Original Article

How Much Disinfected Ground Tooth Do We Need to Fill an Empty Alveolus after Extraction? Experimental *in vitro* Study

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Abstract

Aim and Objectives: The main objective of this study was to evaluate how much crushed, extracted human teeth material can use to fill an empty alveolus of the mandibular anterior teeth. Material and Methods: Fifty-four human teeth were collected from 10 donors due to advanced periodontal disease. The patients were clinically selected, signed informed consent, and receive no financial compensation for participating in this study. Fifty-four teeth were mechanically cleaned, dried, sectioned, and grounded. All teeth are grounded using the Smart Dentin Grinder machine. Cone-beam computed tomography scanners of each patient were done and processed the standard tessellation language images by a three-dimensional (3D) printer, and 3D models were obtained. Results: The mean of each alveolus was 12.1 ± 0.34 mm for lower incisors and 17 ± 0.29 mm for lower canines. The mean values of root material we need for central and lateral incisors alveolus filling were 0.298 ± 0.14 cc, and for lower canines was 1.02 cc. Therefore, we need one root or one and half-crowns must be needed to fill a lower incisor alveolus. A lower canine needs at least one canine root or one canine crown and two lower incisors crown to fill the canine alveoli. Conclusions: Dentin is a helpful graft to fulfill an empty alveolus due to osteoinductive properties, and a ground crown is useful for buccal bone protection due to osteoconductive properties before and after implant placement.

Keywords: Ground crown, ground dentin, grounded tooth graft, Smart Dentin Grinder, teeth particles

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INTRODUCTION

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The autogenous dentin matrix was used for alveolus filling and bone regeneration in different bone defects.^[1] The use of dentin matrix in bone regeneration is based on the biochemical and structural similarities between bone and dentin/enamel.^[2] The dentin has transformed factors to induce undifferentiated mesenchymal cells to grow into bone cells to form new bones.^[3] Immediate bone grafting of extraction sockets was widely used with or without membranes to prevent crestal bone loss during wound healing.^[4] The first description of the human dentin matrix was in 2008 created from extracted human teeth related to its osteoinductive, osteoconductive, and remodeling capacity. The bone and dentin are composed of the weight and volume of collagen, hydroxyapatite, and bone proteins.^[5,6] All those characteristics situated bone and dentin like the same biomaterials consisting of collagen and hydroxyapatite material working as

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scaffolds.^[7] Autogenous bone grafts showed similar resorption rate characteristics as dentin grafts, close to 12 weeks. The resorption rate in the bone interface between dentin/bone graft and original bone showed new bone formation.^[8] Other authors explain that tooth grafts have not got additional benefits over conventional graft materials.^[9] The use in clinical practices will determine its success due to the high variability in resorption time (2–24 weeks), and the risk of graft dehiscence (12.96%–34.38%) has been observed.^[10] Most biomaterial structures need a scaffold to let the new bone establish tooth dentin and cementum structure work directly deposed on membranous surfaces, like ankylosis.^[11,12]

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Another way to preserve extracted teeth is by cryopreservation; this technique will maintain the dentin's hole integrity and mechanical properties compared with noncryopreserved ones.^[13]

The particulate dentin graft is an excellent material for socket preservation and another oral surgical technique. One of the unique characteristics was high bone replacement due to its resorption by the osteoinductive and osteoconductive properties in implant placement techniques.^[14] Dental implants in sites regenerated with dentin matrix showed similar primary stability to xenograft granules and equal clinical- and patient-related outcomes.^[15]

The main goal of our study was to estimate how much ground tooth after extraction we need to fill an empty socket or sockets, maintaining the crestal bone.

MATERIALS AND METHODS

The study protocol with registration number 6781; 21-07-2017, approved by the Catholic University of Murcia Ethics Committee, related to using extraction teeth for *in vitro* studies.

Mandibular anterior human teeth were extracted from 10 patients between 60 and 70 years mean and standard deviation were (64.23 ± 0.28) including six women and four men in the study. The whole group of patients signed informed consent to donate their teeth for the study and did not receive financial compensation. The patient's teeth were diagnosed with periodontal disease in the lower six anterior teeth (number 33 to number 43). The inclusion criteria were healthy patients over 60 years of age who needed an anterior mandibular tooth/teeth extraction for periodontal reasons. Exclusion criteria are patients who do not need an anterior mandibular tooth extraction, systemically complicated patients (American Society of Anesthesiologists III, IV, and V), pregnancy or lactation, and patients who unwillingness to follow-up calls.

A total of 34 teeth were collected from 10 donors, cleaned using carbide-tungsten burs, grinding the ligament fibers attached to the root, and using an air syringe to dry all teeth [Figure 1].

Each tooth was washed, dried, and ground using the "Smart Dentin Grinder Genesis" device (KometaBio Inc., Cresskill, NJ, USA) [Figure 2]. Teeth particles sized at 300 up to 1200 μ m were obtained by sieving the particles into two different compartments. The teeth particles were immersed in a basic alcohol cleanser in a sterile container for 5 min to dissolve all organic waste and bacteria and then washed with sterile saline for 30 seconds twice [Figures 3 and 4].

After 5 minute of teeth particles immersion in dentin cleanser, many periodontal bacteria were eliminated in these disinfectants.^[16-19] We observed ground particles by an optical microscope showing dust in all particles before cleaning and completed clean and transparent after that using an optical microscope (AXIO, Lab. A1 with Axiocam 305 color, Zeiss, Jena, Germany) [Figure 5].



Figure 1: Teeth were cleaned using straight carbide tungsten burs, grinding periodontal ligament, and dried



Figure 2: After being cleaned and dried, the teeth were grinded using the "Smart Dentin Grinder" device (KometaBio Inc., Cresskill, NJ, USA)



Figure 3: (a) Extracted teeth before grinding on a microscale (b) grinding teeth on a microscale

When the material was ready to use, we guessed how much autologous dentin graft we needed to fill each anterior mandibular extracted teeth' empty sockets. A printed mandible was used in the study after a cone-beam computed tomography scan of one of the donor's scanners. A second scanner was

done to get a final model with an empty alveolus. After that, we transformed the standard tessellation language archives into printed files processed by a three-dimensional (3D) printer Formlabs 3B (Somerville, MA 02143, USA) [Figure 6]. After that, we compared the printed mandible and made the width and length measurements with the scanner measurements [Figures 7 and 8]. Finally, the ground root and roots were introduced inside the 3D-printed mandible, as shown in Figure 9.

Each grinding tooth was kept in separate sterile crystal pots, labeled with the patient's data.

Statistical analysis

Statistical analysis of this *in vitro* study was done by PASW Statistics v. 18.0.0 software (SPSS, IBM SPSS Statistics V.18. IBM Inc. Chicago, IL, USA). Analysis of variance one-way test was applied to compare the means and assuming a level of significance of 95% (P < 0.05). In addition, the Mann– Whitney and Kolmogorov–Smirnov tests were used to assume normality. Finally, testing mean and standard deviation were made by a descriptive test.

RESULTS

Human lower central incisors mean measurements were 5.4 ± 0.6 mm in a spread, 1.28 ± 0.45 mm in amplitude, and scaled 0.8 ± 0.16 g, whereas lower lateral incisors mean



Figure 4: (a) Kometabio Smart Dentin Genesis Grinding Machine (b) dentin cleanser (red cap) and Buffered saline (green cap)



Lower mandibular alveoli were measured in 54 places with a caliper and a periodontal probe to get the real dimensions of the alveoli as described in Table 2. With mean values ranged from $10-12 \pm 0.34$ mm to $16-18 \pm 0.21$ mm in deep, $7-7.5 \pm 0.27$ mm to $9-9.5 \pm 0.43$ mm in buccolingual direction, and $5-6 \pm 0.22$ mm to $7.5-8 \pm 0.44$ mm in mesiodistal direction [Table 2].

The alveoli of those six mandibular extracted teeth were used individually to know how many particulate teeth need it to fulfill the hole dental alveolus. Figure 9 shows

Table 1: Descriptive test of the mean and standard						
deviation of each human	tooth	length,	width,	and weight		
of 10 patients						

Human teeth	Mean length±SD (mm)	Mean width±SD (mm)	Mean weight±SD (g)
Lower central incisor	5.4±0.6	1.28 ± 0.45	0.8±0.16
Lower lateral incisor	5.3 ± 0.28	1.17 ± 0.33	$0.7{\pm}0.85$
Lower canine	6.7±0.5	$1.54{\pm}0.88$	1.31 ± 0.75

SD: Standard deviation



Figure 5: (a) Dusty tooth particle after grinding (b) cleaned particles ready to be used



Figure 6: Diagram of evaluation and filling procedure of lower anterior extracted teeth



Figure 7: (a) Original model of the patient who needs lower teeth extraction (b) 3D reconstruction (c) 3D-printed mandibular alveoli

the amount of ground tooth required to fill one, two, or three alveoli.

Human teeth particles showed that disinfected roots and crowns increased double the particles' weight and increasing the volume. In addition, after disinfection, some pores appear all around dentin particles; explained by Calvo-Guirado *et al.* in 2019, 44.48% corresponded to interparticle spaces and 2.533% for particle porosity.^[17]

The mean tooth weight ranged from 0.486 ± 0.6 g for central and lateral incisors and 1.27 ± 0.4 g for lower canines. When we divided crown and root, the cutting mass loss was -0.05 g for central and lateral incisors and -0.07 g for lower canines. The mean of obtained ground root cc and a crown was 0.25 ± 0.2 cc and 0.192 ± 0.3 cc for central



Figure 8: (a) Measurement of mesiodistal site with a caliper (b) deep measurement with a periodontal probe (c) buccolingual measurement with a caliper

and lateral incisors and 0.905 ± 0.4 g and 0.296 ± 0.1 cc for lower canines, respectively. The most critical data were disinfected root and crown, 0.299 ± 0.24 cc, and 0.214 ± 0.16 cc for central and lateral incisors. The amount of disinfected root and crown for canines was 1.02 ± 0.33 cc and 0.411 ± 0.56 cc, respectively [Table 3 and Graph 1].

Every tooth extracted needs the same root or one and a half crown to fill the socket. The canine is the exception because it needs its ground root or crown and two incisor crowns [Figure 10].

If we lose the lower central incisor, we need at least the same root ground or one and a half incisor crowns to stuff the alveoli fully. If we lose two lower incisors (central or lateral), we need the same two ground roots and three incisors' crowns. The last clinical relevance is that we need three roots and four crowns if we lose three teeth. When we lose all lower teeth from left to right canines, only with all particulate roots (total six), we can fulfill all sockets, and ground crowns could be used to protect buccal plates and reduce crestal bone resorption [Figure 11]. The distribution of each part of the tooth inside the alveolus revealed that different density of new bone had been founded in differents sites, the use of dentin graft has a valuable meaning because dentin with low hydroxyapatite crystallinity could be placed at the middle and bottom of all alveoli of its characteristics of osteoinduction. The ground crown with high crystallinity must be placed on top of the bone crest for osteoconduction and bone crest maintenance [Figure 12]. Finally, with one ground disinfected central incisor root



Figure 9: (a) Dentin grinder in one canine alveolus (b) particulate teeth placed in two alveoli (c) dentin graft placed in three alveolus

Table 2: Mean values of teeth alveoli dimensions							
Tooth alveoli	31 (mm)	32 (mm)	33 (mm)	41 (mm)	42 (mm)	43 (mm)	
Deep	10-12±0.34	11-13±0.11	16-18±0.21	10-12±0.34	11-13±0.11	16-18±0.21	
Buccolingual	7-7.5±0.27	7-8±0.54	9-9.5±0.43	7-7.5±0.27	7-8±0.54	9-9.5±0.43	
Mesiodistal	5-6±0.22	5-6±0.22	7.5-8±0.44	5-6±0.22	5-6±0.22	7.5-8±0.44	

Table 3: Evaluation of different teeth measurements described in grams								
Tooth alveolus	Tooth weight (g)	Root weight (g)	Crown weight (g)	Grinded root (g)	Grinded crown (g)	Disinfected root (g)	Disinfected crown (g)	Disinfected crown (g)
31	0.495±0.31	0.286±0.62	1.001±0.12	0.26±0.81	0.159±0.23	0.312±0.44	0.242±0.32	-0.04 ± 0.33
32	$0.506{\pm}0.76$	0.288 ± 0.41	0.197 ± 0.33	$0.256{\pm}0.48$	$0.188 {\pm} 0.55$	0.307 ± 0.21	$0.216{\pm}0.24$	-0.073 ± 0.32
33	1.25 ± 0.22	0.809 ± 0.42	0.367 ± 0.22	0.81 ± 0.51	0.342 ± 0.47	$0.926{\pm}0.25$	0.411 ± 0.34	-0.05 ± 0.43
41	0.496 ± 0.68	0.282 ± 0.52	0.208 ± 0.12	0.273 ± 0.23	0.197 ± 0.27	0.926 ± 0.43	$0.229{\pm}0.71$	-0.045 ± 0.21
42	0.45 ± 0.26	$0.254{\pm}0.28$	0.175 ± 0.35	0.227 ± 0.47	0.25 ± 0.32	0.271 ± 0.22	0.172 ± 0.29	-0.045 ± 0.62
43	1.294 ± 0.44	1.001 ± 0.32	0.285 ± 0.56	0.92 ± 0.65	0.25±0.21	1.114 ± 0.51	0.417 ± 0.32	-0.09 ± 0.33







Figure 11: Root or crowns needed to be placed in every alveolus

 0.312 ± 0.44 cc, we can fill one alveolus; in the same situation to fill an alveolus of lower lateral incisor 0.307 ± 0.21 cc, we can fill one alveolus, but with a lower canine 0.926 ± 0.25 cc, we can fill two and a half central incisor roots.

DISCUSSION

Teeth-derived biomaterial without antigenicity improves bone formation and remodeling capabilities.^[16] We described using ground tooth particles in extraction sockets, finding different types of bone at test sites than at control sites at 30 and 90 days.^[16-19] A considerable range of bone graft materials available in the market has physicochemical properties related to the type of defect filling.^[20]

Other authors used dentin matrix in extraction sockets, demonstrating that it was as effective as organic bovine bone.^[21] For other devices such as tooth transformers, after

175



Graph 1: Teeth measurements obtained in the study

grinding, particles were demineralized and sterilized using six different solutions and then were then collected in sterile 1.5 mL polypropylene tubes and stored at R. T. until use.^[21] The dentin grinder procedure is more straightforward and quicker to get the particles for grafting, avoiding any store. On the other hand, the effect of this clinical procedure has something to explain in detail related to the characteristics of time of resorption. The demineralization process augments dentin grafts' low crystallinity properties, following a similar resorption pattern rate as autogenous bone grafts for 12 weeks.^[22] Tasdemir et al. adding simvastatin on tooth graft enhances osteoinductive properties through bone morphogenetic protein-2 and O. C. expression.^[23] We agree with the authors that the partial demineralization process can enhance bone resorption and determine to the place of dentin grafts at the middle and bottom of the alveolus due to osteogenic properties. Yüceer-Çetiner et al. composite tooth graft with platelet-rich plasma protocol showing that demineralized autogenous dentin graft with platelet rich factor (PRF) increases bone formation capacity.^[24]

Furthermore, this material suffered a dimensional contraction of the postextraction socket but remained stable at 16 weeks after surgery in vertical and horizontal dimensions.^[25] Um *et al.*, showed that the initial resorption during the healing period of auto-DDM applications was at 3 to 6 months.^[26] Furthermore, a scientific explanation related to roots and crowns was explained by Binderman and Pohl, using roots and crowns in different positions depending on the clinical needs. The amount of cc need it for a single root will be approximately the same root ground and disinfected inserted in the same clinical procedure.

CONCLUSIONS

We observed in our study that tooth particle grafts have to be used as a biomaterial for any bone defects due to their similar characteristics to the autologous bone. The amount of tooth we need to fill one alveolus is about one and a half roots to fill one socket. Dentin could be a helpful graft to fulfill an empty alveolus due to its osteoinductive properties because the resorption is quick, and a ground crown specially composed of a high percentage of enamel could be used for buccal bone protection due to osteoconductive properties



Figure 12: Socket filling with ground root and crown

because the resorption is zero before and after implant placement.

Author contributions

The following statements should be used "Conceptualization, Nuria García Carrillo, Jose Luis Calvo-Guirado.; Software, Miguel Garcés-Villalá.; Validation, Jose Manuel Cervera-Maillo; Methodology, Felix de Carlos Villafranca; Formal Analysis: Francisco Martínez Martínez, Nuria García Carrillo, Investigation, Jose Luis Calvo-Guirado. Jose Manuel Cervera-Maillo; Resources: josé Luis Calvo-Guirado; Data Curation: Nuria García Carrillo, Felix de Carlos Villafranca; Writing-Original Draft Preparation, Jose Luis Calvo-Guirado.; Writing-Review and Editing, Nuria García Carrillo, José Luis Calvo-Guirado; Visualization: José Luis Calvo-Guirado., Miguel Garcés-Villalá; Supervision, Jose Luis Calvo-Guirado.; Project Administration, Jose Luis Calvo-Guirado, Francisco Martínez Martínez; Funding Acquisition, Jose Luis Calvo-Guirado, Francisco Martínez Martínez.

Ethical statement

The study passed the Ethical comitee of Universidad Catolica of Murcia 28/02/2020, Cod CE22007.

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NII.

Conflicts of interest

There are no conflicts of interest.

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