Use of dentin as a biomaterial for bone augmentation

The objective of this experimental study was to evaluate the efficacy of new bone formation using particulate teeth, grafted immediately in critical defects of 6 mm compared to sites without, 60-day follow up in New Zealand Rabbits, the results of which show that Autogenous crushed tooth particles should be considered as a new suitable biomaterial for the filling of critical bone defects.

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Introduction

Bone defects appear due to trauma, atrophy or resection of intraosseous lesions.

Bone regeneration using grafts Autologous bone or biomaterials has evolved enormously in the last decade. Various graft materials including autografts, allografts, xenografts and alloplasts, have been used for bone augmentation, each with its benefits and challenges. Synthetic bone, as an example, does not carry the risk of disease transmission but it lacks the capacity to promote osteogenesis and osteoinduction. Still, it is a great scaffolding for the new bone formation. The healing of large bone defects is directly related to size and time elapsed since the trauma. When it elapses longer, the greater the healing and therefore the maturation of bone tissue (1). Osteogenesis is the process whereby new

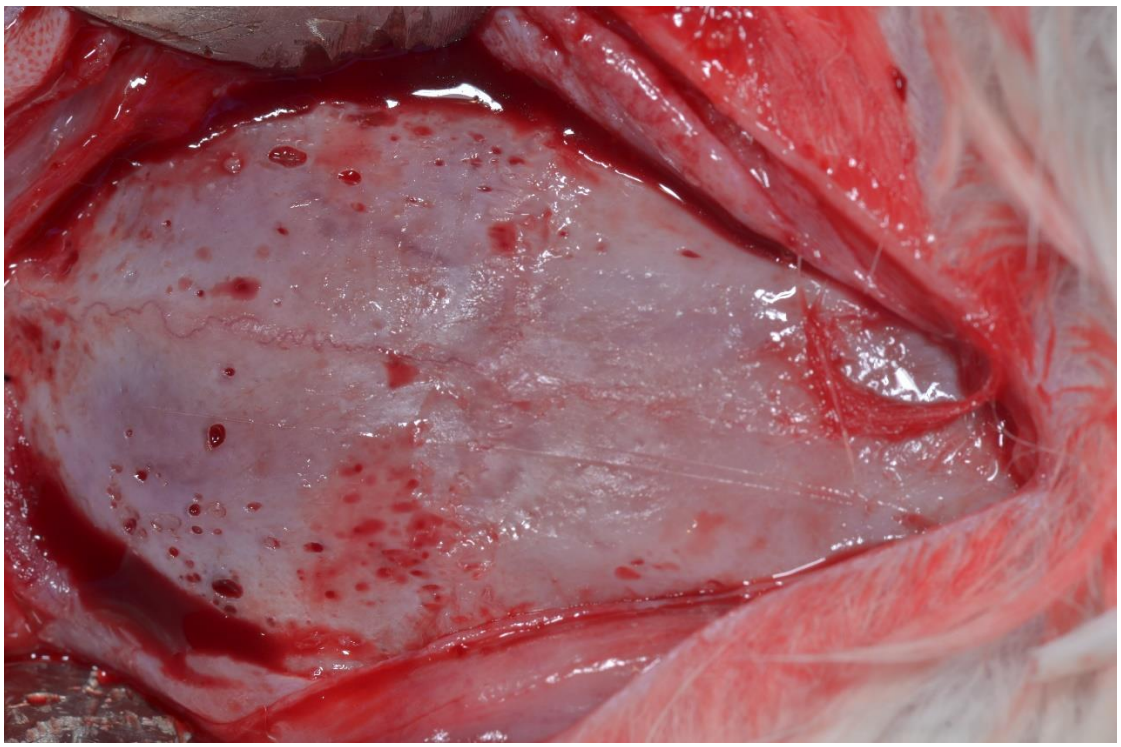

Figure 1. Rabbit calvaria exposed.

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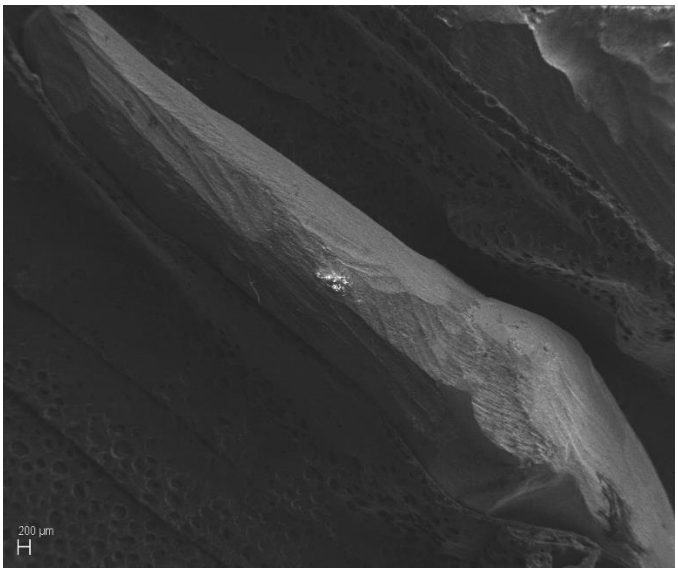


Figure 2. Image of electron microscopy (SEM) of the tooth to be crushed.

bone is formed from osteoprogenitor cells; Osteoinduction is the stimulation and activation of osteoprogenitor cells of the surrounding tissue of the lesion; and osteoconduction is the process which facilitates the development of the blood vessels (2). Autologous grafts also have disadvantages, such as the amount of graft that can be harvested, the morbidity of the donor site, the duration of the surgical procedure, as well as the postoperative discomfort and pain (3). Autograft materials, allograft, xenograft and synthetics have been used as bone substitutes for a long time with great success (4-6).

The human dentin matrix, created from extracted teeth, was introduced in 2008 in Korea and has been evaluated for its osteoinductive and osteoconductive capacity in filling of bonny defects. Dentin and bone are very similar in composition of collagen (30%), hydroxyapatite (60%) and body fluid (10%) by weight (7-8). Dentin is an acellular matrix rich in collagen without blood vessels, while the bone is a cellular tissue, highly vascularized. More specifically enamel has 96% inorganic substances and 4% water, while dentin has 65% inorganic substance, 35% organic substance and water. The alveolar bone has 65% inorganic substances and 35% organic substance (9).

Generally, extracted teeth have been discarded for being considered infectious materials. Today, we can give extracted, non-functional teeth a second chance and use them as a native resource suitable to be grafted in disadvantaged areas of bone. Several authors have shown that the properties of the crushed tooth, could act as a bone substitute induced by dentin and pulp dentin, and studied autologous recyced human teeth as a new graft material for bone regeneration in Japan and Korea (10-13).

The Smart Dentin Grinder was introduced to efficiently grind extracted teeth and sort them into particles of dentin of specific size that range between 300 to 1200 microns. Newly formed bone using this technique has been 75% in experimental animals (14-15). The objective of this study was histological evaluation and histomorphometry of vital bone formation (VB) after the augmentation using crushed tooth graft compared to unfilled areas in rabbits at 60 days of follow-up.

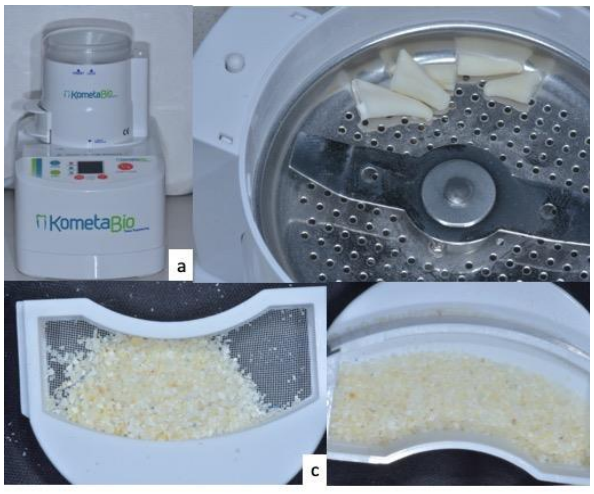


Figure 3.
a) Machine Smart Dentin Grinder; b) Roots of teeth a Crush; c) Compartment top with filter for particles from 600 to 1200 microns; d) Compartment lower for particles of 300 microns.

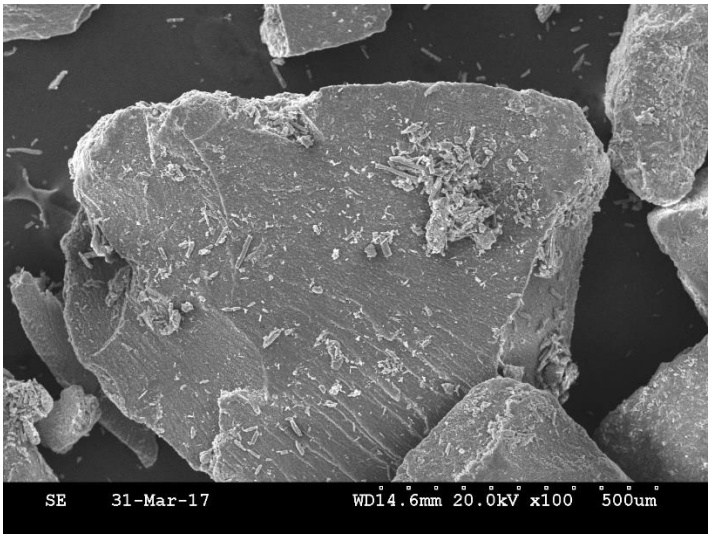


Figure 4. Image of electron microscopy (SEM) of the tooth particle of 300 microns, with collagen on the upper structure.

Materials and methods

Animals

In the study, 21 New Zealand rabbits were used, each with a weight of 3.2 to 4 Kg (average 3.5 Kg). The study protocol was approved by the Ethics Committee of the University of Murcia, Spain (05-09-2012), which followed the established guidelines by the Directive of the Council of the European Union (53/2013, February 1, 2013) for the care and the experimentation of animals. The animals were fed a daily diet of granules ad libitum during the whole study period. The animals received an administered intramuscular injection of 0.5 to 1 mg / kg of acepromazine maleate. Fifteen minutes later, general anesthesia 5 to 8 mg / Kg of ketamine plus chlorbutol with 0.05 mg / Kg of atropine as adjuvant was administered intravenously. The rabbit calvaria was shaved and washed with Sea4 Gums (sea water with hyaluronic acid). The medial sections of the skull were exposed through by an incision in the skin and a careful subperiosteal dissection (Figure 1). Two defects were created of 6 mm in diameter (16). The surgical area was irrigated with sterile physiological saline to eliminate the skeletal remains.

Dental biomaterial

The mandibular premolars and the first molars (P2, P3, P4, M1) of 6 Beagle dogs were removed bilaterally under general anesthesia one week before (Figure 2). The teeth with multiple roots were sectioned in a buccolingual direction at the bifurcation using a carbide tungsten bur so that the roots could be extracted individually, without damaging the socket walls. The clean and dry teeth were immediately crushed using the Smart Dentin Grinder, that is especially designed for

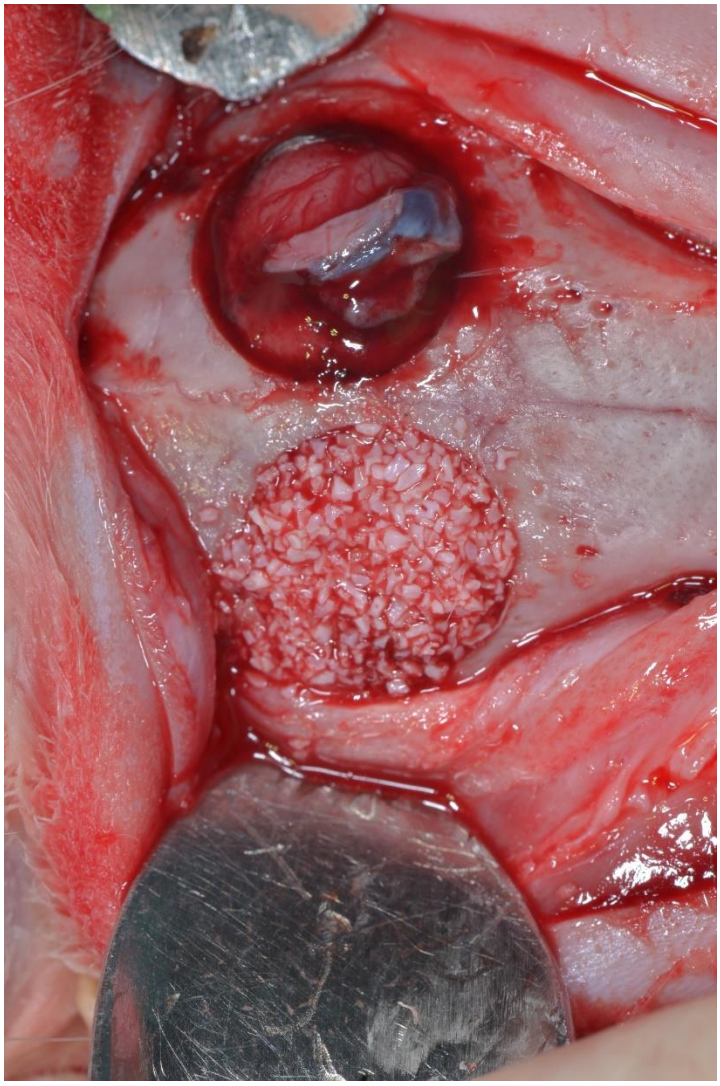


Figure 5. Exposed defect with graft of particulate tooth and control area without filling.

this procedure. The teeth particles that were obtained were 300-600 to 1200 um, which are subsequently sifted through multiple sieves and into two separate compartments as part of the system (Figures 3-4). The particulate teeth were immersed in a Dentin Cleanser of alkali and alcohol solution in a sterile container for 10 minutes in order to dissolve all organic waste and bacteria. Next, the particles were washed with EDTA for 2 minutes to partially decalcify the particles, and finally washed with sterile saline for 3 minutes. Then used to graft the critical defects chosen randomly. At 60 days the animals were sacrificed.

A defect was filled with crushed tooth granules (Group A). The second defect was not filled using it as a control and the defects were covered with a collagen membrane (Group B), (Figure 5). Later, the samples were assigned to the groups test using a scrambling software (www.randomization.com).

Analgesia was administered by injection of Novalgin (50 mg / Kg body weight) and was administered Amoxicillin (0.1 ml / Kg intramuscularly) at the end of surgery. The animals were kept in a room especially designed for experimental animals and they were fed a standard laboratory diet.

Statistic analysis

The values were recorded as mean-standard deviation. For the comparison of the means, a non-parametric Wilcoxon test for samples related was applied, assuming a level of significance 95% ($p < 0.05$). If the distribution of two variables paired in two related samples is the same, then this test takes into account the magnitude of the differences between two paired variables. They were considered as a null hypothesis equal means, while the existence of significant differences between the media acted as an alternative hypothesis.

As significant differences between existing means, the null hypothesis was rejected. All data were expressed as average averages and standard deviation. The t-test was used to analyze the differences between the variables. The statistical analysis was performed using SPSS 15.0 software (SPSS, Chicago, IL, USA). The significance level was established as $p < 0.05$.

Results

Histomorphometry Analysis

The histomorphometry found a total of newly formed bone of 47 ± 4.6% in defects treated with Dentin graft, with significant differences between the control samples (3 ± 1.3%). (Table I). The findings revealed significant differences between the filling material compared to the group of control (Figure 6). There were also significant differences between this period of study and the results obtained at 60 days.

Radiovisiography

In image 7 we can observe a condensation of more homogeneous and stable bone particles that are in the bone with Dentin grafted site at 60 days of its placement (Figure 7)

60 days / Optical microscopy

For groups treated with particulate tooth, the images were characterized by a predominance of mature regenerated bone which is well organized by osteons, although there were still areas of bone disorganized with high cellularity, although in a small proportion of total bone tissue (Figure 8). The control group showed greater organization of connective tissue and a lot of disorganized immature bone (Figure 9).

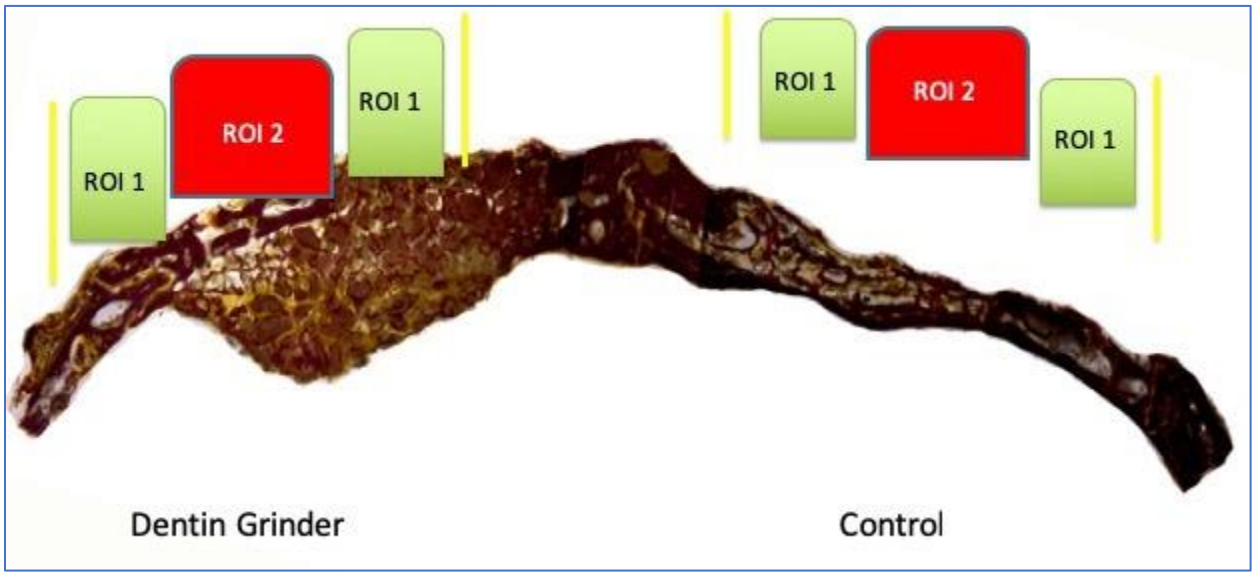


Figure 6. ROI areas of Dentin Grinder and control for histomorphometry

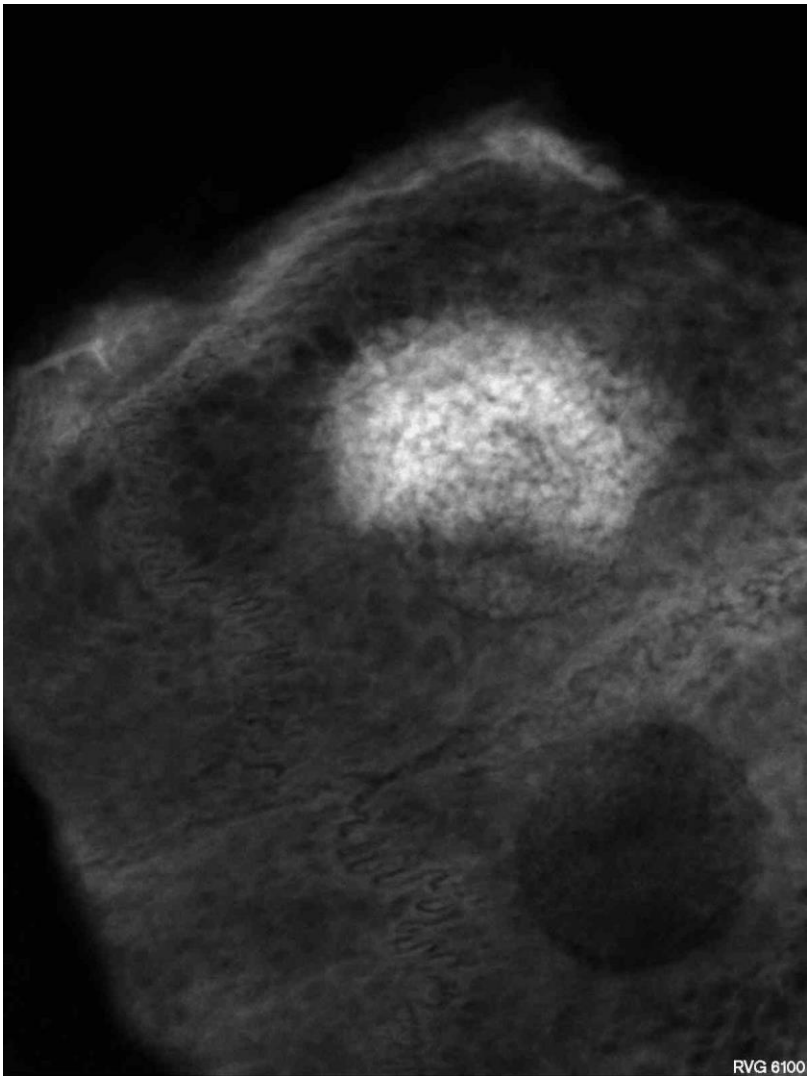
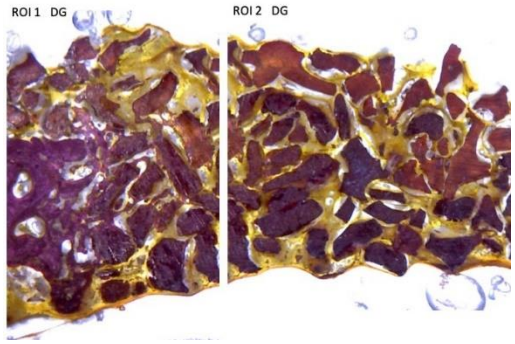


Figure 7. Radiovisiography from defects at 60 days progress.

Figure 10 shows the greater formation of bone and great maintenance of the bony walls of the defect in the right lateral zone (Figure 10)



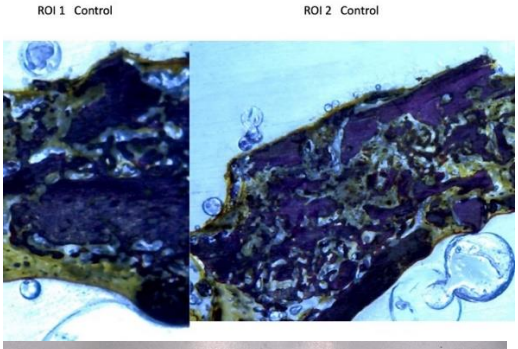


Figure 8. Dentin Grinder biopsy showing a bone new, with mature osteons and new bone around the particles to 90 days. Picrosirius-hematoxylin stain x20.

Figure 9. Biopsy of the control area where one observes a large amount of newly formed bone, immature, disorganized at 60 days of evolution.

Figure 10. Biopsy of the control area where it is observed little bone formation and in the Dentin Grinder area with greater bone formation.

Discussion

We first learn about the ability of teeth to generate bone from the Urist's study in which he examined bone generation after applying the demineralized tooth in different parts of bone. Since then, multiple studies have shown the ability of the tooth to generate bone that is ideal for reconstruction of hard tissue defects. Studies show that dentin has guiding capacity to bone, demonstrates osteoconduction, osteoinduction and osseointegration, and does not trigger foreign body reaction therefore assuring quick healing (17-20).

Our results reveal a similar interaction between mineralized dentin and osteogenic cells that bind and produce the mineralized bone matrix directly on the scaffold consisting of the particulate tooth (14-15).

The bone graft material derived from the tooth has characteristics that does not present antigenicity, improves the bone remodeling abilities stimulating osteoinduction.

Among a variety of available bone graft materials, the choice of graft material should be

dictated by the extension of defects and procedural purposes, the bone graft derived from teeth can be considered as an option, given its autogenous origin and favorable clinical and histological results when the extraction of the teeth is necessary.

Summary

Objectives

The objective of this study was to evaluate the effectiveness and quality of new bone formation using particulate teeth as graft, immediately grafted on 6 mm critical defects compared to the 60-day unfilled sites in New Zealand rabbits.

Methods

Twenty-nine New Zealand rabbits were used. Two defects of 6 mm diameter were caused in the parietal area and filled with crushed tooth of six Beagle dogs. The extracted teeth were crushed using the Smart Dentin Grinder, specially designed for this procedure. The particles of teeth that were obtained were 300-600 and 1200 um. Crushed teeth were arbitrarily grafted into critical defects of 6 mm in diameter. This study evaluated tissue healing and bone formation by histological analysis and histomorphometry at 60 days.

Results

The bone formation around the crushed tooth was observed with greater bone formation in group A compared to the control group at 60 days ($p < 0.05$). The immature bone was lower in the group of Dentin Grinder compared to the control group. There were significant differences between bone formation at 60 days in group A compared to the control group for the concentration of collagen in the site.

Conclusions

The autogenous crushed tooth particles should be considered as a new biomaterial suitable for the filling of critical bone defects.

Demineralized dentin exposes the growth factors of the matrix and the differentiation factors for an effective osteogenesis, newly formed bone but the residual demineralized dentin are weak to support and anchor the implant. Conversely, the SDG procedure (Smart Dentin Grinder) allows the preparation of mineralized and partial demineralized particulate dentin free of bacteria from newly autologous extracted teeth, ready to be used immediately as autologous material in the same session within a few minutes.

Furthermore, despite the inductive properties, the mineralized dentin integrates with newly formed bone, creating a solid site for anchoring of dental implants. In fact, there are authors that describe clinical studies that indicate that Insertion and loading of the implant can be done in lower and upper jaws 2-3 months in a mesh of crushed teeth (20-21).

Conclusions

We consider that autogenous dentin can be considered as a standard for preservation of the alveolar bone and filling of critical defects of 6 mm. Currently, the crushed tooth can be used as a bone graft without losing the capacity for regenerating bone, maintaining space and with minor resorption compared to unfilled defects.

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