

Autologous tooth for bone regeneration: dimensional examination of Tooth Transformer[®] granules

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Abstract. – OBJECTIVE: Since 1967, when the osteoinduction properties of autogenous demineralized dentin matrix were discovered, autologous tooth grafts have been advocated as a viable option to autologous or heterologous bone graft. Tooth graft materials may be extracted from the patient's whole tooth using a granulating device. The aim of this study was to examine the size of granules obtained by the Tooth Transformer (TT)[®] device, using a laser instrument with high precision.

MATERIALS AND METHODS: The TT[®] device can obtain bone graft material in a short period from an extracted tooth. The resulting material can act as an osteoconductive scaffold, providing a mineral substrate during resorption, including platelet growth factors and morphogenetic proteins. Different studies have investigated the dimension and behavior of various graft material particles, since the size of the grafted particles may play a role in osteogenesis and bone regeneration.

RESULTS: Different dimensions of granules are available: small (< 400 µm), medium (400 µm-1,000 µm) and large (1,000 µm-2,000 µm). From 4.03 µm to 100 µm the percentage of granules was 14.52 ± 1.93%. A larger part of the granules was up to 100 µm, while 85.47 ± 1.93% of the granules were from 100 µm to 1,000 µm.

CONCLUSIONS: 85% of the granules produced were in accordance with the dimensions suggested in the literature.

Key Words:

Autologous tooth, Biomaterials, Dentin graft, Granules, Grow factors, Human dentin matrix, Osteogenesis, Tooth regeneration, Tooth transformer.

Abbreviations

ABBM: anorganic bovine bone matrix; BMP: bone morphogenetic proteins; DBM: demineralized bone matrix; DBBM: deproteinized bovine bone mineral; SFE: sinus floor elevation; TT[®]: tooth transformer; RUNX2: run-related transcription factor 2; BGLAP: bone gamma-carboxyglutamate protein.

Introduction

For over a century, autologous bone grafts have been widely used in regenerative medicine, being considered the gold standard in healing bone defects^{1,2}.

Graft materials must exhibit a three-dimensional structure that promotes cell attachment and bone formation. These properties are influenced by the chemical composition, micro surface area, crystallinity, and size of the crystals. Bone graft materials must be safe and biocompatible, with no immunological responses or disease transmission. They must have a porous structure to allow body fluids, cells, and blood vessels to penetrate inside and surface roughness to allow osteoblasts to adhere and proliferate, and must provide space for nutrients^{3,4}.

The bone substitute should be retained during new bone formation to support osteoblast function. Sensitivity to enzymatic or chemical dissolution also greatly influences the space-making

capability; if passive chemical dissolution is too fast, bone substitutes disappear before new bone formation, leading to defective space formation. Traditionally, in terms of biocompatibility, bone graft materials are classified as biotolerant, bioinert, or bioactive. Biotolerant implant materials remain in the body with fibrous encapsulation, evoking a tissue reaction. Bioinert implant materials have direct contact with the adjacent bone tissue without any chemical reaction. Bioactive implants establish chemical bonds with adjacent bone tissue, which leads to the direct deposition of bone matrix on the implant material. This conceptual classification is based on histological observations of local effects after implantation into bone tissue. Several bone substitutes are available on the market and according to their origin, they are mainly divided into natural and synthetic substitutes. The synthetic and natural substitutes both display osteoconductive properties^{5,6}.

Autologous bone remains the replacement material for osteoconductive, osteoinductive, and remodeling capabilities. Homologous bone tissue can be taken from the iliac crest, femur, tibia, humerus, ribs, and vertebrae.

Heterologous bone substitutes, on the other hand, are biologically derived animal grafts, that is, from natural bone (usually bovine or equine) that has been decellularized and stripped of all antigenic elements. They generally have slower resorption kinetics than normal turnover. Finally, among the natural grafts, there are corals, from calcified seaweed, which consist mainly of calcium carbonate in the form of porous aragonite. They occur in granules or blocks and are materials with excellent osteoinductive capacity with very long resorption, up to 3 years. Synthetic bone replacements come in a variety of materials such as hydroxyapatites, calcium phosphates and sulphates, and so on, and have extremely heterogeneous properties⁷.

In recent years, the possibility of using the tooth as a graft material has emerged. The tooth, like bone, consists of an inorganic part consisting of hydroxyapatite and an organic part consisting of collagen and noncollagenic proteins⁸.

Both demineralized bone matrix (DBM) and demineralized dentin matrix (DDM) include type I collagen, growth hormones, and bone morphogenetic proteins (BMP-2) after demineralization⁹.

Dentin matrix has been considered^{10,11} an excellent alternative to autologous or heterologous bone graft because this material displays osteoconductive and osteoinductive properties.

The dentin graft is considered a bioactive material. Bioabsorption of bone substitutes should involve the replacement of the implanted material by newly formed bone tissue *via* bone remodeling, i.e., “regeneration” and not “reconstruction”. Bioabsorption during the bone formation phase is associated with space-making capability and biocompatibility and is predominantly mediated by the passive chemical dissolution of the bone substitute^{10,11}.

The dentin tubules increase after the demineralization. This space affects the flow of blood and interstitial fluid in local bone tissue¹². Blood and interstitial fluid transport oxygen and nutrients into the local tissue, being essential for bone regeneration¹³.

Different procedures for demineralizing teeth have been investigated¹⁴⁻¹⁸, with similarly disparate outcomes in bone tissue development.

When comparing tooth-crushing systems, the following must be taken into account: the degree of sterilization, the repeatability of the system, the liquids and their concentration, the size of the granules, the amount of residual protein after treatment, the wettability and plasticity of the granules, and the ergonomics of the system¹⁹.

The preparation technique for transforming autologous teeth into suitable grafting material represents the fundamental step of the procedure of TT[®] (*via* Washington 59, 20146, Milan, Italy) (Figure 1).

Since the device creates granules, the sizing of the granules is important to facilitate the formation of the bone during healing. The tooth is very similar to the bone and contains many growth factors²⁰.

A critical factor determining the bioabsorption rate during the remodeling phase is the chemical composition and water solubility of the bone substitute. Osteoclasts can degrade bone substitutes by hydrolysis by secreting hydrogen ions. *In vivo* histological studies^{21,22} have confirmed that osteoclasts and osteoclast-like cells can form resorption pits

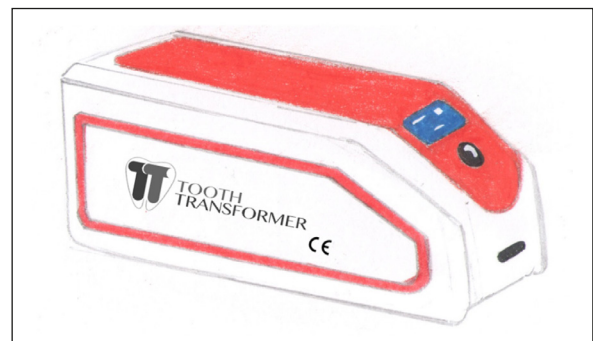


Figure 1. The Tooth Transformer[®] (CE).

on pure water-soluble calcium phosphate. Osteoclast-like multinucleated giant cells appear to prefer small particles (< 1 mm) in both autogenous bone and bone substitutes, such as bovine bone minerals.

Many studies^{23,24} analyze the correct dimensions of the graft materials granules. The dimension of the graft granules is critical information about a graft material because the reabsorption is influenced by three different factors: the chemical composition, the cell adhesion, and the volume. The use of autologous material cannot change the first two aspects but it is possible to change the third²⁴.

Since there are different particle sizes available for use in clinical practice and the size of the grafted particles may play a role in osteogenesis and bone repair, it is very important to take into consideration the dimensions of the granules^{25,26}.

Many studies^{1,27,28} have found that the particle size of bone graft materials plays an important role in triggering osteoconduction and influencing the quality of new bone.

Shapoff et al²⁵ compared small particles (100-300 µm) with larger particles (1,000-2,000 µm). The authors found that the osteogenesis associated with the smaller particles was significantly greater than that with the larger particles²⁵. Pallesen et al²⁶ reported that particles 0.5 to 2.0 mm were preferable to particles of 10 mm, allowing more rapid bone remodeling²⁶. Rivault et al²⁹ stated that small particles of 100 microns in autogenic bone resulted in a quicker and larger osteogenic activity than bigger particles that could occasionally cause localized inflammatory reactions²⁹.



Figure 2. Cleaning of the teeth.



Figure 3. Fractionated tooth.

This study aims to investigate if the granule dimensions made from the TT® are coherent with those suggested in the literature.

Materials and Methods

After the extraction of the teeth, a cleaning of the carious processes is performed. This step is done with the use of a diamond bur mounted on a turbine (Figure 2). Teeth are fractionated, making these ready for grinding (Figure 3).

After inserting the sectioned and cleaned teeth inside the grinder, it is closed and placed in the device (Figure 4). The disposable part contains a cartridge with liquids and a cylinder with a cup for collecting the granulate (Figure 5).



Figure 4. Tooth fragments are positioned in the Tooth Grinder® (CE).

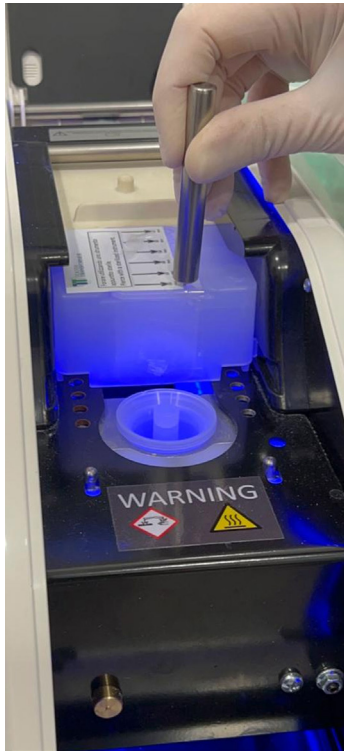


Figure 5. The liquids are inserted, and the cartridge is pierced before starting the transformation cycle.

Both are inserted into the device in their respective slots, the cartridge is activated by piercing, and then, once the lid is closed and the button is pressed, the process starts. The procedure is completely automatic and repeats the same steps each time. The first phase of grinding at low speed causes the granules to fall into the collection basket. The six liquids present in the cartridge tank fall by gravity after the automatic perforation of the lower membrane of the cartridge and start the process.

The liquids are three different solutions in six different compartments of the same single-use cartridge. Two of these are active liquids, constituted by 0.1 m hydrochloric acid, 10% hydrogen peroxide, the other liquid is demineralized water. The four compartments full of mineralized water are used to remove, in four different procedure phases, the acid residues.

The granules are subjected to UVA rays and ultrasonic vibrations with temperature variations always below 43° to avoid damage to the proteins.

After only 25 minutes, the teeth processing is completed and the tooth-derived graft can be used (Figure 6). At the end of the process, the used and contaminated liquids remain inside the cylindrical container, which can be disposed in medical waste disposal^{18,30}.

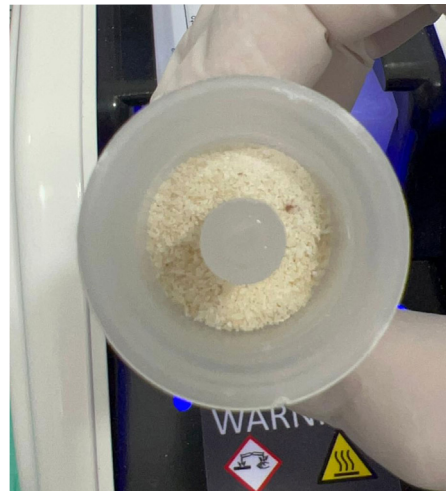


Figure 6. Tooth-derived graft.

Ten extracted natural teeth were used. Each tooth was cleaned with a diamond bar, sectioned, and inserted in the TT[®] grinder. The medical device TT[®] from Tooth Transformer[®] Srl (via Washington 59, 20146 Milan, Italy) was used (Figure 7).

The device was the normal device sold on the market, and the standard grinder contained in the starting kit. The starting kit consists of a shock-proof plastic suitcase containing the device, the electrical cables to connect it to the electricity grid, the multi-use perforator that will be used to punch the top of the tank containing the six disposable liquids of single-use, and the grinder which is composed of three parts. The three parts are assembled by inserting the blades and closing the lid. After each use, it is pos-



Figure 7. TT[®] device is able to granulate, demineralize and decontaminate the tooth automatically.



Figure 8. The Mastersizer 3000 is able to analyze the size of the granules using laser diffraction.

sible to disassemble the disposable components and sterilize the rest for later use. The grinder consists of two concentric conical blades that are assembled before the grinding phase and present a calibrated lower lumen. The distance between the two blades at the bottom determines the size of the granules. The structure of the grinder allows the assembly to be always identical to produce granules of the same size. Our test was to establish if, in repeated cycles, the granules' dimensions are always similar.

In order to compare the size of the granules after the different grinding processes, we used a device that can analyze and measure the size of each individual granule produced by the TT®. The instrument used for analyzing the size of the granules was the Mastersizer 3000 for Malvern Instruments Limited (Grove Wood Road, Malvern, Worcestershire, UK, WR14 1XZ) (Figure 8).

Results

The results of the Mastersizer analysis are in a graph showing the distribution of granules on the two axes x and y. The x axis shows the size of the granules, and the y axis shows the volume of the granules (Figure 9). The same results are then shown in Table I. The curve resulting from the 10 different teeth analyzed by the Mastersizer 3000 for Malvern Instruments Limited (Grove Wood Road, Malvern, Worcestershire, UK, WR14 1XZ) can be divided into three sections that allow evaluating the percentage granule dispersion and the compared data.

The Mastersizer 3000 calculates an impressive particle size range from 10 nm up to 3.5 mm using a single optical measurement path.

The instrument uses a sequential combination of measurements with red and blue light sources to measure across the entire particle size range.

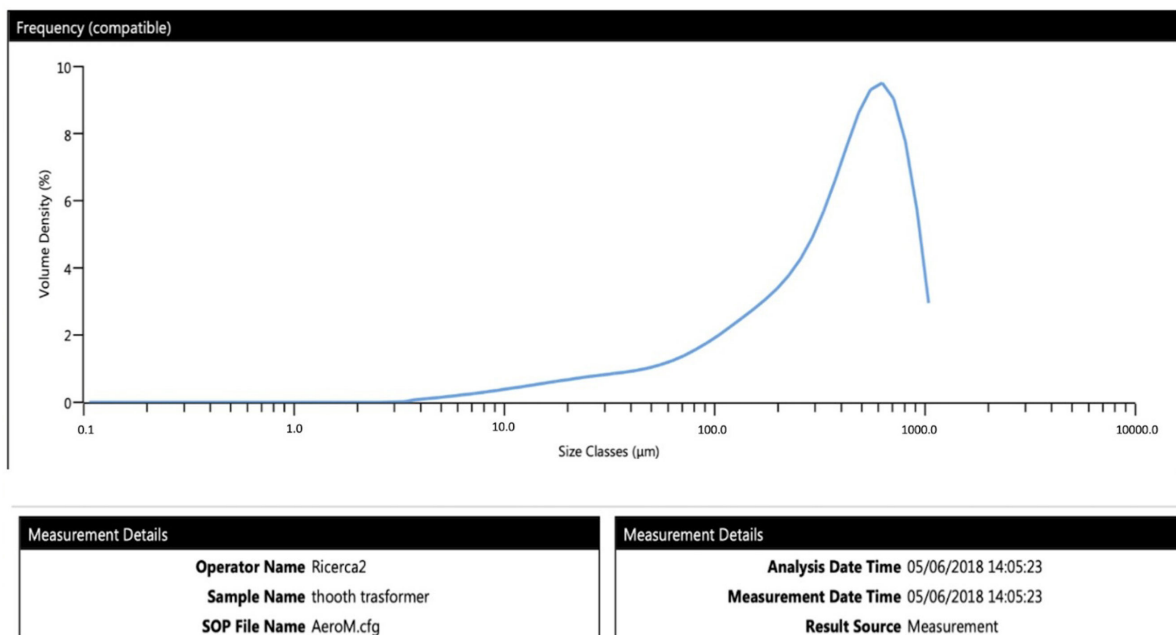


Figure 9. Graph of the results obtained by the Mastersizer with data indicating the size of the granules (this figure shows the results of test No. 1).

Table I. Values of test No. 1 performed by the Mastersizer 3000. The left column shows the size of the granules analyzed. The right column indicates the % of volume corresponding to the sum of the volume of particles of that size added to that of the previous ones.

Size (µm)	% Volume	Size (µm)	% Volume	Size (µm)	% Volume	Size (µm)	% Volume
0.0995	0.00	1.28	0.00	16.4	2.98	211	28.25
0.113	0.00	1.45	0.00	18.7	3.49	240	31.39
0.128	0.00	1.65	0.00	21.2	4.05	272	34.93
0.146	0.00	1.88	0.00	24.1	4.65	310	39.00
0.166	0.00	2.13	0.00	27.4	5.29	352	43.73
0.188	0.00	2.42	0.00	31.1	5.96	400	49.26
0.214	0.00	2.75	0.00	35.3	6.67	454	55.65
0.243	0.00	3.12	0.00	40.1	7.42	516	62.85
0.276	0.00	3.55	0.00	45.6	8.21	586	70.64
0.314	0.00	4.03	0.00	51.8	9.06	666	78.62
0.357	0.00	4.58	0.16	58.9	9.98	756	86.20
0.405	0.00	5.21	0.28	66.9	11.01	859	92.73
0.461	0.00	5.92	0.43	76.0	12.16	976	97.52
0.523	0.00	6.72	0.61	86.4	13.46	1,110	100.0
0.594	0.00	7.64	0.82	98.1	14.94		
0.675	0.00	8.68	1.08	111	16.60		
0.767	0.00	9.86	1.37	127	18.48		
0.872	0.00	11.2	1.71	144	20.57		
0.991	0.00	12.7	2.09	163	22.88		
1.13	0.00	14.5	2.51	186	25.44		

After 25 minutes, the TT[®] had granulated the teeth and it was possible to recover the granules from a basket named “creator” from the TT[®]. The granules were inserted in the Mastersizer 3000. Ten different teeth were used for this test.

There were no granules under 4.03 µm in any of the samples (Table II).

Discussion

On the market, there are some other devices that allow the use of the tooth as a graft, and the procedures are different³¹⁻³⁴. The only device with a CE mark as an electromedical device is the Bon Maker[®] (from Korean Dental Solution, Busan, Korea), which

Table II. Average values of all the tests (%) carried out by the Mastersizer 3000 divided into three sections. The first section indicates the presence percentage of the 0/4.03 µm granulometry in each sample. The second column indicates the percentage presence of the 4.03/100 µm granulometry in each sample. The third column indicates the presence percentage of the 100/1,000 µm granulometry in each sample. From 4.03 µm to 100 µm the percentage of granules was $14.52 \pm 1.93\%$. A part of the granules were up to 100 µm. The majority, $85.47 \pm 1.93\%$, of the granules were from 100 µm to 1,000 µm.

	0/4.03 µm	4.03/100 µm % presence	100/1,000 µm % presence
Test 1	0	0.16/14.94%	14.94/100 85.86%
Test 2	0	0.16/15.12%	15.12/100 84.88%
Test 3	0	0.16/12.78%	12.78/100 87.22%
Test 4	0	0.16/14.11%	14.11/100 85.89%
Test 5	0	0.16/16.08%	16.08/100 83.92%
Test 6	0	0.16/13.22%	13.11/100 86.89%
Test 7	0	0.16/13.87%	13.87/100 86.13%
Test 8	0	0.16/18.13%	18.13/100 81.87%
Test 9	0	0.16/15.74%	15.74/100 84.26%
Test 10	0	0.16/11.23%	11.23/100 88.77%
Average value (%)	0	$14.52 \pm 1.93\%$	$85.47 \pm 1.93\%$

uses different procedures. The Bon Maker® device crushes the tooth using a hammer and pestle. Next, the tooth is placed in a nonsterilizable high-speed mill and the granules are separated by a manual sieve equipped with two different filters: the larger 850 µm granules are blocked by the first filter, while the finer 450 µm granules pass through the second filter into the lower plate. The granules are manually inserted into a sterilizable plastic cylinder (Bonbin), housed in a slot at the top front of the machine where a bottle is filled with saline. The liquids are emptied manually into their respective cavities according to a color code. The material is extracted after 26 minutes, at the end of the treatment. Spent and contaminated liquids are collected in a glass flask at the front of the device that must be removed after a few uses⁷.

An advantage of the TT® is that it avoids tooth substance loss in pulverization thanks to a low-speed, multipurpose sterilizable system. Furthermore, only the TT®, allows to automatically carry out all the steps of the procedure. It allows automatic crushing of the tooth with high and low-speed milling. Only with the TT®, the automatic recovery of contaminated liquids is possible⁷.

This study aimed to investigate if the dimensions made from human extracted teeth are of the same dimension as declared by the company and corresponding to the literature indications. The dimension of the graft granules is critical information about a graft material because the reabsorption is influenced by three different factors: the chemical composition, the cell adhesion, and the volume. The use of this autologous material cannot change the first two aspects but allows for a change of the third. The production of grafting materials allows the industrialization of the process by deciding size, chemical composition, and wet ability, and this influences the results. In literature, several authors have analyzed the relationship between size and results in bone regeneration. Actually, data regarding optimal granule size are conflicting and derived primarily from *in vitro* studies. Although still controversial, the results of studies⁴ reported that the smaller the particle size of the material, the greater the bone formation of bone, due to the fact that the smaller particles increase the available surface area and promote the secretion of more growth factors of various types to induce the differentiation of mesenchymal cells into osteoblasts responsible for the production of new bone and facilitate the formation of new blood vessels.

Furthermore, it has been stated³⁵ that the particle spacing should be higher than 100 µm in order to promote adequate vascularization and bone formation.

In bone formation tests using hydroxyapatite, an interconnected porous structure between particles was discovered to be a key determinant for osteoconduction^{36,37}. As a result, both the structure porosity and particle size are crucial elements because they improve bone formation and influence the resorption time³⁴.

In the study by Nam et al³⁴, bone defects in rabbits were grafted with DDM using four different particle sizes and densities. The authors found that DDM with particles of 0.25 to 1.0 mm in size with 200 µm of space were effective in promoting osteogenesis.

Clinicians have at their disposal two particle sizes when using the majority of xenografts: a large particle size of 1.0 to 2.0 mm and a small particle size of 0.25 to 1.0 mm. The histomorphometric results in a study by Testori et al²³ indicated a statistically significant increase in vital bone formation when the larger particle size was used.

According to these results, Carano and Filvaroff²⁴ suggested the utilization of the larger particles because these leave more space for vascular ingrowth, which is essential for bone formation in the augmented volume, while small particles disappeared in a short time²⁴.

In contrast to the studies previously described, Dozza et al²⁸ suggested using medium particle size. In this study²⁸, demineralized bone matrix (DBM) powder of three different particle sizes was prepared according to the protocol by Reddi and Huggins³⁸. From sheep bone, three different particle size fractions were obtained by stratification with a nested column of sieves. Each sieve in the column had a wire mesh cloth with openings smaller than the sieve above. They obtained the following fractions with particles of discrete size ranges, namely large particle size (L), 1-2 mm, medium particle size (M), 0.5-1 mm, small particle size (S), < 0.5 mm. They placed the granules in a bone hole and made a radiograph of each fraction to assess the demineralization process. This study demonstrated that DBM collagen structure is affected by particle size, with medium particles being altered but not as much as small particles. Medium particle size allows more efficient results, because it promotes higher cell adhesion and regulates the expression of genes involved in osteogenic differentiation, decreasing the levels of runt-related transcription factor 2 (RUNX2) levels and increasing the levels of bone gamma-carboxyglutamate protein (BGLAP). The authors²⁸ suggested that their results should guide researchers to use DBM par-

ticles of 0.5-1 mm size range in bone grafts. The use of medium (0.5-1 mm) particles *in vivo* is preferable when DBM is implanted alone, while medium and small (< 0.5 mm) particles are recommended when DBM is implanted in association with mesenchymal cells (MSC). Koga et al⁹ also carried out an analysis using three different sizes of human teeth particles (large, 1-2 mm, small, 0.5 mm, and medium, 0.5-1 mm), obtaining the best results with particles ranging from 0.5 to 1 mm. Smaller particles were resorbed too quickly to allow for sufficient space retention over time and bone formation.

Reabsorption is more efficient using dentin because the process of reabsorption free numerous growth factors and this process is influenced by the granules' size. The bone morphogenetic proteins (BMP)-2 need to have a carrier, which is the dentin^{17,39}.

The growth factors are blocked in the dentin and preserved during the time, so during the reabsorption from osteoclast, the proteins (in particular BMP-2 proteins) are freeing and stimulating the mesenchymal cells⁴⁴ (to be transformed into osteocytes) and osteocytes to produce new bone. From the literature, the suggested correct dimension is the medium-size particles (450- to 1,000 μm)⁴⁰.

According to Kluppel et al⁴¹, anorganic bovine bone matrix (ABBM) of small-size granules leads to a greater amount of osteoid tissue, and the particles were almost totally reabsorbed within 60 days of implantation. The authors made 4 holes in rabbits' calvaria, and they filled these with small-size anorganic bovine bone matrix (ABBM) particles (< 450 μm), medium-size ABBM particles (450 to 749 μm), large-size ABBM particles (750 to 1,000 μm) and control with autogenous bone. The defects filled with large particles of anorganic bovine bone matrix (750 to 1,000 μm) presented a radiographic pattern very similar to the surrounding cranial bone. Interestingly, decreasing the size of the particles caused an increase in the radiolucency of the defects⁴¹.

Differently, the results from Jensen et al⁴² showed no differences between two sizes of particles of deproteinized bovine bone mineral (DBBM, Bio-Oss[®], Geistlich Pharma, Wolhusen, Switzerland, a porous bovine-derived bone mineral with osteoconductive properties), when DBBM was used for a bilateral sinus elevation with simultaneous implant placement in minipigs. In the same minipigs, one side received the small particle size (0.25-1 mm) and the other received the bigger particle size (1-2 mm) of DBBM.

Small-size DBBM showed a higher osteoconduction after 6 weeks than large-size DBBM. After 12 weeks, this difference was compensated. Their conclusion was that small and large particle sizes were equally predictable⁴².

From the literature analysis, it can be deduced that homogeneous and 0.5-1 mm granules ensure optimal performance because medium-sized granules are large enough to create sufficient space for vascular growth and avoid reabsorption and small enough to promote a high cell adhesion. The total surface area of granules also affects bone regeneration and it can be guaranteed only by homogeneous granule sizes. High-speed trituration results in granules of different sizes and shapes, while low-speed trituration, performed by TT[®], results in similar granules of similar size and shape^{9,10,28,43}.

Our test showed that the granules produced by the TT[®] mill, despite the teeth differences in size and shape, always result in the range suggested by the literature.

Conclusions

Further studies should be performed to validate the better dimension using dentin granulation in graft procedures. The TT[®] device allows to obtain a demineralized, disinfected material, with a pasty consistency. The results of this study showed that the TT[®] can granulate the teeth creating size granules from 400 μm to 1,000 μm .

Some aspects of the use of this autologous material are still unknown and therefore, further research could lead to considering different sizes determined by different tooth treatments.

Authors' Contributions

Conceptualization, E.M., A.P. (Andrea Palermo), A.P. (Assunta Patano), F.I., G.D., E.D.R., A.M.C. A.M.I. and A.M.; Methodology, E.M., A.P. (Andrea Palermo), F.V., A.D.I., G.D., A.M.I., F.I. and S.S.; Software, E.M., F.V., A.M., and G.D.; Validation, G.M., E.M., F.V., A.D.I., F.I., G.D. and F.I.; Formal analysis, E.M., A.P. (Andrea Palermo), A.P. (Assunta Patano) and A.M.; Writing-original draft preparation, E.M., A.P. (Assunta Patano), F.I., A.D.I., A.M.C., E.D.R., G.M., A.M.I., G.D. and S.S.; Writing-review and editing, A.P. (Andrea Palermo), A.P. (Assunta Patano), E.M., F.I., G.D. and A.M.I.; Visualization, A.M.C., G.M., E.D.R., A.M. and S.S.; Supervision, E.M., G.D. and F.I.; Project administration, E.M., A.P. (Andrea Palermo), A.M.I., G.D. and F.I. All authors have read and agreed to the published version of the manuscript.

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Ethics Approval

Not applicable.

Informed Consent

Not applicable.

Data Availability

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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