A new procedure for processing extracted teeth for immediate grafting in post-extraction sockets. An experimental study in American Fox Hound dogs[☆]

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A R T I C L E   I N F O

A B S T R A C T

Objectives: To investigate freshly extracted dental particulate used to graft post-extraction sockets in dogs, comparing new bone formation at experimental and control sites.

Materials and methods: Bilateral premolars P2, P3, P4 and first mandibular molars were extracted atraumatically from six American Fox Hound dogs. The teeth were ground immediately using a 'Smart Dentin Grinder'. The dentin particulate was sieved to ensure a grain size of 300–1200 μm and immersed in an alcohol cleanser to dissolve organic debris and bacteria, followed by washing in sterile saline buffer solution.

The animals were divided into two groups randomly: group 'A' (control) samples were left to heal without any extraction socket grafting procedure; group 'B' (experimental) sockets were filled with the autogenous dentin particulate graft. The rate of tissue healing and the quantity of bone formation were evaluated using histological and histomorphometric analyses at 60 and 90 days post-grafting. The type of bone generated was categorized as woven (immature bone) or lamellar bone (mature bone).

Results: Substantially more bone formation was found in Group B (experimental) than Group A (control) at 60 and 90 days ($p < 0.05$). Less immature bone was identified in the dentin grafted group (25.7%) than the control group (5.9%). Similar differences were also observed at 90 days post grafting.

Conclusion: Autogenous dentin particulate grafted immediately after extractions may be considered a useful biomaterial for socket preservation, protecting both buccal and lingual plates, generating large amounts of new woven bone formation after 60 days, and small amounts of lamellar bone after 90 days healing.

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1. Introduction

A variety of materials have been commercialized for use in different procedures in the fields of maxillofacial, periodontal, and dental implant surgery. In particular, bone grafts are used to stabilize blood clotting, to increase alveolar bone volume and to restore its original structure, function and appearance. As alter-

natives to autogenous bone grafts, a range of biomaterials have been developed for grafting and bone regeneration, which offer a good osteoinductive capacities but suffer some risk of viral infection; synthetic materials offer osteoconductive capacities and can be supplied in unlimited quantities (Kim, 2012; Kim et al., 2001a,b,c). Allografts, xenografts, and synthetic materials as bone substitutes for grafting small and large defects all have a long history that has established their usefulness and efficacy (Pérez-Sánchez et al., 2010). Hydroxyapatite is chemically comparable to biological apatite crystals and is considered bioactive as a calcium phosphate ceramic (Gauthier et al., 2004; Maté-Sánchez de Val et al., 2012; Maté-Sánchez de Val et al., 2014). Variations in the physico-chemical properties of bone substitute materials influence the degradation process so that the resorption of deproteinized bone particles proceeds slowly enough to allow sufficient time for bone maturation to take place (Ramírez Fernández et al., 2017a,b,c).

Bone graft materials derive from four sources, each with its particular advantages and disadvantages. Synthetic bone grafts are relatively inexpensive and run no risk of disease transmission, but they lack a capacity for promoting early osteogenesis and osteoinduction, so their use is limited to applications requiring the long-term formation of woven and lamellar bone. Xenografts derive from non-human sources such as pig, cow or horse bone. They resorb slowly and typically act as a good scaffold for bone regeneration processes, but they do not integrate well with newly formed bone. Alografts are sourced from human bone, usually from long bone harvested from a cadaver donor. Alografts tend to resorb too quickly and so do not provide the scaffold function required for long-term bone remodeling. They do, however, integrate well with newly formed bone. Autografts are harvested from the patient and are considered the 'gold standard' in bone grafting procedures. Autogenous bone grafts exhibit bioactive cell instructive matrix properties and are non-immunogenic and non-pathogenic but suffer the disadvantage of the need to harvest bone from a donor site and therefore possible morbidity (Kim et al., 2010).

Tooth extraction is one of the most widely performed procedures in dentistry. The procedure yields teeth that are discarded as clinical waste (Horowitz et al., 2012). But teeth could be recycled to produce an effective graft material (Kim et al., 1993; Kim et al., 1994; Kim et al., 1996a; Kim et al., 1996b; Kim et al., 1997; Kim, 1998; Kim et al., 2001a,b,c; Kim et al., 2004a,b). Dentists have been transplanting teeth for many years and tooth ankylosis is a well-studied principle. In recent years, several research teams have assessed the potential uses of extracted teeth.

Autogenous dental bone graft material (AutoBT, Korea Tooth Bank Co., Seoul, Korea), a human demineralized dentin matrix derived from extracted human teeth, was first developed in 2008 and its osteoinductive, osteoconductive and remodeling capacities in implant dentistry have been evaluated.

The chemical compositions of teeth – especially dentin – and bones are very similar. Cementum is made up of 45–50% inorganic substances, 50–55% organic substances, and water. Alveolar bone has 65% inorganic and 35% organic substances (Kim et al., 2010). Bone and dentin consist of fluid (10% weight volume), collagen (20%) and hydroxyapatite (70%) (Bhaskar, 1980; Murata et al., 2000; Murata et al., 2002; Akazawa et al., 2006; Murata et al., 2007; Min, 2007; Nanci, 2008). Dentin particulate is of medium-hard consistency and is easily handled making it suitable as a grafting material, especially in light of the growth factors and bone morphogenetic proteins (BMPs) contained in fresh dentin matrix.

Both demineralized dentin matrix (DDM) and demineralized bone matrix (DBM) are predominantly composed of type I collagen fibers (95%) with the remainder comprising non-collagenous proteins (NCPs) including small amounts of growth factors. Consequently, DDM and DBM can be defined as acid-insoluble collagen bound with BMPs, including members of the transforming growth factor-beta (TGF-β) super-family that enhances bone formation (Nanci, 2008). Dentin xenografts offer the potential to be incorporated in bone without producing inflammation, where they are gradually resorbed and replaced by new bone (Andersson, 2010; Nakashima, 2005, Hassan et al., 2003).

The present work describes a clinical procedure that uses freshly extracted teeth, recycling them into a bacteria-free particulate of autogenous mineralized dentin for immediate grafting. A 'Smart Dentin Grinder^{TM}' device was adapted to grind extracted teeth into dentin particles of a specific size. A chemical cleanser was used to leave the particulate bacteria-free in little more than 15 min. The aim of the study was to assess the efficacy of the particulate graft material filling extraction sockets by histological and histomorphometrical evaluation of vital bone (VB) formation, in comparison with non-grafted sockets.

2. Materials and methods

The study used six American Fox Hound dogs aged approximately 1-year, each weighing 14–15 kg. The Ethics Committee for Animal Research at the University of Murcia (Spain) approved the study protocol, which followed guidelines established by the European Union Council Directive of February 1st 2013/53/CEE. Clinical examination determined that the dogs were in good general health. Animals were quarantined for application of anti-rabies vaccine and vitamins. The animals were kept in kennel cages pre and postoperatively, receiving appropriate veterinary care with free access to water and standard laboratory nutritional support throughout the study period. All animals presented intact dental arches, without any oral, viral or fungal lesions.

The animals were pre-anesthetized with 10% zolazepam at 0.10 ml/kg and acepromazine maleate (Calmo-Neosan$^®$, Pfizer, Madrid, Spain), 0.12–0.25 mg/kg, and medetomidine 35 lg/kg (Medetor 1 mg, Virbac, CP-Pharma Handelsgesellschaft GmbH, Germany). The mixture was injected intramuscularly in the femoral quadriceps. Animals were then taken to the operating theater where, at the earliest opportunity, an intravenous catheter was inserted (diameter 22 or 20 G) into the cephalic vein, and propofol was infused at the rate of 0.4 mg/kg/min as a slow constant infusion rate. Anesthetic maintenance was performed using volatile anesthetics, and the animals were submitted to tracheal intubation with a Magill probe for adaptation of the anesthetic device and for administration of oxygen-diluted volatile isoflurane (2 V%). Additionally, local anesthesia (Articaine 40 mg; 1% epinephrine, Normon$^®$, Madrid, Spain) was administered at the surgical sites. These procedures were carried out under the supervision of a veterinary surgeon.

Bilateral mandibular premolars and first molars (P2, P3, P4, M1) were extracted (Fig. 1) under the local and general anesthesia described above. Multi-rooted teeth were sectioned in buccolingual direction at the bifurcation using a tungsten-carbide bur so that the roots could be individually extracted without damaging the remaining bony walls.

Bucco-lingual (3.9 ± 0.37 mm) and mesio-distal (4.3 ± 0.45 mm) dimensions of the entrance to each fresh extraction socket was measured using sliding calipers, determining mean alveolar ridge measurements. Extraction sockets' mean alveolar ridge measurements were as follows: 3.8 ± 0.21 mm(P2), 4.0 ± 0.5 mm (P3), 4.1 ± 1 mm (P4), 5.6 ± 0.07 mm (M1).

Tooth roots were prepared after cutting the crown way with a tungsten carbide dental cutter (Zekrya FG Maillefer – Dentsply, USA), and cleaned using an ultrasonic device, grinding the root surfaces to eliminate ant remaining periodontal ligament (Fig. 2). The roots were then ground with a Smart Dentin Grinder (Bioner Sistemas Implantológicos, Catalonia, Spain) (Fig. 3). The 'Smart Dentin
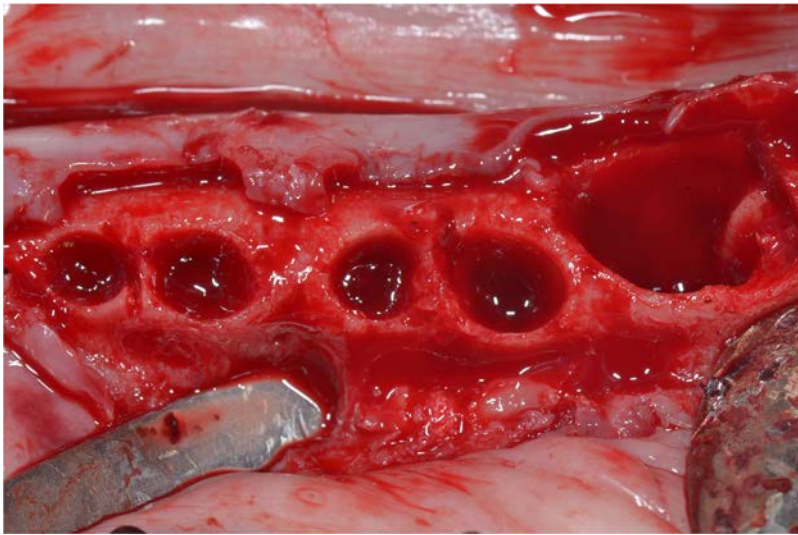
Fig. 1. Post extraction sites of mandibular premolars and firs molar.

Fig. 2. Smart dentin grinder chamber with roots extracted from the dog.

Grinder'TM was developed to grind extracted teeth and sort the resulting dentin particulate into two specific sizes of particle. This sorting process enjoys 95% efficiency. A basic alcohol cleanser was used to rid the dentin particulate of any bacteria, which was then washed twice in sterile phosphate buffered saline (PBS). The whole process was completed in 15–20 min. This procedure can be applied to teeth extracted for periodontal reasons, teeth restored with fixed prostheses (eliminating the prostheses beforehand) and partially or totally impacted teeth. However, teeth that have undergone previous root canal procedures should not be used in this way as they may suffer contamination by foreign materials. Alternatively, the wet particulate can be placed on a hot plate (140 °C) for 5 min and the dry bacteria-free particulate can then be used for immediate or future grafting (Binderman et al., 2014).

The ground dentin material was analyzed by scanning electron microscopy (SEM) to evaluate root morphology (Figs. 4–6). For SEM study, both test and control samples were placed in liquid nitrogen for approximately 2 min and then split longitudinally; the other halves were cut in the middle with a diamond-coated water-cooled band saw. The freshly formed and cleaned surfaces were coated with carbon film (BalTec CED 030; BalTec, Balzers,

Liechtenstein) for SEM analysis at ×10 magnification. The resolution was (0.8 nm @ 15 kV; 1.4 nm @ 1 kV; 0.6 nm @ 30 kV (STEM mode); 3.0@ 20 kV at 10 nA; and WD 8.5 nm using Gemini II Electron Optics (Carl Zeiss Microscopy Gmbh, Jena, Germany) fitted with detectors for secondary electrons and backscattered electrons in order to allow exploration of the different biological processes involved in tissue healing and to identify morphological changes in the cellular components of different materials. The primary electron beam generates secondary electrons and backscatter electrons. The difference in energy ensures that secondary electrons and backscatter electrons are displayed on different trajectories by the Beam Booster. Secondary electrons precipitate directly from the topmost nanometers of the sample with energy of less than 50 eV and show the topography of the surface. The electric field of the SEM column attracts these electrons and the excitation lens directs them to the annular InLens detector. Depending on the sample's surface condition, it detects the secondary electrons over a wide angular range. Backscatter electrons are generated below the surface. Their energy level is similar to that of the primary electrons hitting the sample. Their number is greater than that of the secondary electrons and they provide highly specific information

Fig. 3. (a) Smart dentin grinder device, (b) root graft with 1200 μ, (c) root graft with 3 μ, (d) dentin graft after cleaning.
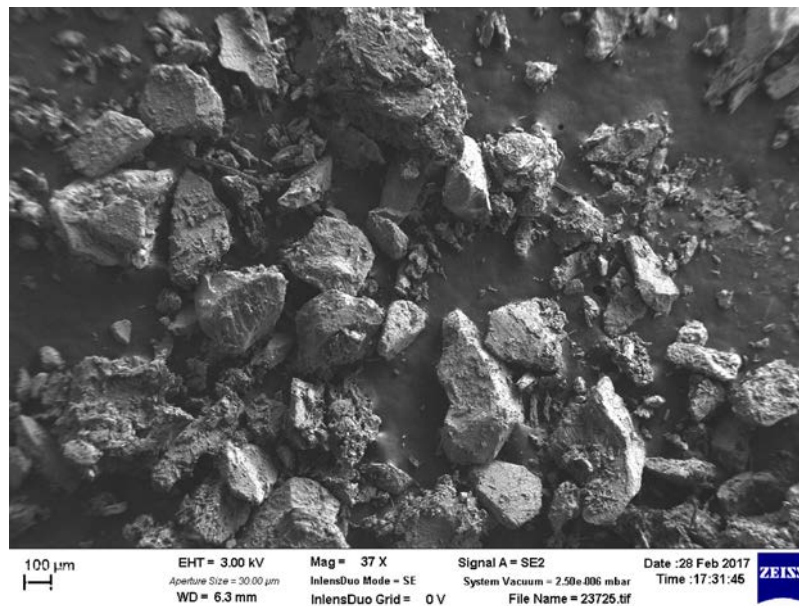

Fig. 4. Scanning electromicroscopy image of different particles size.

about the sample material's characteristics. Small-angle backscatter electrons provide highly specific information about the sample's crystalline structures (Carr and Toner, 1981; Mangano et al., 2013; Eberle et al., 2015; Ribeiro da Silva et al., 2016; Arshad et al., 2017).

Post-extraction Sockets at P3, P4 and M1 were selected randomly on both left and right sides (Group B, experimental sites), and filled with the freshly extracted dentin particulate (Fig. 7), while P2 post-extraction sockets on both sides remained untreated and were left to heal naturally (Group A, control sites). Simple resorbable sutures were used (3–0 TB-15. Lorca Marin®, Murcia, Spain) so that the gums completely covered the sockets. The sutures were removed after 2 weeks.

The anterior sectors from canine to canine were conserved in order to maintain minimum masticatory function. No intervention was performed in the upper maxilla. All surgery was performed under the supervision of the vet assigned to the University Animal Research Unit.

Throughout the surgical procedure the intravenous line was hydrated with glucose-saline (250 cm³) to aid the animals' post-surgical recovery. The following anti-inflammatory, analgesic and antimicrobial medication was administered: Metacam® anti-inflammatory: 1–2 ml intramuscularly; Alsir® antibiotic (enrofloxacin): 2 ml intramuscularly.

The antibiotics and anti-inflammatories were administered after surgery and then every two days for four days to prevent post-operative infection and inflammation, following guidelines established by animal research ethics committees.

After surgery, the dogs were moved to a kennel where they recovered under veterinary supervision. During the following days, post-surgical care of the post-extraction wounds was performed to
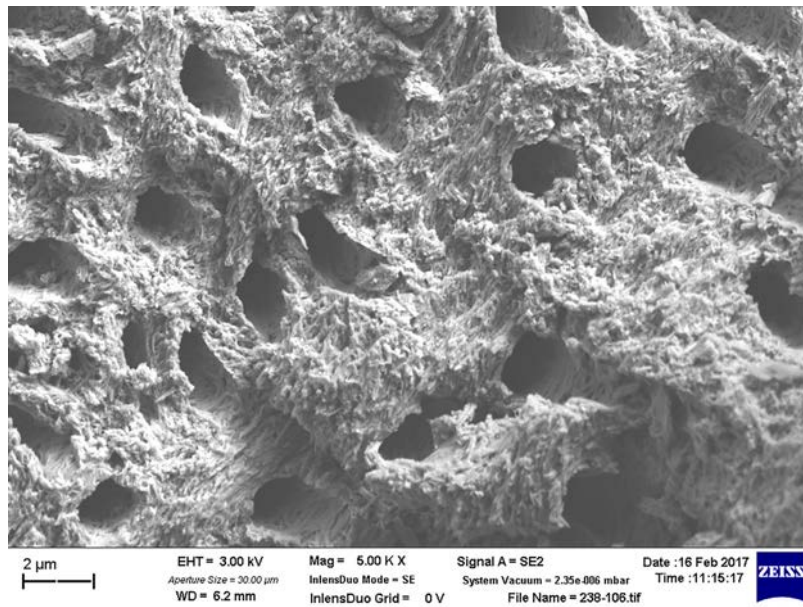
Fig. 5. Scanning electron microscopy of the dentin grinded surface after demineralization (×10,000).

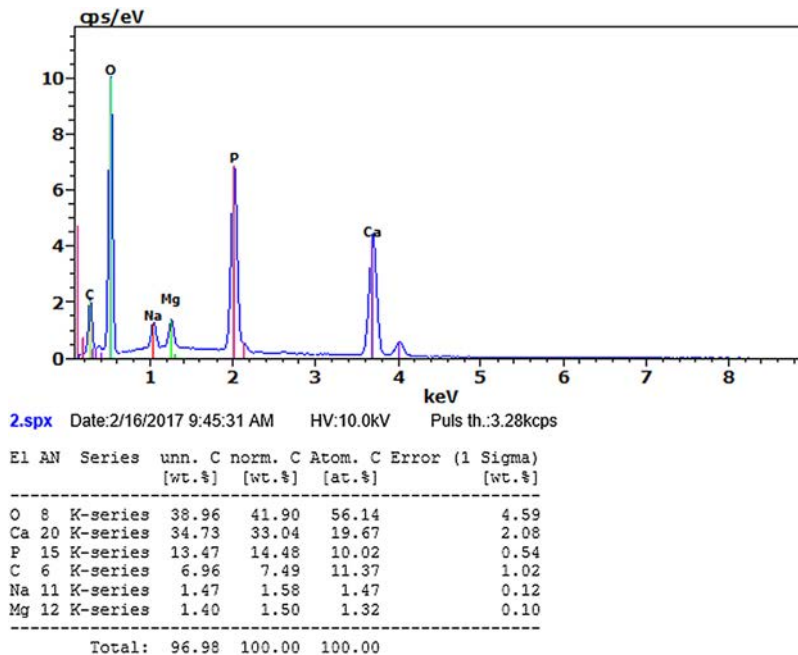| El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	38.96	41.90	56.14	4.59
Ca	20	K-series	34.73	33.04	19.67	2.08
P	15	K-series	13.47	14.48	10.02	0.54
C	6	K-series	6.96	7.49	11.37	1.02
Na	11	K-series	1.47	1.58	1.47	0.12
Mg	12	K-series	1.40	1.50	1.32	0.10
		Total:	96.98	100.00	100.00	

Fig. 6. EDX image of dentin graft.

avoid infection and the animals' general health was monitored. The animals were fed ad libitum with a soft food and water diet. The oral mucosa was cleaned using gauzes impregnated with a new mouth-rinse made from sea water (Sea 4 Encias®, Blue Sea Laboratories, Alicante, Spain). Local anesthetic was administered to the animals' vestibular and lingual gums, making supercrestal incisions from canines to second molars.

The bone was left to heal for either 60 or 90 days. A full-thickness flap was lifted (Fig. 5) and biopsies of control sites and experimental sites were removed using a trephine bur (2.75 mm diameter) from left or right sides respectively (Fig. 8).

One sample core corresponding to each period of time in the control and test groups were analyzed by micro CT (Bimodal SPECT-CT, single Photon Emission Computed Tomography, Albira Brunker,

Massachusetts, USA) and the sample subsequently embedded in light curing epoxy (Technovit 7200 VLC, Heraeus Kulzer, Austria). The Micro-CT technique made evaluations at a size of 18 mm^3 and 20,000 voxels per sample.

2.1. Histology and sample preparation

Bone cores were obtained and conserved individually in 4% formaldehyde for 15 days. The samples were decalcified for 30 days using TBD-2 (Anatomical Pathology International, Runcorn, Cheshire, U.K.). Half of the samples underwent dehydration and inclusion in paraffin, creating 8 μm sections (thinner cuts allow better light passage and so allow better structure evaluation). Afterwards, the sections were deparaffinized, hydrated with dis-

Fig. 7. Surgical image of dentin graft in premolars and molar alveolus.
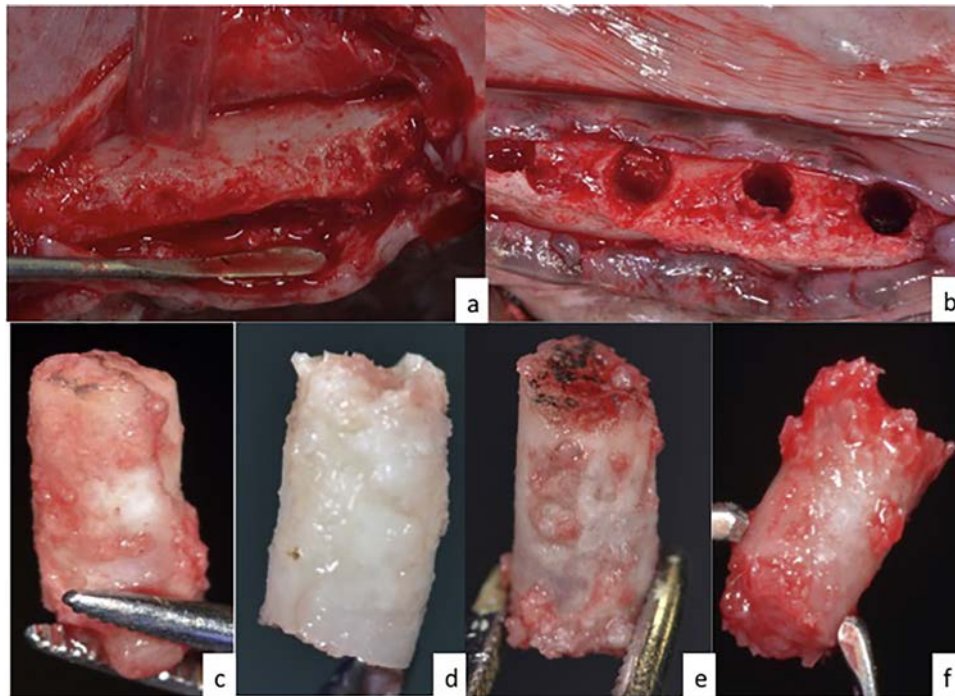

Fig. 8. (a) Healed bone with dentin graft after 3 months of healing, (b) trephine areas of grafted sites, (c) bone core of control sites at 60 days of healing, (d) bone core of dentin grafted sites at 60 days of healing, (e) bone core of control sites at 90 days of healing, (f) bone core of dentin grafted sites at 90 days of healing.

tilled water and stained with toluidine blue for 2–3 min. The other half of the samples were dehydrated in a decreasing alcohol series (100–50%) and embedded in histological resin (Technovit 7200-VLC, Kulzer, Wehrheim, Germany). After polymerization, the samples were sectioned longitudinally along the block axis using a metallographic cutter in sections of 100 μm, and then ground down to 30 μm. The sections were then immersed in $AgNO_3$ for 30 min and exposed to sunlight, washed under tap water, dried, and immersed in basic fuchsin for 5 min, washed again, and then mounted. For histomorphometric analysis, images were magnified x20, analyzing the samples digitally with ten fields per sample (DP12, Olympus, Nagano, Japan). Microimage 4.0 software (Media Cybernetics, Silver Spring, Maryland, USA) was used for image analysis. All analyses were performed by the same technician who

was unaware of each sample's group assignment (experimental or control). Only the area of newly formed bone was evaluated; connective tissue was disregarded. The percentage of immature bone within the total bone area was determined. Immature bone was characterized by images of totally disorganized mineralized bone, with high indices of cellularity and large medullary cavities, differentiating this from mature bone characterized by images showing a predominance of osteones made up of bone laminas organized concentrically around Haversian canals.

2.2. Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS, Chicago, IL, USA). Descriptive statistics were calculated

Table 1
Mean values ± standard deviation of immature bone quantity at each study time; the level of significance was set at $P < 0.05$.

	Quantity of immature bone	
	Dentin grinder (%) MEAN ± SD	Control (%) Mean ± SD
60 days	25.7 ± 0.11[*]	55.98 ± 0.16
90 days	12.6 ± 0.34[*]	34.8 ± 0.1

[*] $P < 0.05$.

Histometric measurements of quantity of immature bone (mean mm + SD) at 60 and 90 days of healing.

Table 2
Mean values ± standard deviation of new bone and connective tissue quantity at 60 days study time; the level of significance was set at $P < 0.05$.

Time of measurement 60 days	Dentin grinder Mean ± SD%	Control	P value
New bone	82.22 ± 1.7[*]	57.29 ± 0.11	<0.022[*]
Connective tissue	18.67 ± 0.1	30.56 ± 0.3	<0.562

Histometric measurements of quantity of new bone (mean mm + SD) at 60 days of healing.

[*] $p < 0.05$.

Table 3
Mean values ± standard deviation of new bone and connective tissue quantity at 90 days study time; the level of significance was set at $P < 0.05$.

Time of measurement 90 days	Dentin grinder Mean ± SD%	Control	P value
New bone	91.32 ± 0.8	65.89 ± 0.6	<0.123[*]
Connective tissue	8.38 ± 0.9	23.67 ± 1.1	<0.150

Histometric measurements of quantity of new bone (mean mm + SD) at 90 days of healing.

[*] $p < 0.05$.

(mean and standard deviation). The non-parametric Wilcoxon test for related samples was applied for comparison of mean values, assuming a significance level of 95% ($P < 0.05$). Equal means were regarded as the null hypothesis, while the existence of significant differences between means acted as an alternative hypothesis. As significant differences between the means were identified, the null hypothesis was rejected. Student's t-test was used to analyze differences between variables. Significance was established as $p < 0.05$. Mean percentages of new bone formation, residual graft material, and non-mineralized connective tissue at each evaluation period are shown in Tables 1–3.

3. Results

3.1. MicroCT evaluation

Fig. 9 shows a micro-CT image of a dentin-grafted site at 90 days presenting mean 828 ± 13 Hounsfield units (max value 1210 Hounsfield units), similar to D1 type bone, while the control site at 90 days obtained 773.6 ± 15 Hounsfield units of and similar bone quality.

3.2. Results at 60 days

3.2.1. Optical microscopy

The control group showed large quantities of newly formed bone covering the bone defects, but this was seen to consist entirely of immature (woven) bone, represented by images of highly disorganized tissues with high indices of cellularity and large medullary cavities. The groups treated with dentin graft were characterized by the presence of newly formed bone including areas of irregular

arrangement with high levels of cellularity, but in a lesser proportion than the control group. The dentin graft-treated group showed remains of dentin particulate, which had been partially resorbed and showed osteoinduction on and between the surfaces of the dentin particles (Fig. 10).

3.2.2. Histomorphometric analysis

Histomorphometry found a total of immature bone (woven bone) of 25.7 ± 0.11% in defects treated with dentin graft with significant differences in comparison with control samples (55.98 ± 0.16%) (Table 1). After 60 days, new bone formation was 82.22 ± 1.7% in defects treated with dentin grafts, while at untreated defects new bone was 57.29 ± 0.11% with statistically significant difference ($p < 0.05$) (Table 2).

3.3. 90 days

3.3.1. Optical microscopy

The control group showed more newly formed bone compared with the first study time (60 days); bone defects were covered, and images showed concentric laminas forming osteons, while other areas still showed disorganized immature (woven) bone. Images of samples treated with dentin graft were characterized by a predominance of mature newly formed (lamellar) bone, which was well organized as osteons, showing blood vessel development, although areas of disorganized bone with high cellularity remained, albeit as a small proportion of the total bone tissue (Fig. 11).

3.3.2. Histomorphometric analysis

Histomorphometric analysis found a mean proportion of marrow bone of 12.6 ± 0.34% in defects treated with dentin graft, with significant difference in comparison to control samples (34.8 ± 0.1%) (Table 1). Significant differences between the 60-day and 90-day study times were found (Table 1). After 90 days, new bone represented 91.32 ± 0.8% in defects treated with dentin graft, while new bone was 65.89 ± 0.6% in the control group with statistically significant difference ($p < 0.05$) (Table. 3).

4. Discussion

Hydroxyapatite crystals are identical in both structures. Of course, the tissues show differences in mechanical structure and in the cells present: unlike bone, dentin is acellular. Dentin presents a water content of 10%, all of which is located in tubules. Undoubtedly it decreases with age as peritubular and intratubular dentin occupies the tubules and squeezes the water content out. Given the hydrodynamic theory of pain in dentin, this explains why (in combination with other multi-factorial reasons) the perception of pain decreases significantly among the elderly. When dentin particulate is used as a graft material, its behavior is based on the principle of ankylosis; the process is uncompromised due to the water content in its organic matrix, as the hydrogen bridges remain almost unchanged throughout its entire life and beyond. This explains why teeth are often used as a reliable source of information in forensic investigation. The presence of free amino acids in the dentin treated by the method described above was checked, finding very few, although the most important RDG peptides (arginine, glycine, aspartate) remained undamaged. Of course, this was after the grinding and ethanol cleaning processes described. The main reason for this is that the mineral is extremely dense and covers the matrix. Moreover, the dentin was not heated or treated with (strong) acids allowing the host osteoclasts to do their work. In this sense, the present method worked perfectly regardless of the considerable loss of free water content.

The safety of autogenous tooth bone graft material has already been established. It is a useful material that can act as a substi-
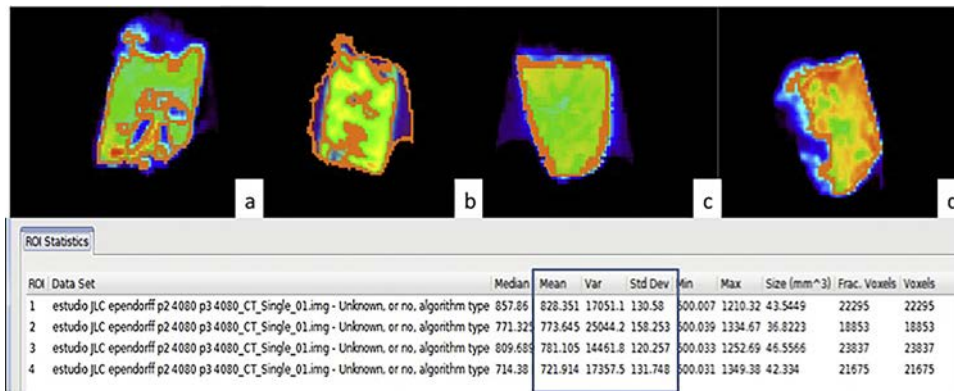
Fig. 9. (a) MicroCT image of dentin grafted site at 90 days with Hounsfield units of 828 ± 13, (b) MicroCT image of control site at 90 days with Hounsfield units of 7736 ± 15, (c) MicroCT image of dentin grafted site at 60 days with Hounsfield units of 781 ± 1, (d) MicroCT image of dentin grafted site at 60 days with Hounsfield units of 721 ± 9.
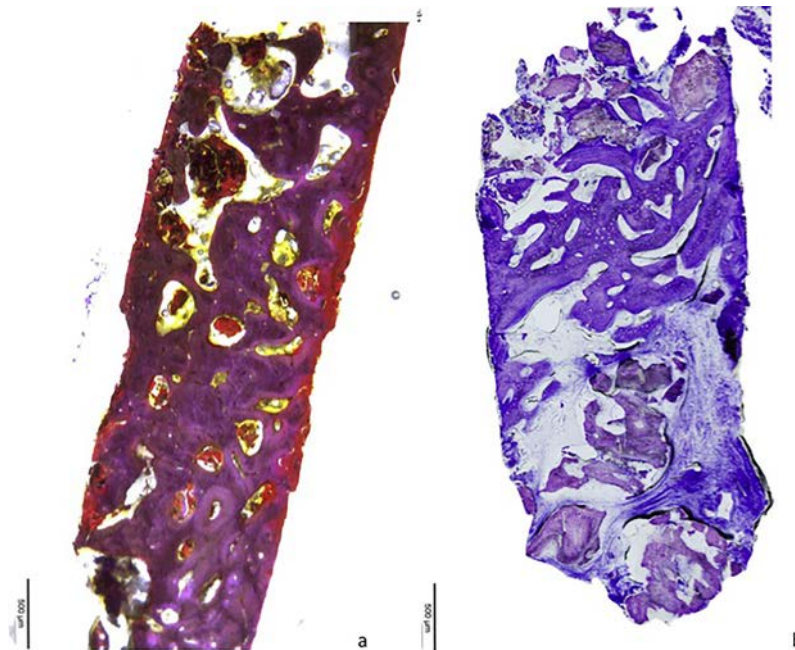

Fig. 10. (a) Control group core showed large quantities of newly formed bone covering the bone defect but this was seen to be completely immature (woven bone), (b) dentin graft core showed newly formed bone included areas of irregular arrangement with high levels of cellularity ($\times 10$ magnification).

tute for free autogenous bone graft, showing good bone healing properties thanks to excellent osteoinduction and osteoconduction derived from both its organic and inorganic components. When the amount of available bone graft material is insufficient, other bone graft materials can be introduced to make up the shortfall. A great deal of research is underway into osteoinductive and osteoconductive bone graft materials using homogenous and xenogenous teeth, to develop ideal scaffolds to convey stem cells and growth factors, in endodontic treatment and as tooth restoration materials (Kim, 2012).

The water content of dentin present in microscopic pores, which increase in number and size with age, also exist within the extracellular matrix of bone tissue. The distribution of water in bone appears to change throughout life. It has been reported that water in bone tissues decreases with skeletal growth (Jonsson et al., 1985) and with progressive mineralization. It can be presumed that the distribution of water within the tissue of bone – the amount of water bound to collagen, to mineral, and the mobile water in the vascular and lacunar-canalicular cavities – may dictate the bone's mechanical behavior.

Autogenous teeth have been routinely transplanted into extraction sockets for over 40 years. It is evident that transplanted teeth that are ankylosed in the jaw bone undergo replacement resorption by bone over 5–8 years (Sperling et al., 1986). It is also well documented that avulsed teeth that are implanted back into their sockets undergo firm reattachment by bone which is formed directly onto root dentin or cementum, leading to ankylosis (Andersson et al., 1989). However, the capacity of dental material to generate bone has not been investigated further. Without doubt, autogenous bone is the ideal graft material for reconstructing hard tissue defects. It has bone forming, osteoconduction, osteoinduction, and osseointegration capacities, and does not trigger foreign body reactions; it also undergoes rapid healing (Urist, 1965; Urist et al., 1973, 1982).

An ankylosed root is continuously resorbed and replaced by bone, so that eventually the entire root is resorbed; the alveolar process is preserved during this resorption period and beyond. In a recent review, Malmgren (2013) affirmed that ankylosed teeth that are treated by decoronation help to maintain the alveolar ridge
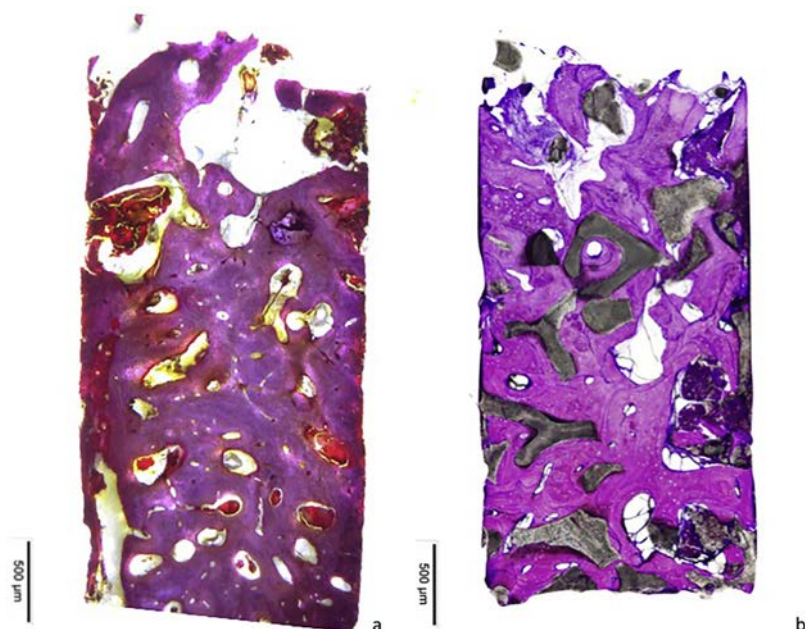
Fig. 11. (a) Control group core showed increased newly formed bone organization showed disorganized immature bone (woven bone), (b) dentin graft core showed predominance of mature newly formed bone (lamellar bone) well organized as osteons (×10 magnification).

in buccal-palatine direction, while vertical height often increases (Malmgren, 2013; Park et al., 2007)

Given the wide range of bone graft materials available, choosing the right one can be challenging. The choice of graft material should be dictated by the extent of the defect to be filled and the procedure's specific purpose. When tooth extraction is necessary, tooth-derived bone grafts can be considered a viable option thanks to their autogenous origin and favorable clinical and histological outcomes (Kim et al., 2013). Although demineralized dentin exposes matrix-derived growth and differentiation factors that produce effective osteogenesis, the newly formed bone and residual demineralized dentin are too weak to support implant anchorage. But the Smart Dentin Grinder procedure assayed in the present work makes it possible to prepare bacteria-free, mineralized dentin particulate from freshly extracted autologous teeth, which can be used immediately as an autogenous graft material in the same session.

Mineralized dentin particles have the advantage of maintaining mechanical stability, allowing early loading after grafting in fresh sockets and bone defects (Kim, 2012). Moreover, although inductive properties are somewhat delayed, the mineralized dentin is firmly integrated into newly formed bone, creating a solid site for anchoring implants. In fact, the clinical data obtained in the present study suggests that implant insertion and loading could be performed in both mandible and upper maxilla within 2–3 months after grafting with dentin particulate (Yeomans and Urist, 1967). The present results show that dentin grafts acted as an excellent scaffold for new bone formation, remodeling much more slowly than cortical bone or most of the available biomaterials, in terms of long-term alveolar crest and mucoperiosteum structure and esthetics (Andersson, 2010; Binderman et al., 2014).

Research into bone graft materials for replacing autogenous bone has been extensive. In particular, Kim et al. (2014) developed a bone graft material using extracted auto-teeth aimed at overcoming the disadvantages of allografts, xenografts, and synthetic materials (Kim et al., 2014). The authors claimed that auto-tooth bone graft was an innovative material, and very useful in clinical situations, with all the advantages of autogenous bone thanks to the similarity of its components to bone. It also overcomes

the distaste expressed by patients when an allograft or xenograft is proposed. It offers excellent biocompatibility without causing an immune response, foreign material reaction, or cross contamination. In addition, it has osteoinduction, osteoconduction, and creeping substitution capacities (replacement resorption or remodeling) and can be manufactured in various shapes and sizes (Kim et al., 2001a,b,c, 2002, 2004a,b). Teeth can be used even when the root remains in the alveolar bone. In fact, some surgical protocols deliberately leave the tooth root intact in order to preserve the alveolar bone (Sperling et al., 1986; Andersson et al., 1989).

Kim et al. reported that 90% of a tooth's organic component is type I collagen, which is very important in bone calcification (Urist, 1965; Urist et al., 1973, 1982; Malmgren, 2013; Park et al., 2007; Kim et al., 2014). Teeth and jawbone show great affinity, sharing a similar chemical structure and composition. Therefore, taking immediate advantage of extracted non-functional teeth or periodontally involved teeth instead of discarding them is a valid proposal and would avoid any delay-related immune response to dentin graft material (Kim, 2012; Kim et al., 2017). Kim experimented with special devices for treating dental material to produce either blocks or particulate that were then successfully assayed in clinical and animal studies. In the present dog study, woven bone formation was observed at 60 days and a small amount of lamellar bone was observed at 90 days. According to these findings, autogenous dentin particulate would appear to be a viable treatment as a bone repair material especially when autogenous bone is not available for socket preservation.

5. Conclusions

Autogenous dentin particulate grafted immediately after extractions may be considered a useful biomaterial for socket preservation, protecting both buccal and lingual plates, generating large amounts of new woven bone formation after 60 days, and small amounts of lamellar bone after 90 days healing.

The present results suggest that autogenous mineralized dentin particulate can be considered an alternative graft material for bundle bone preservation in socket preservation procedures, bone augmentation in the sinus, or for repairing bone defects.

Acknowledgements

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