

The most suitable system to grind the whole tooth to use it as graft material

Elio Minetti^{1†} , Gianna Dipalma^{2†*} , Andrea Palermo³ , Alessio Danilo Inchingolo² , Fabio Viapiano² , Angelo Michele Inchingolo² , Francesco Inchingolo^{2*} 

¹Department of Biomedical, Surgical and Dental Science, University of Milan, 20122 Milan, Italy

²Department of Interdisciplinary Medicine, University of Bari Aldo Moro, 70124 Bari, Italy

³College of Medicine and Dentistry, Ulster University, B4 6BN Birmingham, UK

[†]These authors share the first authorship.

***Correspondence:** Gianna Dipalma, giannadipalma@tiscali.it; Francesco Inchingolo, francesco.inchingolo@uniba.it.

Department of Interdisciplinary Medicine, University of Bari Aldo Moro, 70124 Bari, Italy

Academic Editor: Gaetano Isola, University of Catania, Italy

Received: October 23, 2023 **Accepted:** November 22, 2023 **Published:** January 12, 2024

Cite this article: Minetti E, Dipalma G, Palermo A, Inchingolo AD, Viapiano F, Inchingolo AM, et al. The most suitable system to grind the whole tooth to use it as graft material. *Explor Med.* 2024;5:1–16. <https://doi.org/10.37349/emed.2024.00202>

Abstract

Aim: In regenerative dentistry, the success is influenced by the graft material, which should act as an osteoconductive scaffold. It provides a mineral substrate during resorption and induces the activity of osteoinductive cells capable of producing new bone, platelet growth factors, and cell differentiation factors that guide the differentiation of undifferentiated mesenchymal cells. Given that dentin shares many biochemical characteristics with bone tissue, it has recently attracted considerable interest as a biomaterial for bone repair. The aim of this study is to compare two grinder types to determine the optimal method for producing dentinal particles using a mechanical grinder.

Methods: A sample of 40 natural human teeth without restorations, prostheses, or root canal treatments was used and divided into two groups subjected to two different grinder speeds (high-speed and low-speed).

Results: The high-speed showed a greater dispersion ($53.5\% \pm 9.89\%$ of the tooth) due to the pulverisation (highly thin granules) of part of the tooth. The low-speed grinder did not pulverize the dentin and the percentage of tooth loss is $9.16\% \pm 2.34\%$.

Conclusions: The low-speed grinder allows to save a major part of the tooth and has a maximum quantity of graft material but requires more time. Further studies must be promoted to optimise the grinding procedures.

Keywords

Autogenous dentin particulate, bone regeneration, dental biomaterials, growth factors, high-speed grinder, low-speed grinder, tooth graft, tooth transformer



Introduction

In recent decades, the field of regenerative medicine has experienced significant growth and advancement, with a notable contribution from biotechnology. Within this context, researchers have shown a keen interest in the regeneration of bone tissue, a topic particularly relevant in dentistry [1–6]. Studies have emphasized the importance of considering the size of bone defects when aiming for effective regeneration. Various types of bone defects have emerged, presenting significant challenges in clinical practice and highlighting the pressing need for materials that can facilitate bone repair [3, 7, 8].

The development of bone regeneration techniques involved both autologous grafts and xenografts, with the aim of increasing the bone tissue for implant purposes [9]. In the context of bone grafting, it is crucial for the graft material to be decontaminated and to serve as an osteoconductive scaffold. This scaffold should provide a mineral substrate during resorption, stimulate the activity of osteoinductive cells capable of generating new bone, and release growth factors such as bone morphogenetic proteins (BMPs). BMPs play a pivotal role in transforming undifferentiated mesenchymal cells into osteogenic cells (osteoblasts) [10–14].

Recently, dentin has garnered substantial attention as a potential biomaterial for bone repair. Its biochemical composition has been likened to that of bone tissue, with both consisting of approximately 61% inorganic material (hydroxyapatite crystals) and 39% biological material. The organic component, primarily composed of collagen, imparts strength and flexibility to the structure, enhancing its resistance to fractures [15]. Non-collagen proteins, representing around 10% of the total composition, include various proteins such as osteopontin (OPN), dentin sialoprotein (DSP), dentin glycoprotein (DGP), bone sialoprotein, osteocalcin, and more. Notably, these proteins are shared between dentin and bone [16–21].

The concept of tooth grafting was introduced over 50 years ago by Yeomans and Urist [22, 23], who discovered the osteoinductive potential of demineralized dentin matrix (DDM). Schmidt-Schultz et al. [24] conducted pioneering research on the use of teeth as a biomaterial for grafting, isolating growth factors like insulin-like growth factor-II (IGF-II), BMP-2, and transforming growth factor (TGF) from teeth dating back thousands of years [25, 26]. More recently, Bessho et al. [27] demonstrated the presence of BMPs in the human dentin matrix, with thirteen different BMPs identified [28]. BMP-2, in particular, plays a pivotal role in promoting mesenchymal cell differentiation into osteoblasts [29, 30]. BMP-3 and BMP-7 also contribute to bone growth and osteoblast differentiation [31].

The process by which demineralized dentin stimulates bone regeneration closely mimics the mechanism of autologous bone factors [32–34]. This similarity is observed in both demineralized bone matrix (DBM) and DDM, both of which contain type I collagen and growth factors, with a particular emphasis on BMP-2 [35–37].

Key growth factors for bone regeneration are found in both bone and dentin matrices and serve as a rich source of BMPs and bioactive growth factors like TGF- β , which play vital roles in the bone repair process [38–40]. In dentin, according to Bono et al. tests [41], there are 200 pg/g of BMP-2 after treatment with acids. In bone, according to tests conducted by Wildemann et al. [42] using different extraction methods, the range is between 400 and 3,778 μ g/g of BMP-2. The extraction method determines the quantity of BMP-2 present, and in the literature, tests have been carried out using non-comparable systems. However, the crucial aspect is the presence of BMP-2 and its usability. The demineralization process, which removes mineral content, has been shown to provide superior bone augmentation compared to non-demineralized dentin [43, 44]. Furthermore, the chemical composition of bone and dentin is nearly identical, featuring an inorganic component of hydroxyapatite and an organic component primarily composed of type I collagen and other secondary proteins [45]. For over 35 years, heterologous or alloplastic grafting materials have been used in bone augmentation treatments, serving primarily as mechanical scaffolds for host cells without offering osteoinductive stimuli [12, 46–49]. Recent studies have confirmed the effectiveness and safety of on-site preparation of autogenous partially DDM for clinical use in implant dentistry procedures such as socket preservation, alveolar ridge augmentation, and maxillary sinus floor augmentation [4, 39, 50, 51].

Various methods of tooth demineralization have been investigated, each yielding varying outcomes in bone tissue development. When comparing different tooth crushing systems, factors such as the degree of sterilization, system repeatability, liquid types and concentrations, degree of demineralization, granule size, residual protein content, wettability, plasticity of granules, and system ergonomics must all be taken into account [44, 52].

A recent prospective study has validated the use of teeth as grafting materials in socket preservation procedures [53]. Bianchi et al. [54] emphasized the importance of understanding how human cells react to different dentinal derivative grafting materials in various clinical scenarios. Their study evaluated human periodontal ligament fibroblasts (hPLF) exposed to different dentinal derivative particles, including mineralized dentine, deproteinized and demineralized dentine, and demineralized dentine, along with deproteinized bovine bone as a control material [55, 56]. Observations revealed the expression of proliferative markers and cytoskeletal elements involved in the adhesion process [57]. Notably, signals for vinculin and integrin were particularly strong in the sample exposed to the demineralized dentine material, confirming the biocompatibility and high conductivity and inductivity properties of dentinal derivatives, which are crucial for the regenerative process [54, 58].

Clinical and histological assessments have shown the absence of inflammation or adverse reactions following the use of teeth treated as grafting materials. Clinical tests have demonstrated seamless dentin integration during the regeneration process [59]. However, further research is needed to delve into the intricacies of the regeneration stage. Histomorphometric analysis of tooth derivative materials used as bone substitutes in socket preservation procedures indicated an average of 38% new bone formation and only 7% graft residue after just 5 months [60–63].

Grinding serves as the initial step in preparing teeth for use as graft materials, and it is imperative to develop a protocol that enables the consistent production of particles while preserving osteoinductive capabilities [64]. In the surgical application of teeth, meticulous care must be exercised to prevent the dispersion of this irreplaceable sample (the extracted tooth) during grinding processes [65, 66]. Disadvantages of improper grinding techniques include the creation of non-uniformly sized particles, often resulting in material loss, xenograft material, even though it has irregular particles, still falls within a range of sizes. In this case, within the same range, there remains little material. Hence, achieving uniform particle size, as close as possible to optimal weights, is essential. There are two primary types of systems for pulverizing solid materials: high-speed mills and low-speed grinders [26, 67].

To the best of our knowledge, this study represents the first in the literature to comprehensively analyze the two grinder types and establish a procedure for producing dentinal particulates through mechanical grinding.

The aim of this study is to evaluate which grinding system is more effective in terms of reduced substance loss and homogeneity (similar particle sizes) of the produced particulate matter.

Materials and methods

In the effort of trying to understand which was the system that guaranteed the best performance, two different grinding systems (Figure 1) were evaluated, one at high-speed (testing two different grinders), similar to a coffee grinder, and one at low-speed, with concentric conical blades comparing the amount of material produced. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethical Committee for Biomedical Research of Chieti and Pescara (Protocol Number 1869/21.03.2019).

A literature review was conducted using the following keywords: “high-speed grinder” or “low-speed grinder” and “tooth.” The results from the last 10 years are as follows:

PubMed: 0 results, Web of Science (WOS): 50 results, Scopus: 14 results. All the articles found are not relevant to dentistry.

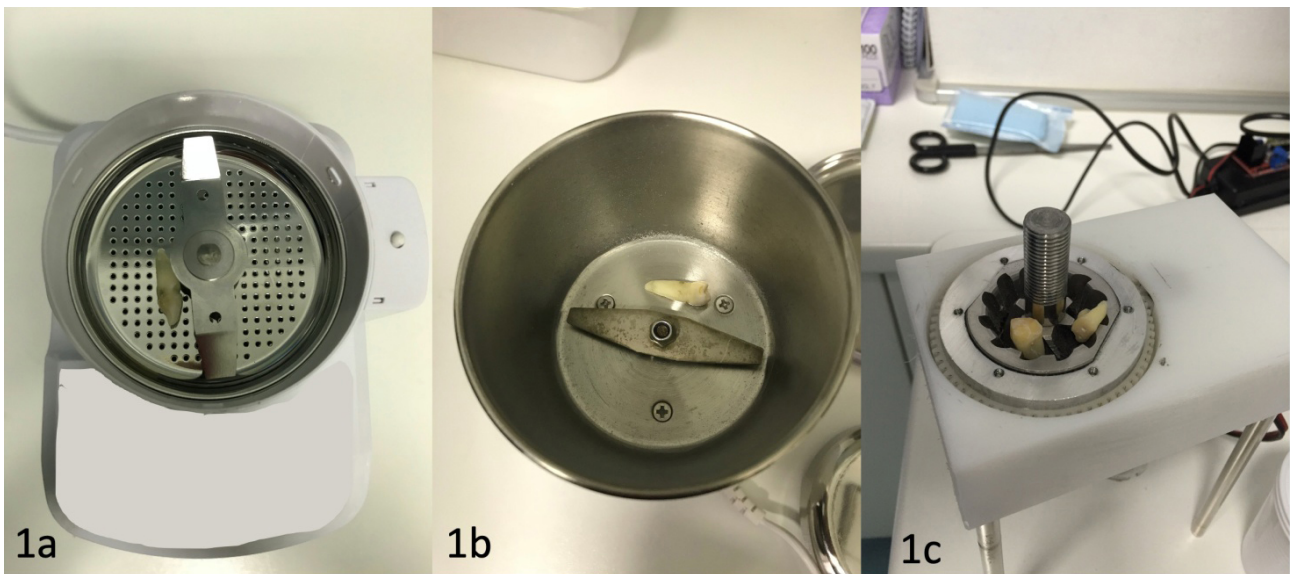


Figure 1. The high-speed blades are composed of a rotating hammer moving at high-speed along its axis, all contained within a metal cylinder with a lid that contains the fragments propelled into the surrounding space by the impacts with the high-speed hammer. The low-speed blades consist of two concentric conical blades that drag the material to be fragmented downwards. The granule sizes are determined by the distance between the two blades, and the resulting granules fall downward due to gravity. (a) and (b) two high-speed grinders; (c) the low high-speed grinder

The reasons for this could be attributed to the development of a new research field in regenerative dentistry that has gained momentum in recent years, focusing on the use of teeth as graft materials.

The study was conducted by dividing the subjects into two groups. Group 1 used the high-speed grinder, while group 2 used the low-speed grinder. Each group was provided with 20 natural teeth (without restorations, prosthetics, or root canal treatments) to be reduced into particulate matter.

The difference between high-speed and low-speed is in the revolutions per minute (RPM). Basically, it is how many times in a minute the burrs rotate. The speed is the measurement of one complete revolution of the blades. Most high-speed grinders have a hammermill that spins anywhere between 1,100 RPM to 1,200 RPM. These machines are typically assisted with some sort of feeding system. On the other hand, low-speed shredders spin at 30 RPM to 40 RPM and are often built without an assisted feeding mechanism. Concentric blades do not require a sieve due to their design. Low-speed concentric blades fragment the tooth, pushing the residues downward. The granule sizes are determined by the distance between the blades.

Low-speed: These are at a lower RPM for two reasons. They usually have smaller burrs, and most are conical shaped. The lower RPM grinders usually have less heat buildup. Low-speed shredder blades are conical concentrics with sharp edges. They have an accumulation zone of the material to be ground called the upper chamber and a lower one. The distance between the blades in the lower chamber determines the size of the granulate.

High-speed: These usually have flat burr sets, and beefier motors. The flat burrs provide a more consistent grind and the higher RPM grind the teeth faster. High-speed grinders are direct drive, which means fewer gears to wear or break, and are quieter. The blades of the high-speed shredder are shaped like a specular hammer, rotating on a central pin placed inside a sealed drum. The tooth is placed inside the drum and only after rapid rotation of the hammer, the granulate is produced that then must be filtered to the right size of the granules [68, 69].

A tooth was triturated, and then analyzed under a microscope (ESEM Zeiss EVO50, Carl Zeiss, Milan, Italy) linked to a secondary electron detector for energy dispersive X-ray spectroscopy (EDS) analysis was used to analyze the surface morphology of sample particles to reveal the shape of the produced granules (Figure 2), filtered through calibrated sieves (Figure 3). A sieve analysis (or gradation test) is a practice or procedure used to assess the particle size distribution (also known as gradation) of a granular material by

passing it through a series of sieves with progressively smaller mesh sizes and weighing the amount of material caught by each sieve as a fraction of the total mass [70, 71].

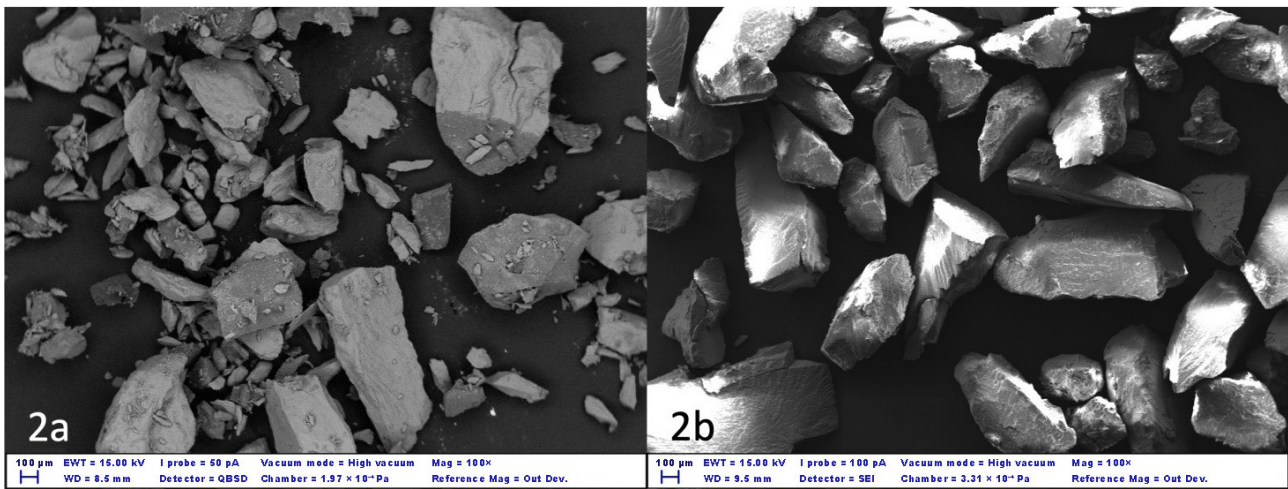


Figure 2. Morphology and particle size of teeth subjected to different grinding treatments. (a) Granules are produced with the high-speed grinder. Different sized granules can be seen, many of which are very small; (b) granules are produced with a low-speed grinder, similar in size and similar in shape. EWT: energy-weighted transmission; WD: working distance; I Probe: current probe; QBSD: quantitative backscatter detector; SEI: secondary electron imaging; Mag: magnification; Out Dev.: output device



Figure 3. Calibrated sieve able to differentiate the granule sizes

The procedure to sieve is: the vertical throwing action is combined with a small circular motion, resulting in sample dispersion throughout the whole sieve surface. The particles are vertically propelled (thrown upwards). When they fall back, they do free rotations in the air and interact with the holes in the sieve mesh. Particles that are smaller than the openings pass past the sieve. They are tossed if they are bigger. The spinning motion while hung increases the likelihood that the particles will have a different orientation to the mesh when they fall back, and so may pass through the mesh.

Finally, granules with a size between 0.5 mm and 1 mm were weighed.

Tests were carried out to understand the percentage dispersion in terms of the dry weight of a ground tooth and then filtered through a sieve (Figure 3), to keep only the granules with a size between 0.5 mm and 1 mm. The granules were produced using a grinder (low- and high-speed) and then filtered by size using metal sieves (Figure 3, Filtra, Seneco S.r.l., Milan, Italy) and divided into three groups: i) group A: particles from 200 µm to 900 µm; ii) group B: particles < 200 µm; iii) group C: particles > 900 µm. Only granules from group A were used for the tests.

The two mesh sieves have dimensions of 850 μm for the upper and 425 μm for the lower sieve. The tooth was weighed using the Tanita super precision Mini weighing scale (TANITA, Arlington Heights, IL, USA), before grinding and after grinding.

A sample of 40 natural human teeth (Figure 4) was used without restorations, prostheses, or root canal treatments. In the case of vital teeth vascular and nervous tissues should not be removed, whereas residues of fillings or tartar need to be removed, as well as the periodontal tissue, since periodontal tissue is more challenging to eliminate during preparation.



Figure 4. The forty natural human teeth used

In the case of the low-speed grinder, the teeth were sectioned due to problems of insertion into the space between the blades and this resulted in a weight loss caused by the cuts.

However, it was decided not to weigh the teeth after sectioning because this represents one of the limitations of this system and it must be fully evaluated. The whole teeth were inserted into the high-speed grinder.

The granules filtered through a sieve were then inserted into a device (Mastersizer 3000, Malvern Panalytical Ltd, Malvern, UK) allowing the quantification of both dry and wet granules through laser diffraction, to understand if the granules' dimensions were in line with the requisite.

The laser diffraction test indicated that the average particle size varied between 406 μm and 815 μm with peaks up to 1,110 μm .

Results

The results of the two different grinding systems have been recorded in the following table (Table 1). The results were determined by calculating the average for the 20 teeth ground using each grinding system and showing the two types of grinding and the corresponding tooth weight before grinding and after grinding. The weight difference is shown in the percentage of tooth loss.

Table 1. Average tooth weight before and after test. Results of the two different grinding systems

Type of test	Average tooth weight before test \pm SD (g)	Average tooth weight after test \pm SD (g)	Lost tooth weight \pm SD (%)
High-speed	1.20 \pm 0.53	0.56 \pm 0.29	53.50 \pm 9.89
Low-speed	1.44 \pm 0.62	1.32 \pm 0.58	9.16 \pm 2.34

SD: standard deviation

Discussion

In recent years, several researchers have investigated the use of particles derived from autologous teeth as bone grafting material. In the study by Kim et al. [72], once the soft tissues, tartar, and foreign materials were removed, the tooth elements were divided and crushed, obtaining particles between 0.5 mm and 1.0 mm in size. The particles of the crushed teeth were immersed in distilled water and hydrogen oxide solution, dehydrated with ethyl alcohol solution, and degreased with ethyl ether solution. Then, after the freeze-drying procedure, they were sterilized with ethylene gas and packaged [72]. Jun et al. [73] also created the material in the form of a powder with particles of 0.5–1.0 mm, while, in another study, sample teeth were pulverized into powder, with each particle having a diameter of 0.4–0.8 mm [74, 75].

Murata et al. [76] described that the teeth were crashed in liquid nitrogen, washed in 1 mol/L sodium chloride, and demineralized in HCl solution at pH 2.0. The tooth particles were thoroughly rinsed in cold distilled water before being lyophilized into 0.4 mm and 0.8 mm particles [76]. Nampo et al. [77] obtained the graft material by removing the crown portions of the extracted teeth with scissors and trimming the root portions of the remaining teeth as close to 500 μ m as possible. The trimmed tooth was then mixed with a measured amount of β -tricalcium phosphate [77]. Finally, in the study by Kim et al. [78], the particle size ranged from 0.2 mm to 1.2 mm.

Various studies have investigated the effect of anorganic bovine bone matrix (ABBM) particle size on bone healing in order to define the perfect dimension of granules for the optimal use in regeneration surgery (Table 2). Histological and radiographic studies were carried out to understand if bone repairing could be influenced by the particle size [72–74, 76–86].

Table 2. Graft granules' dimensions reported by different studies. The table indicates what is the size of the granules commonly considered optimal in regeneration and then has a reference

Authors	Granules' dimensions (mm)
Kim et al., 2014 [72]	0.5–1.0
Kim et al., 2013 [74]	0.4–0.8
Murata et al., 2005 [76]	0.4–0.8
Nampo et al., 2010 [77]	0.5
Kim et al., 2010 [78]	0.2–1.2
Jun et al., 2014 [73]	0.5–1.0
Binderman et al., 2014 [85]	0.3–1.2
Dozza et al., 2017 [79]	0.5–1.0
Testori et al., 2013 [81]	1.0–2.0
Klüppel et al., 2013 [86]	0.2–0.4

In the study by Klüppel et al. [86], 18 male New Zealand rabbits were employed, and four cavities were drilled and filled with varying particle sizes of ABBM. The first cavity had small particles (under 450 μ m), the second cavity included medium particles (450 to 749 μ m), and the third cavity contained giant particles (750 to 1,000 μ m). Particulated autogenous bone was used to fill the fourth cavity (control group). The animals were suppressed for 15, 30, and 60 days following surgery. Before the decalcification process and histological assessment, radiographs of the cranial vault were taken. The authors concluded that ABBM particle size affects the bone healing process: Smaller particles resorb faster and induce more bone neoformation than bigger particles [86].

Shapoff et al. [83] studied freeze dried bone allograft (FDBA), tiny particles (100–300 µm) combined with bone marrow, and big particles (1,000–2,000 µm) mixed with bone marrow in six rhesus monkeys. The findings indicated that the tiny particles produced more bone. The authors indicate the possible superiority of the smaller graft for some reasons: The increase in surface area, the release of a large amount of calcium salts by hydrolytic enzymes, and the increase in the number of pores all encourage bone formation [83, 87].

Different results were reported in the study by Testori et al. [81]. The authors compared vital bone formation after maxillary sinus augmentation using two different particle sizes of ABBM, finding a statistically significant increase in vital bone formation in the larger particle grafts [81].

Kon et al. [88] employed twenty-four rabbit cranial bones to evaluate the augmentation process of two distinct autogenous bone graft particle sizes: large bone (1–2 mm) and small bone (150–400 µm) particles were used. Autogenous bone is thought to be the gold standard for bone augmentation in the clinical setting. Nevertheless, the capacity to enhance may vary depending on particle size. By 8 weeks, the small bone had shrunk to 51.3% and 51.0% of its initial volume and height, respectively, while the large bone had maintained its volume. Finally, they proposed to use big autologous bone particles [88].

In another work demineralized FDBA (DFDBA) was processed and crushed into two sizes: 250–500 µm and 850–1,000 µm. Ten individuals with intrabony defects were chosen at random and, for each defect, soft and hard tissue measurements were taken. The bone defect fill was computed as a depth reduction from a given position: For the tiny particle group, it was 1.32 mm, while for the big particle group, it was 1.66 mm. This difference did not result as being statistically significant [89].

The osteoconductive capacity of deproteinized bone particles of two distinct diameters (300–500 and 850–1,000 µm) in rabbits was compared in the study of Xu et al. [90]. The deproteinized bone was made from white rabbit limbs. The cortical bone was soaked in water for 10 h before being immersed in 1 mol/L HCl. Finally, the bone was sintered in an electric furnace for 3.5 h at 600°C and 3.5 h at 1,100°C. In a bone mill, the bone was crushed into two particle groups: big particles (850–1,000 µm) and small particles (300–500 µm). Finally, particles were used to perform the sinus lift. Small particle groups produced superior outcomes [90].

In sheep femoral condyles, larger particles of silicate-substituted calcium phosphate (diameters of 250–500 µm or 1,000–2,000 µm, respectively) tended to preserve the volume of early bone formation better than smaller particles (90–120 µm) [91, 92]. Larger particles tended to be retained in newly produced bone tissue, owing to the longer time required for dissolution or remodeling [84, 93]. After successful engraftment, an autogenous bone block graft demonstrated a lower bone resorption rate than the particulates [94].

Koga et al. [95] compared DDM, partial DDM, and mineralized dentinal matrix, using three different sizes of graft particles for each group. Defects in the sheep's cortical bone were realized by inserting scaffolds of different sizes (large, 1–2 mm; small, < 0.5 mm; medium, 0.5–1.0 mm); subsequently clean, pulverized human teeth were divided into three groups according to sizes. The best result was obtained from dimensions ranging between 0.5 mm and 1.0 mm. Smaller particles are too quickly resorbed to ensure sufficient space retention over time and to allow for bone formation. It is therefore obvious that the best performances are obtained when the dimensions of the granules are homogeneous and they are between 0.5 mm and 1.0 mm [95].

Dozza et al. [79] analyzed the DBM collagen-based biomaterial. A cortical sheep bone was ground and three different granule graft sizes were inserted and analyzed: small (< 0.5 mm), medium (0.5–1.0 mm), and large (1–2 mm). The authors concluded that medium particles were the best condition for cytocompatibility and recommended the use of an average size of 0.5 mm to 1.0 mm [79].

Some authors stated that osteoclast-like multinucleated giant cells appear to prefer small particles (< 1 mm) in both autogenous bone and bone substitutes, such as bovine mineralized bone [88, 96].

Larger bone replacement particles, on the other hand, can provide a greater quantity of bone augmentation. Larger autogenous bone particles (diameter, 1–2 mm) produced a greater augmented bone volume than smaller particles in a vertical bone augmentation model in rabbit calvaria utilizing polytetrafluoroethylene chambers (diameter, 150–400 mm) [97].

DDM resorbs faster than mineralized dentin, producing the best osteoconduction results, but very small parts may result in rapid graft resorption and failure to preserve volume [63, 72].

Dimensions under 400 µm are reabsorbed from osteoclasts in a short time. Conversely, dimensions over 1,000 µm are impossible to be reabsorbed. For this reason, granules produced by the two different speed grinders are sieved and the granule dispersion is analyzed using a master sizer 3000, laser diffraction particle size analyzer (Mastersizer 3000, Malvern Panalytical Ltd, Malvern, UK). This first analysis, despite the limitations due to the number of elements analyzed and the tools used, made it possible to establish that the grinding at low-speed, resulted in a more homogeneous grinding and was more efficient, allowing a greater percentage of the tooth to be available for use.

These findings suggest that bigger particles (1 mm) have stronger mechanical resistance as a mass for space-making than smaller particles (0.4 mm) and that space-making capability is more critical for early bone formation than the balance of bone resorption and formation. Nevertheless, dentin differs from the other graft materials in several ways. One of the most significant aspects is that no volume is lost during the preparation since it is unable to add (extract) additional material, and this process is based on the use of a removed tooth, thus it makes no sense to remove another tooth for graft usage. This element, which should be a limitation, should instead push to evaluate every single part of the preparation in order to avoid losing any component of the removed and useful teeth for producing grafting material.

As a result, several researchers have investigated the tooth's weight and typical volume [98]. A total of 205 removed teeth were weighed and measured with a millimeter-level syringe using a professional digital micro scale. The average weight varied from 0.68 g to 1.88 g, while the average volume was 0.38 mL to 0.96 mL. The volume is sufficient to accomplish the majority of the graft operation, but it is impossible to add more material in the same procedure if production is low. The findings indicated that the material produced from teeth might be adequate to be used as bone grafting material. However, the varying grinding from different machines may alter the volume available for regeneration [98].

The evaluations of the authors are exactly the point and purpose of this study. The only usable engineering solutions for the fragmentation of a solid structure transforming it into granular are: The first is high-speed by means of flat blades that rotate inside a capsule container and the structure to be fragmented is placed inside the capsule itself, the second is a concentric conical blades that rotate on the same axis and at low-speed where the solid structure must be inserted in the space between the conical blades.

The test analyzed three different data:

average tooth weight before the test: Each tooth was weighed to be able to compare the weight after the treatment;

average tooth weight after the test: Each pulverized tooth was weighed after the treatment to be able to compare with the same tooth weight before the treatment;

lost tooth weight (%): The difference between before and after the treatment was measured in the percentage of weight loss.

After sieving, the two different speed grinders produced the same particulate. The high-speed showed a greater dispersion. The dispersion was due to the pulverization (highly thin granules) of part of the tooth. The shape and the dimension of the granules were very different, so to obtain optima granules for the regeneration surgery a lot of parts of the tooth could be lost (Figure 2a). The percentage of the mean tooth lost with this high-speed grinder is $53.50\% \pm 9.89\%$ of the tooth load.

The low-speed grinder does not pulverize the dentin and creates a regular dimension of the tooth granules. The percentage of teeth loss is $9.16\% \pm 2.34\%$.

The hypothesis is that low-speed grinding is more effective, even though it requires a longer tooth preparation. The working hypothesis has thus been confirmed by the obtained data. This investigation conducted a comparative analysis between a low-speed grinder and a high-speed grinder, yielding disparate outcomes. Despite both methods yielding similar particle sizes, a marginal dispersion of tooth material was observed in the low-speed grinder group. This observation underscores the clinical and ethical imperative to preserve a substantial portion of the tooth, ensuring the maximal quantity of graft material. It is crucial to recognize that the tooth, being a non-commercial graft material, is irreplaceable, and procurement of additional graft material is not feasible if the production proves insufficient. Consequently, optimization strategies are imperative to maximize the volume derived from a single extracted tooth. While the low-speed grinder aligns with this imperative by minimizing material dispersion, it concurrently extends the duration of the procedural timeline. To address this trade-off, future endeavors should focus on the development of an innovative system capable of amalgamating the expeditious nature of high-speed grinding with the meticulous preservation of volume inherent in low-speed procedures. This proposed system would not only uphold the ethical mandate to conserve tooth material but also enhance procedural efficiency. Anticipating the evolution of dental technology, further investigations are warranted to explore and refine methodologies that balance speed and volume preservation. The advancement of such technologies is pivotal in advancing the field of dental grafting, ensuring both clinical efficacy and ethical responsibility in the utilization of precious biological resources. Consequently, the promotion of additional studies is imperative to propel the realization of these technological advancements and their integration into clinical practice.

Abbreviations

ABBM: anorganic bovine bone matrix

BMPs: bone morphogenetic proteins

DDM: demineralized dentin matrix

RPM: revolutions per minute

Declarations

Author contributions

EM, GD, ADI, and FI: Conceptualization, Methodology, Writing—original draft, Writing—review & editing. AP: Conceptualization, Methodology, Writing—original draft. FV and AMI: Conceptualization, Methodology, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethical Committee for Biomedical Research of Chieti and Pescara (Protocol Number 1869/21.03.2019).

Consent to participate

Informed consent was obtained from all subjects involved in the study.

Consent to publication

Not applicable.

Availability of data and materials

Data from the present manuscript will be made available upon reasonable request, and the corresponding author will provide the data upon request.

Funding

Not applicable.

Copyright

© The Author(s) 2024.

References

1. Buser D, Dahlin C, Schenk R. Guided bone regeneration in implant dentistry. Batavia (IL): Quintessence Publishing; 1994.
2. Romasco T, Tumedei M, Inchingolo F, Pignatelli P, Montesani L, Iezzi G, et al. A narrative review on the effectiveness of bone regeneration procedures with OsteoBiol® collagenated porcine grafts: the translational research experience over 20 years. *J Funct Biomater*. 2022;13:121.
3. Inchingolo F, Hazballa D, Inchingolo AD, Malcangi G, Marinelli G, Mancini A, et al. Innovative concepts and recent breakthrough for engineered graft and constructs for bone regeneration: a literature systematic review. *Materials (Basel)*. 2022;15:1120.
4. Inchingolo AD, Inchingolo AM, Bordea IR, Xhajanka E, Romeo DM, Romeo M, et al. The effectiveness of osseodensification drilling protocol for implant site osteotomy: a systematic review of the literature and meta-analysis. *Materials (Basel)*. 2021;14:1147.
5. Inchingolo F, Tatullo M, Marrelli M, Inchingolo AM, Inchingolo AD, Dipalma G, et al. Regenerative surgery performed with platelet-rich plasma used in sinus lift elevation before dental implant surgery: an useful aid in healing and regeneration of bone tissue. *Eur Rev Med Pharmacol Sci*. 2012;16:1222–6.
6. Inchingolo AD, Malcangi G, Semjonova A, Inchingolo AM, Patano A, Coloccia G, et al. Oralbiotica/oralbiotics: the impact of oral microbiota on dental health and demineralization: a systematic review of the literature. *Children (Basel)*. 2022;9:1014.
7. Tang G, Liu Z, Liu Y, Yu J, Wang X, Tan Z, et al. Recent trends in the development of bone regenerative biomaterials. *Front Cell Dev Biol*. 2021;9:665813.
8. Mummolo S, Mancini L, Quinzi V, D’Aquino R, Marzo G, Marchetti E. Rigenera® autologous micrografts in oral regeneration: clinical, histological, and radiographical evaluations. *Appl Sci*. 2020;10:5084.
9. Chisci G, Hatia A, Chisci E, Chisci D, Gennaro P, Gabriele G. Socket preservation after tooth extraction: particulate autologous bone vs. deproteinized bovine bone. *Bioengineering (Basel)*. 2023;10:421.
10. Linkhart TA, Mohan S, Baylink DJ. Growth factors for bone growth and repair: IGF, TGF beta and BMP. *Bone*. 1996;19:S1–12.
11. Canalis E, Pash J, Varghese S. Skeletal growth factors. *Crit Rev Eukaryot Gene Expr*. 1993;3:155–66.
12. Gargiulo Isacco C, Ballini A, Paduanelli G, Inchingolo AD, Nguyen KCD, Inchingolo AM, et al. Bone decay and beyond: How can we approach it better. *J Biol Regul Homeost Agents*. 2019;33:143–54.
13. Del Corso M, Vervelle A, Simonpieri A, Jimbo R, Inchingolo F, Sammartino G, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 1: periodontal and dentoalveolar surgery. *Curr Pharm Biotechnol*. 2012;13:1207–30.
14. Simonpieri A, Del Corso M, Vervelle A, Jimbo R, Inchingolo F, Sammartino G, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: bone graft, implant and reconstructive surgery. *Curr Pharm Biotechnol*. 2012;13:1231–56.

15. Sieverts M, Obata Y, Rosenberg JL, Woolley W, Parkinson DY, Barnard HS, et al. Unraveling the effect of collagen damage on bone fracture using *in situ* synchrotron microtomography with deep learning. *Commun Mater*. 2022;3:78.
16. Bhaskar SN, Orban BJ. *Orban's oral histology and embryology*. 9th ed. Bhaskar SN, editor. Saint Louis (MO): Mosby; 1980.
17. Butler WT, Ritchie H. The nature and functional significance of dentin extracellular matrix proteins. *Int J Dev Biol*. 1995;39:169–79.
18. Chen J, Shapiro HS, Sodek J. Development expression of bone sialoprotein mRNA in rat mineralized connective tissues. *J Bone Miner Res*. 1992;7:987–97.
19. Ganss B, Kim RH, Sodek J. Bone sialoprotein. *Crit Rev Oral Biol Med*. 1999;10:79–98.
20. Zhang J, Tu Q, Chen J. Applications of transgenics in studies of bone sialoprotein. *J Cell Physiol*. 2009;220:30–4.
21. Inchingolo F, Tatullo M, Abenavoli FM, Marrelli M, Inchingolo AD, Gentile M, et al. Non-syndromic multiple supernumerary teeth in a family unit with a normal karyotype: case report. *Int J Med Sci*. 2010;7:378–84.
22. Yeomans JD, Urist MR. Bone induction by decalcified dentine implanted into oral, osseous and muscle tissues. *Arch Oral Biol*. 1967;12:999–1008.
23. Bang G, Urist MR. Bone induction in excavation chambers in matrix of decalcified dentin. *Arch Surg*. 1967;94:781–9.
24. Schmidt-Schultz TH, Schultz M. Intact growth factors are conserved in the extracellular matrix of ancient human bone and teeth: a storehouse for the study of human evolution in health and disease. *Biol Chem*. 2005;386:767–76.
25. Sassano P, Gennaro P, Chisci G, Gabriele G, Aboh IV, Mitro V, et al. Calvarial onlay graft and submental incision in treatment of atrophic edentulous mandibles: an approach to reduce postoperative complications. *J Craniofac Surg*. 2014;25:693–7.
26. Inchingolo AM, Patano A, Di Pede C, Inchingolo AD, Palmieri G, de Ruvo E, et al. Autologous tooth graft: innovative biomaterial for bone regeneration. Tooth Transformer[®] and the role of microbiota in regenerative dentistry. A systematic review. *J Funct Biomater*. 2023;14:132.
27. Bessho K, Tanaka N, Matsumoto J, Tagawa T, Murata M. Human dentin-matrix-derived bone morphogenetic protein. *J Dent Res*. 1991;70:171–5.
28. Di Pompo G, Liguori A, Carlini M, Avnet S, Boi M, Baldini N, et al. Electrospun fibers coated with nanostructured biomimetic hydroxyapatite: a new platform for regeneration at the bone interfaces. *Biomater Adv*. 2023;144:213231.
29. Ten Dijke P, Miyazono K, Heldin CH. Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. *Curr Opin Cell Biol*. 1996;8:139–45.
30. Li Y, Fu G, Gong Y, Li B, Li W, Liu D, et al. BMP-2 promotes osteogenic differentiation of mesenchymal stem cells by enhancing mitochondrial activity. *J Musculoskelet Neuronal Interact*. 2022;22:123–31.
31. Ripamonti U, Ramoshebi LN, Matsaba T, Tasker J, Crooks J, Teare J. Bone induction by BMPs/OPs and related family members in primates. *J Bone Joint Surg Am*. 2001;83:S116–27.
32. Burchardt H. The biology of bone graft repair. *Clin Orthop Relat Res*. 1983:28–42.
33. Lorusso F, Inchingolo F, Dipalma G, Postiglione F, Fulle S, Scarano A. Synthetic scaffold/dental pulp stem cell (DPSC) tissue engineering constructs for bone defect treatment: an animal studies literature review. *Int J Mol Sci*. 2020;21:9765.
34. Vermesan D, Prejbeanu R, Haragus H, Poenaru DV, Mioc ML, Tatullo M, et al. Clinical relevance of altered bone immunopathology pathways around the elbow. *Eur Rev Med Pharmacol Sci*. 2014;18:2846–50.

35. Arafat Kabir M, Murata M, Kusano K, Mohammad Zakaria S, Hena Mohammad Noor A, Khuda F, et al. Radiological evaluation of human dentin autografts in Bangladesh. *J Hard Tissue Biology*. 2014;23: 363–70.
36. Isacco CG, Nguyen KCD, Pham VH, Di Palma G, Aityan SK, Tomassone D, et al. Searching for a link between bone decay and diabetes type 2. *Endocr Metab Immune Disord Drug Targets*. 2022;22: 904–10.
37. Cantore S, Mirgaldi R, Ballini A, Coscia MF, Scacco S, Papa F, et al. Cytokine gene polymorphisms associate with microbiological agents in periodontal disease: our experience. *Int J Med Sci*. 2014;11: 674–9.
38. Nakashima M. Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. *Cytokine Growth Factor Rev*. 2005;16:369–76.
39. Ballini A, Cantore S, Scacco S, Perillo L, Scarano A, Aityan SK, et al. A comparative study on different stemness gene expression between dental pulp stem cells vs. dental bud stem cells. *Eur Rev Med Pharmacol Sci*. 2019;23:1626–33.
40. Scarano A, Inchingolo F, Murmura G, Traini T, Piattelli A, Lorusso F. Three-dimensional architecture and mechanical properties of bovine bone mixed with autologous platelet liquid, blood, or physiological water: an *in vitro* study. *Int J Mol Sci*. 2018;19:1230.
41. Bono N, Tarsini P, Candiani G. Demineralized dentin and enamel matrices as suitable substrates for bone regeneration. *J Appl Biomater Funct Mater*. 2017;15:236–43.
42. Wildemann B, Kadow-Romacker A, Pruss A, Haas NP, Schmidmaier G. Quantification of growth factors in allogenic bone grafts extracted with three different methods. *Cell Tissue Bank*. 2007;8:107–14.
43. Rijal G, Shin HI. Human tooth-derived biomaterial as a graft substitute for hard tissue regeneration. *Regen Med*. 2017;12:263–73.
44. Hazballa D, Inchingolo AD, Inchingolo AM, Malcangi G, Santacroce L, Minetti E, et al. The effectiveness of autologous demineralized tooth graft for the bone ridge preservation: a systematic review of the literature. *J Biol Regul Homeost Agents*. 2021;35:283–94.
45. Rapone B, Inchingolo AD, Trasarti S, Ferrara E, Qorri E, Mancini A, et al. Long-term outcomes of implants placed in maxillary sinus floor augmentation with porous fluorohydroxyapatite (Algapore® FRIOS®) in comparison with anorganic bovine bone (Bio-Oss®) and platelet rich plasma (PRP): a retrospective study. *J Clin Med*. 2022;11:2491.
46. Boyne PJ. Experimental evaluation of the osteogenic potential of bone graft materials. *Annu Meet Am Inst Oral Biol*. 1969:13–21.
47. Mellonig JT, Bowers GM, Cotton WR. Comparison of bone graft materials: Part II. New bone formation with autografts and allografts: a histological evaluation. *J Periodontol*. 1981;52:297–302.
48. Colnot C, Romero DM, Huang S, Helms JA. Mechanisms of action of demineralized bone matrix in the repair of cortical bone defects. *Clin Orthop Relat Res*. 2005;435:69–78.
49. Araújo MG, Sonohara M, Hayacibara R, Cardaropoli G, Lindhe J. Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *J Clin Periodontol*. 2002;29:1122–31.
50. Minamizato T, Koga T, I T, Nakatani Y, Umebayashi M, Sumita Y, et al. Clinical application of autogenous partially demineralized dentin matrix prepared immediately after extraction for alveolar bone regeneration in implant dentistry: a pilot study. *Int J Oral Maxillofac Surg*. 2018;47:125–32.
51. Kim SY, Kim YK, Park YH, Park JC, Ku JK, Um IW, et al. Evaluation of the healing potential of demineralized dentin matrix fixed with recombinant human bone morphogenetic protein-2 in bone grafts. *Materials (Basel)*. 2017;10:1049.
52. Libonati A, Marzo G, Klinger FG, Farini D, Gallusi G, Tecco S, et al. Embryotoxicity assays for leached components from dental restorative materials. *Reprod Biol Endocrinol*. 2011;9:136.

53. Sah A, Baliga SD. Clinical application of autogenous tooth as bone graft material in extraction socket- a prospective study. *Clin Epidemiology Glob Health*. 2022;16:101063.
54. Bianchi S, Mancini L, Torge D, Cristiano L, Mattei A, Varvara G, et al. Bio-morphological reaction of human periodontal ligament fibroblasts to different types of dentinal derivatives: *in vitro* study. *Int J Mol Sci*. 2021;22:8681.
55. Minetti E, Berardini M, Trisi P. A new tooth processing apparatus allowing to obtain dentin grafts for bone augmentation: the tooth transformer. *Open Dent J*. 2019;13:6–14.
56. Adina S, Dipalma G, Bordea IR, Lucaciu O, Feurdean C, Inchingolo AD, et al. Orthopedic joint stability influences growth and maxillary development: clinical aspects. *J Biol Regul Homeost Agents*. 2020;34:747–56.
57. Bono N, Tarsini P, Candiani G. BMP-2 and type I collagen preservation in human deciduous teeth after demineralization. *J Appl Biomater Funct Mater*. 2019;17:2280800018784230.
58. Nibali L, Buti J, Barbato L, Cairo F, Graziani F, Jepsen S. Adjunctive effect of systemic antibiotics in regenerative/reconstructive periodontal surgery—a systematic review with meta-analysis. *Antibiotics (Basel)*. 2021;11:8.
59. Minetti E, Palermo A. Comparison between the bone regeneration using tooth graft with or without tooth transformer in sheep. *BAOJ Dent*. 2019;5:54.
60. Minetti E, Corbella S, Taschieri S, Canullo L. Tooth as graft material: histologic study. *Clin Implant Dent Relat Res*. 2022;24:488–96.
61. Minetti E, Palermo A, Savadori P, Barlattani A Jr, Franco R, Michele M, et al. Autologous tooth graft: a histological comparison between dentin mixed with xenograft and dentin alone grafts in socket preservation. *J Biol Regul Homeost Agents*. 2019;33:189–97.
62. Minetti E, Celko M, Contessi M, Carini F, Gambardella U, Giacometti E, et al. Implants survival rate in regenerated sites with innovative graft biomaterials: 1 year follow-up. *Materials (Basel)*. 2021;14:5292.
63. Minetti E, Giacometti E, Gambardella U, Contessi M, Ballini A, Marenzi G, et al. Alveolar socket preservation with different autologous graft materials: preliminary results of a multicenter pilot study in human. *Materials (Basel)*. 2020;13:1153.
64. Santos A, Botelho J, Machado V, Borrecho G, Proença L, Mendes JJ, et al. Autogenous mineralized dentin *versus* xenograft granules in ridge preservation for delayed implantation in post-extraction sites: a randomized controlled clinical trial with an 18 months follow-up. *Clin Oral Implants Res*. 2021;32:905–15.
65. Oguić M, Čandrić M, Tomas M, Vidaković B, Blašković M, Jerbić Radetić AT, et al. Osteogenic potential of autologous dentin graft compared with bovine xenograft mixed with autologous bone in the esthetic zone: radiographic, histologic and immunohistochemical evaluation. *Int J Mol Sci*. 2023;24:6440.
66. Marrelli M, Tatullo M, Dipalma G, Inchingolo F. Oral infection by *Staphylococcus aureus* in patients affected by White Sponge Nevus: a description of two cases occurred in the same family. *Int J Med Sci*. 2012;9:47–50.
67. Inchingolo AD, Malcangi G, Inchingolo AM, Piras F, Settanni V, Garofoli G, et al. Benefits and implications of resveratrol supplementation on microbiota modulations: a systematic review of the literature. *Int J Mol Sci*. 2022;23:4027.
68. Schutyser MA, Briels WJ, Rinzema A, Boom RM. Numerical simulation and PEPT measurements of a 3D conical helical-blade mixer: a high potential solids mixer for solid-state fermentation. *Biotechnol Bioeng*. 2003;84:29–39.
69. Hansen SH, Holmfred E, Cornett C, Maldonado C, Rønsted N. An efficient, robust, and inexpensive grinding device for herbal samples like *Cinchona* bark. *Sci Pharm*. 2015;83:369–76.

70. Saarinen T, Antikainen O, Yliruusi J. Simultaneous comparison of two roller compaction techniques and two particle size analysis methods. *AAPS PharmSciTech*. 2017;18:3198–207.
71. Ndiaye M, Terranova L, Mallet R, Mabilieu G, Chappard D. Three-dimensional arrangement of β -tricalcium phosphate granules evaluated by microcomputed tomography and fractal analysis. *Acta Biomater*. 2015;11:404–11.
72. Kim YK, Kim SG, Yun PY, Yeo IS, Jin SC, Oh JS, et al. Autogenous teeth used for bone grafting: a comparison with traditional grafting materials. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2014;117:e39–45.
73. Jun SH, Ahn JS, Lee JI, Ahn KJ, Yun PY, Kim YK. A prospective study on the effectiveness of newly developed autogenous tooth bone graft material for sinus bone graft procedure. *J Adv Prosthodont*. 2014;6:528–38.
74. Kim YK, Lee J, Um IW, Kim KW, Murata M, Akazawa T, et al. Tooth-derived bone graft material. *J Korean Assoc Oral Maxillofac Surg*. 2013;39:103–11.
75. Vermesan D, Inchingolo F, Patrascu JM, Trocan I, Prejbeanu R, Florescu S, et al. Anterior cruciate ligament reconstruction and determination of tunnel size and graft obliquity. *Eur Rev Med Pharmacol Sci*. 2015;19:357–64.
76. Murata M. Bone engineering using human demineralized dentin matrix and recombinant human BMP-2. *J Hard Tissue Biology*. 2005;14:80–1.
77. Nampo T, Watahiki J, Enomoto A, Taguchi T, Ono M, Nakano H, et al. A new method for alveolar bone repair using extracted teeth for the graft material. *J Periodontol*. 2010;81:1264–72.
78. Kim YK, Kim SG, Byeon JH, Lee HJ, Um IU, Lim SC, et al. Development of a novel bone grafting material using autogenous teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109:496–503.
79. Dozza B, Lesci IG, Duchi S, Della Bella E, Martini L, Salamanna F, et al. When size matters: differences in demineralized bone matrix particles affect collagen structure, mesenchymal stem cell behavior, and osteogenic potential. *J Biomed Mater Res A*. 2017;105:1019–33.
80. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, et al. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. *J Mater Sci Mater Med*. 2014;25:2445–61.
81. Testori T, Wallace SS, Trisi P, Capelli M, Zuffetti F, Del Fabbro M. Effect of xenograft (ABBM) particle size on vital bone formation following maxillary sinus augmentation: a multicenter, randomized, controlled, clinical histomorphometric trial. *Int J Periodontics Restorative Dent*. 2013;33:467–75.
82. Carano RA, Filvaroff EH. Angiogenesis and bone repair. *Drug Discov Today*. 2003;8:980–9.
83. Shapoff CA, Bowers GM, Levy B, Mellonig JT, Yukna RA. The effect of particle size on the osteogenic activity of composite grafts of allogeneic freeze-dried bone and autogenous marrow. *J Periodontol*. 1980;51:625–30.
84. Pallesen L, Schou S, Aaboe M, Hjørting-Hansen E, Nattestad A, Melsen F. Influence of particle size of autogenous bone grafts on the early stages of bone regeneration: a histologic and stereologic study in rabbit calvarium. *Int J Oral Maxillofac Implants*. 2002;17:498–506.
85. Binderman I, Hallel G, Nardi C, Yaffe A, Sapoznikov L. A novel procedure to process extracted teeth for immediate grafting of autogenous dentin. *J Interdiscipl Med Dent Sci*. 2014;2:154.
86. Klüppel LE, Antonini F, Olate S, Nascimento FF, Albergaria-Barbosa JR, Mazzonetto R. Bone repair is influenced by different particle sizes of anorganic bovine bone matrix: a histologic and radiographic study *in vivo*. *J Craniofac Surg*. 2013;24:1074–7.
87. Dohan Ehrenfest DM, Del Corso M, Inchingolo F, Sammartino G, Charrier JB. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in human cell cultures: growth factor release and contradictory results. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110:418–21.
88. Kon K, Shiota M, Ozeki M, Yamashita Y, Kasugai S. Bone augmentation ability of autogenous bone graft particles with different sizes: a histological and micro-computed tomography study. *Clin Oral Implants Res*. 2009;20:1240–6.

89. Fucini SE, Quintero G, Gher ME, Black BS, Richardson AC. Small *versus* large particles of demineralized freeze-dried bone allografts in human intrabony periodontal defects. *J Periodontol.* 1993;64:844–7.
90. Xu H, Shimizu Y, Asai S, Ooya K. Experimental sinus grafting with the use of deproteinized bone particles of different sizes. *Clin Oral Implants Res.* 2003;14:548–55.
91. Coathup MJ, Cai Q, Champion C, Buckland T, Blunn GW. The effect of particle size on the osteointegration of injectable silicate-substituted calcium phosphate bone substitute materials. *J Biomed Mater Res B Appl Biomater.* 2013;101:902–10.
92. Grassi FR, Ciccolella F, D’Apolito G, Papa F, Iuso A, Salzo AE, et al. Effect of low-level laser irradiation on osteoblast proliferation and bone formation. *J Biol Regul Homeost Agents.* 2011;25:603–14.
93. Prieto EM, Talley AD, Gould NR, Zienkiewicz KJ, Drapeau SJ, Kalpakci KN, et al. Effects of particle size and porosity on *in vivo* remodeling of settable allograft bone/polymer composites. *J Biomed Mater Res B Appl Biomater.* 2015;103:1641–51.
94. Gultekin BA, Bedeloglu E, Kose TE, Mijiritsky E. Comparison of bone resorption rates after intraoral block bone and guided bone regeneration augmentation for the reconstruction of horizontally deficient maxillary alveolar ridges. *Biomed Res Int.* 2016;2016:4987437.
95. Koga T, Minamizato T, Kawai Y, Miura K, I T, Nakatani Y, et al. Bone regeneration using dentin matrix depends on the degree of demineralization and particle size. *PLoS One.* 2016;11:e0147235.
96. Chackartchi T, Iezzi G, Goldstein M, Klinger A, Soskolne A, Piattelli A, et al. Sinus floor augmentation using large (1–2 mm) or small (0.25–1 mm) bovine bone mineral particles: a prospective, intra-individual controlled clinical, micro-computerized tomography and histomorphometric study. *Clin Oral Implants Res.* 2011;22:473–80.
97. Kon K, Shiota M, Ozeki M, Kasugai S. The effect of graft bone particle size on bone augmentation in a rabbit cranial vertical augmentation model: a microcomputed tomography study. *Int J Oral Maxillofac Implants.* 2014;29:402–6.
98. Minetti E, Corbella S, Taschieri S. The weight of permanent teeth: an exploratory study on a total of 205 teeth. *Int J Oral Maxillofac Implants.* 2019;34:85–9. Italian.