to the present results, ATG for regenerating bone defects after ILTM removal may offer a viable alternative biomaterial for this type of treatment that achieves adequate periodontal health, in contrast to spontaneous healing process, with pathological PD measurements after 3 years' follow-up. Furthermore, the 3-year results obtained with this graft material

suggest that ATG can be used routinely as an alternative option not only in oral surgery procedures, but also in guided bone regeneration procedures in implant dentistry.³³

One disadvantage of the treatment is of course the longer surgical time required to prepare the ATG (about 15 minutes). However, this prolongation was not significantly reflected in increased swelling or postoperative pain.²¹

A possible limitation of this study is the different preoperative ILTM positions in 9 of the 22 patients, especially considering that a split-mouth study should ideally have similar preoperative conditions on both sides. However, the preoperative situation was almost identical in all patients (21 out of 22) and no relevant baseline differences in preoperative PD were found in any of the recorded sites.

The study also suffered other limitations, particularly the fact that it is a clinical and radiological study and did not include any histological/histomorphometric correlation of the outcomes obtained. It would also be interesting to compare the efficacy of ATG with autogenous bone or other bone substitutes in a split-mouth design, with adequate follow-up periods, to compare clinical behavior and possible differences in complication rates.

Within the limitations of the present study, ATG may be a valid option for improving periodontal healing in bone defects after ILTM removal. The results obtained showed better clinical and radiological behavior compared with a conventional approach without grafts. Moreover, no significant changes in PD were observed after 36 months' follow-up and no major complications occurred.

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