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Comparing the effect of using calcified autogenous nano dentin particles versus micro dentin particles in the healing of mandibular bony defects in New Zealand rabbits

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Abstract

Objective This study aimed to compare the regenerative effect of autogenous micro-dentin and nano-dentin particles on bone regeneration in rabbits' mandibular defects. Sixty adult New Zealand rabbits were randomly divided into three groups: a control group, a micro-dentin group, and a nano-dentin group. A critical-sized bony defect was created at the lower border of the mandible. Bone regeneration was evaluated at two, four, and eight weeks using light microscopy, cone beam computed tomography (CBCT) scans, and histomorphometric analysis.

Results Nano-dentin significantly enhanced bone density and defect closure, as evidenced by CBCT and histological analyses. At eight weeks, it promoted extensive new bone formation, nearly bridging the defect, with minimal residual graft material compared to the micro-dentin group. Histomorphometric analysis confirmed its superior osteogenic potential, demonstrating enhanced bone regeneration and graft resorption. These findings highlight nano-dentin as a highly effective biomaterial for mandibular bone repair.

Keywords Bone regeneration, Micro-dentin particles, Nano-dentin particles, Critical size bone defect, Nanotechnology, CBCT, EDX

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Background

Bony defects in the oral and maxillofacial region arise from various etiologic factors, including tooth loss, periodontal disease, trauma, and pathological conditions such as cysts and oral cancer. These defects often result in the loss of both soft and hard tissues, leading to compromised function, aesthetics, and structural integrity of the jawbone [1, 2]. The severity and morphology of these defects vary, ranging from minor alveolar resorption to extensive bone loss affecting surrounding structures' stability [3, 4]. Given these challenges, bone regeneration techniques are essential for restoring lost tissue and reestablishing a functional and aesthetically pleasing dentition [5]. Guided bone regeneration (GBR) [6, 7], bone grafting procedures [5, 8], and the use of biomaterials such as growth factors and scaffolds play a crucial role in stimulating new bone formation and improving treatment outcomes [9, 10].

Bone grafts should meet specific criteria for optimal performance, including an unlimited supply without harming the donor site, biocompatibility without immune rejection, and the ability to promote osteogenesis, osteoinduction, and osteoconduction [11]. Osteoinduction stimulates new bone formation by inducing osteocompetent cells, while osteoconduction supports bone growth along a scaffold. Additionally, grafts should allow rapid revascularization and be entirely replaced by new bone of comparable quality to the host tissue [12, 13].

Autogenous bone has long been regarded as the gold standard for grafting due to its biocompatibility and ability to promote osteoinduction and osteogenesis [5, 14]. It can be sourced from both intraoral locations (such as the chin symphysis and mandibular ramus) and extraoral sites (like the iliac crest and calvaria) [15–17]. However, numerous limitations and potential complications associated with its use. These limitations encompass the necessity for additional surgical interventions, which can lead to further complications, as well as aesthetic and functional impairments at the donor site [18, 19]. Consequently, these challenges have prompted researchers to investigate alternative treatment modalities.

Among these alternatives are allogenic, xenogenic, and alloplastic graft materials. However, these materials have drawbacks, including limited bone formation, increased risks of infectious diseases, prolonged healing times, and elevated costs [20]. An ideal substitute for autogenous bone grafts should effectively replicate its functional capabilities by stabilizing the initial blood clot and providing a biomechanically robust, three-dimensional scaffold that can release growth factors in a temporally and quantitatively appropriate manner. These characteristics facilitate cell migration, proliferation, and differentiation into osteoblasts [21].

Dentin, histologically, emerges as a natural tissue remarkably analogous to bone in developmental, physical, and chemical properties [22]. The composition of dentin mirrors that of bone, consisting of approximately 65% of inorganic material (hydroxyapatite) and 35% of organic material (collagen proteins and bone morphogenetic protein-BMP), making it similar in its three-dimensional scaffold architecture [23, 24]. Both components facilitate osteoconductive and osteoinductive processes [25, 26]. Furthermore, dentin particles contribute to the healing of mandibular bone defects by providing a scaffold and releasing growth factors that support bone regeneration. Dentin contains different combinations of bioactive molecules, including platelet-derived growth factor that enhances bone regeneration [27].

Moreover, dentin contains growth factors and non-collagenous proteins akin to those found in bone, such as insulin-like growth factor II (IGF-II), bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-β), osteopontin, bone sialoproteins, dentin sialoproteins, osterix, and osteocalcin [28, 29]. Notably, dentin lacks the fat content and marrow spaces present in bone, simplifying its fabrication as a graft material [30].

Dentin has been reported as a grafting material in cases of alveolar socket preservation, correction of bone defects, and regeneration of periodontal defects [24, 31]. Nonetheless, undemineralized dentin does not readily induce new bone formation, necessitating partial demineralization as a preparatory step for its use as a graft material. This process, while effective, introduces its own set of challenges [32].

Biobased nanomaterials are naturally derived substances, including chitin, chitosan, starch, gellan gum, hyaluronic acid, hydroxyapatite, and polylactic co-glycolic acid. They are crucial in tissue engineering and are used for wound dressings, bone implants, scaffolds, cell seeding substrates, and skin disease treatments [33]. Nanomaterial research has gained traction in regenerative medicine in recent years, offering innovative possibilities. Nanoparticles can be synthesized from various materials, with multiple fabrication technologies available to produce nanomaterials featuring diverse nanotopographies [34].

This study evaluated the comparative effects of nano-dentin particles versus micro-dentin particles on the healing of mandibular bony defects in New Zealand white male rabbits.

Materials and methods

Animals

The study used sixty adult New Zealand white male rabbits with an average weight of 7–7.5 pounds. The animals were acquired from Tanta University, Egypt, and housed in the central animal facility at Tanta University, Tanta, Egypt. Ethical approval for the protocol was obtained, and the study was conducted following the animal welfare guidelines outlined by the Research Ethics Committee, Faculty of Dentistry, Tanta University # R-OB-11–22-3. Teeth were collected from six rabbits for graft material preparation, while the remaining rabbits were divided into three groups: a control group, a micro-dentin group, and a nano-dentin group, each consisting of eighteen animals.

Dentin powder preparation

Freshly extracted rabbit incisors were utilized to prepare dentin particles. The teeth were washed and cleaned using distilled water and 70% ethanol, followed by removing pulp tissue. Enamel and cementum tissues were removed by grinding the labial and lingual surfaces to isolate pure dentin. The dentin was crushed into small particles, washed multiple times with distilled water, and milled using a fast mill with ethanol as a solvent. The solution was filtered using filter paper, air-dried for 24 h, and sieved to obtain micro-dentin particles with a size of 500 µm. For nano-sized particles, the tooth powder in ethanol solution was milled for an additional 5 h, followed by rotation with a magnetic stirrer for 48 h. The resulting solution was air-dried for 24 h [18].

Sterilization of graft material

The prepared dentin particles were sterilized using gamma radiation at five kGy at the Atomic Energy Authority, Nasr City [25].

Characterization of dentin powder

The particle size and elemental composition, specifically the calcium and phosphate content, were confirmed using a scanning electron microscope (JSM 5200 LV SEM) and energy-dispersive X-ray analysis (EDX).

Surgical procedures

The animals were anesthetized via intramuscular injection of atropine sulfate (0.4 mL/kg), followed by ketamine hydrochloride 10% (0.5 mL/kg) and xylazine 2% (0.2 mL/kg). A submandibular approach was used to access the right lower mandibular border. The surgical site was shaved, disinfected with povidone-iodine, and incised layer by layer with careful hemostasis. The lower mandibular border was exposed, and the periosteum was retracted to create a standardized defect using a surgical bur attached to a high-torque surgical motor under saline irrigation. The defect measured 8 mm in length, 3 mm in width, and 4 mm in depth, just below the inferior alveolar nerve canal. Defects were left untreated in the control group (Fig. 1A), while the micro-dentin and nano-dentin groups received 200 mg of respective graft particles.

Radiographic analysis

Bone density at the defect sites was measured using a SCANORA® 3Dx CBCT unit (Soredex, Helsinki,

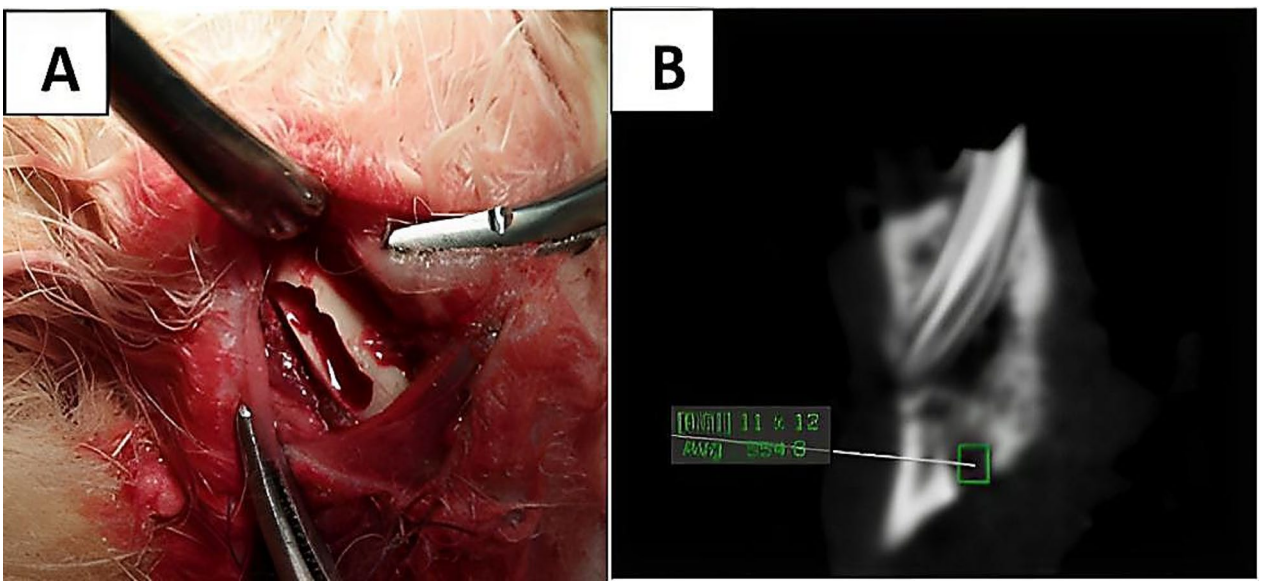

Fig. 1 **A** Showing surgical defect at the lower border of the mandible. **B** Showing CBCT images of group 3 defect at 2nd week

Finland). CBCT images were exported in DICOM format, and SimPlant Pro 13.0 software (Materialise HQ, Leuven, Belgium) was used to calculate bone density in Hounsfield units (HU) (Fig. 1B).

Preparation of tissues

At designated intervals (2-, 4-, and 8-weeks post-implantation), the animals were euthanized via cervical dislocation, and tissue specimens were harvested. Specimens were processed for histological examination using hematoxylin and eosin (H&E) staining.

Histomorphometric analysis of newly formed bone

Prepared slides were examined under a light microscope (Leica DM500 with Leica ICC50 HD Camera system) at 40× magnification. Images of H&E-stained sections were analyzed using ImageJ software (ImageJ 1.48s) to measure the newly formed bone area and graft material remnants. Six sections were selected per group, with approximately 200 µm between sections. For accuracy, the calculated areas were expressed in square micrometers [35].

Statistical analysis

Statistical analyses were conducted using ANOVA, independent t-tests, and Tukey tests. Results were reported as means and standard deviations. The statistical software SPSS 26 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Significance levels were categorized as follows: very highly significant ($p \leq 0.001$), highly significant ($p \leq 0.01$), significant ($p \leq 0.05$), and non-significant ($p > 0.05$).

Results

Characterization of dentin powder

Analysis of scanning electron microscopy (SEM) images revealed that the average particle sizes of the dentin powders were 2.68 ± 0.35 µm and 109.45 ± 11.25 nm for the micro-dentin and nano-dentin particles, respectively (Fig. 2). Energy-dispersive X-ray spectroscopy (SEM–EDX) analysis indicated calcium-to-phosphorus (Ca/P) ratios of 2.08 and 1.87 for the micro-dentin and nano-dentin particles, respectively.

Gross observations

All animals tolerated the surgical procedures well and demonstrated rapid recovery from anesthesia. Slight edema was observed bilaterally at the surgical sites. By the end of the first postoperative week, gross examination revealed evidence of surgical site healing in all animals, with no signs of infection.

CBCT scanning measurements

Cone beam computed tomography (CBCT) scans demonstrated progressive healing and repair of the lower border defects over time. Bone density within the groups increased gradually across the follow-up periods. Notably, there was a significant difference in bone density between the nano-dentin group and the other two groups, as summarized in Tables 1 and 2.

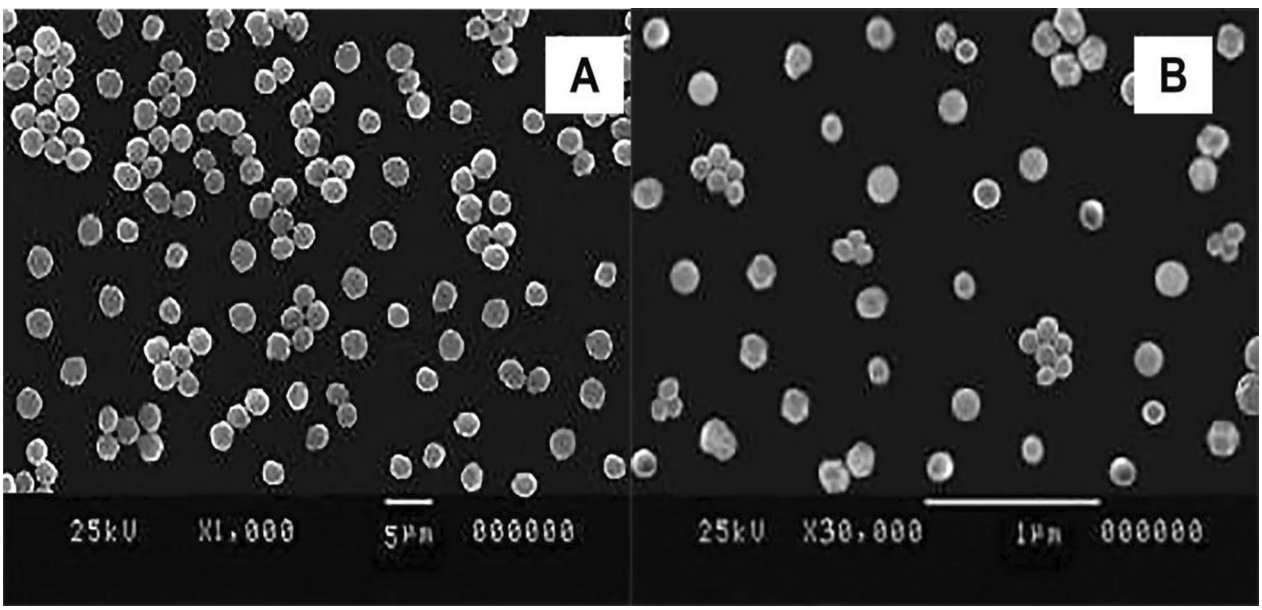

Fig. 2 SEM images of **A** micro-dentin and **B** nano-dentin particles, respectively

Table 1 Analyses newly formed bone density measurement through different durations in all groups

Bone density in Hounsfield Unit scores					
Duration	**Group I (control)**	**Group II (Micro dentin P.)**	**Group III (Nano dentin P.)**	**F**	**P-value**
After two weeks	22.5 ± 9.09	47.66 ± 10.74	68.33 ± 12.27	27.186	0.000***
After 4 weeks	100 ± 29.25	209.66 ± 86.39	363.33 ± 110.75	15.298	0.000***
After 8 weeks	468.33 ± 134.56	815 ± 119.07	1241.66 ± 454.33	11.31446	0.001**

There is a highly significant at P-value < 0.001 (**), and extremely significant at P-value < 0.0001 (***)

Table 2 Multiple comparisons (Tukey test) show the difference between each two groups in all durations to measure newly formed bone density

Bone density within the groups TUKEY TEST			
durations	**G1 vs. G2**	**G1 vs. G3**	**G2 vs. G3**
After two weeks	0.002*	0.000***	0.012**
After4 weeks	0.087	0.000***	0.015**
After 8 weeks	0.117	0.000***	0.047*

There is a significant at P-value < 0.05 (*), highly significant at P-value < 0.001 (**), and extremely significant at P-value < 0.0001 (***)

Light microscopy results

At two weeks postoperatively, light microscopic (LM) examination revealed that the control bone defects were mainly filled with connective tissue and fine bone trabeculae at the defect margins (Fig. 3). The defects in both the nano- and micro-dentin groups were demarcated and primarily filled with highly cellular granulation tissue, graft material, and newly formed bone trabeculae extending from the defect margins toward the center.

The control group showed limited fine woven bone trabeculae formation at four weeks in certain defect areas.

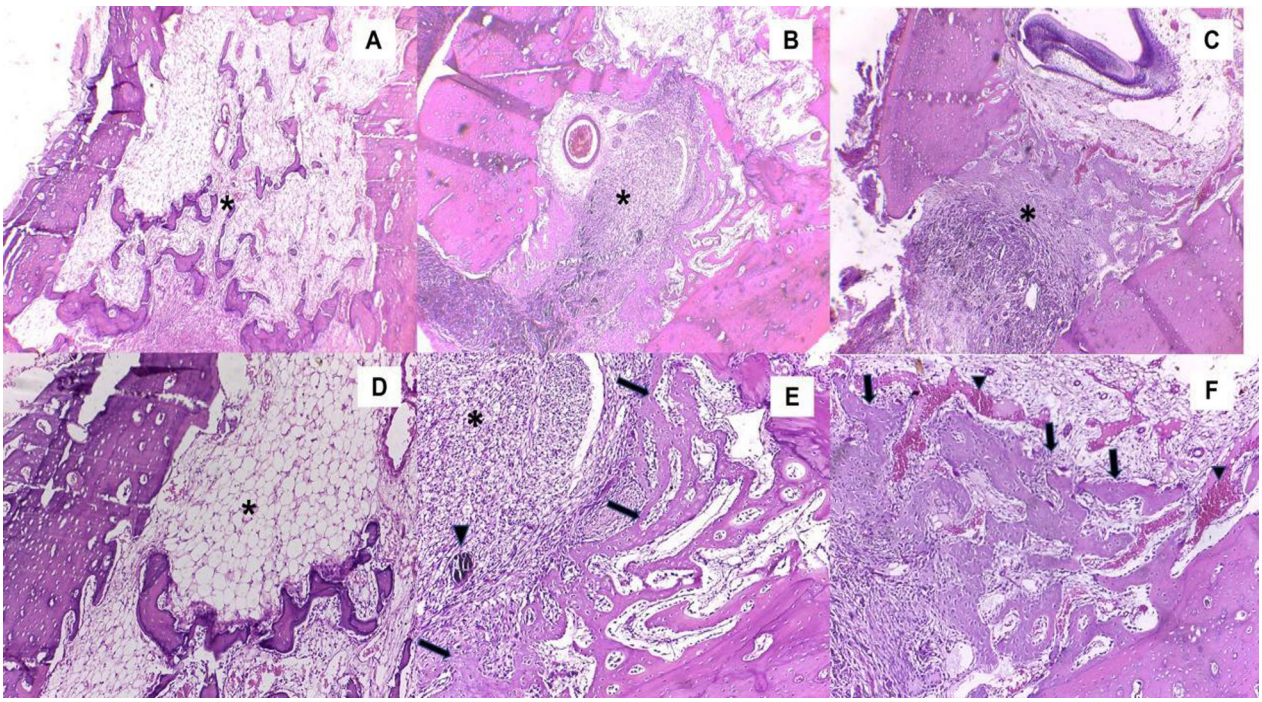

Fig. 3 Microphotograph of the bony defect of group I (control group) (**A**, **D**) at two weeks filled with granulation tissue. No bony tissue is observed at the center of the bony defect (*). Microphotograph of the bony defect of group II (micro-dentin group) (**B**, **E**) and of the bony defect of group III (nano-dentin group) (**C**, **F**) showing graft material (arrow heads) with new bone formation (black arrows) at the defect margins that extends toward the defect center. (H&E, orig. mag., A, B, C × 40 & D, E, F × 100)

The micro-dentin group formed osteoid tissue around resorbed graft material, with new bone observed at the defect center. New bone trabeculae intermingled with the graft material in the nano-dentin group, bridging and effectively closing the defect surface. Resting lines and osteoclasts adjacent to pre-existing bone margins indicated active remodeling (Fig. 4).

At eight weeks, the control group defects remained incompletely filled with bone. The micro-dentin group showed prominent new bone tissue formation, with reversal lines between old and new bone. However, significant graft material remained. In contrast, the nano-dentin group exhibited extensive new bone formation, nearly bridging and filling the defect area. Newly formed bone-enclosed osteocytes lined with active osteoblasts was evident. Residual graft material was present but in significantly smaller quantities than the micro-dentin group (Fig. 5).

Histomorphometric analysis

Histomorphometric analysis quantified new bone formation using mean and standard deviation (SD). Statistically significant differences were observed between groups at each interval and across intervals within each group. However, no significant difference was found between Groups I and II at the two- and eight-week intervals (Tables 3, 4, 5).

Residual graft particles significantly decreased in Group III across all time points, with significant reductions observed between consecutive intervals, except between weeks 4 and 8 in Group II and between weeks 2 and 4 in Group III (Tables 6, 7).

Discussion

Various regenerative bone graft materials have been employed to address skeletal defects. An ideal bone graft material should exhibit osteoinductive and osteoconductive properties to facilitate effective bone regeneration [36]. Bone graft options include xenogeneic bone, allogenic bone, alloplastic materials, and bioactive glass. Degradable bioactive ceramics, such as tricalcium phosphate (TCP) and hydroxyapatite (HA), are commonly utilized [37].

However, calcium phosphate ceramics like TCP are associated with prolonged healing times due to their limited osteoinductive properties [38, 39] and high resorption rates [40]. Consequently, composites of HA and TCP are often developed, as HA is less prone to resorption and exhibits superior osteoinductive properties [41]. Dentin is a promising alternative graft material due to

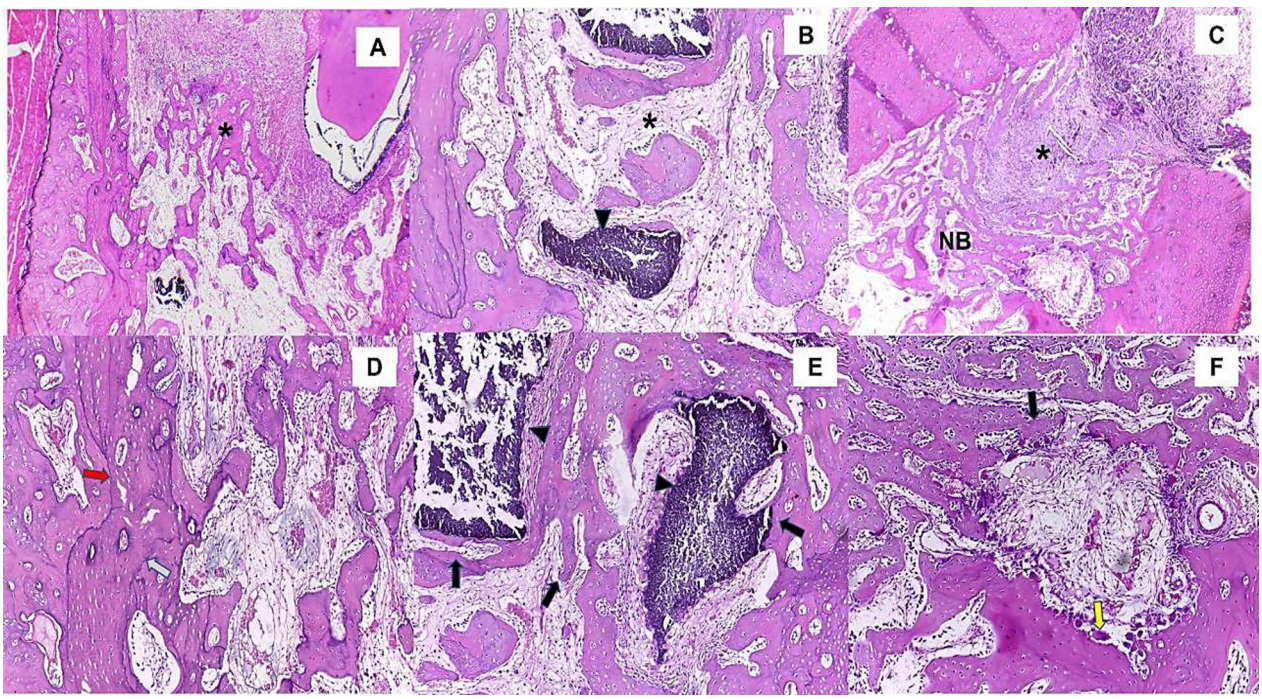

Fig. 4 Microphotograph of the bony defect of group I (control group) at 4 weeks with fine trabeculae of woven bone covering areas of the center of the defect (*) **(A**, **D)**. In group II (micro-dentin) remnants of the graft material (arrow heads) is still evident with new bone trabeculae (black arrows) extending toward the center of the defect (**B**, **E**). In group III (**C**, **F**) the newly formed bone trabeculae form a bridge which closed the defect surface (NB), osteoclasts (yellow arrows) presence indicated remodelling of the old bone surface. Resting lines (red arrows) were evident denoting the successive increments of bone formation and reversal lines (white arrow). (H&E, orig. mag., A, B, C × 40 & D, E, F × 100)

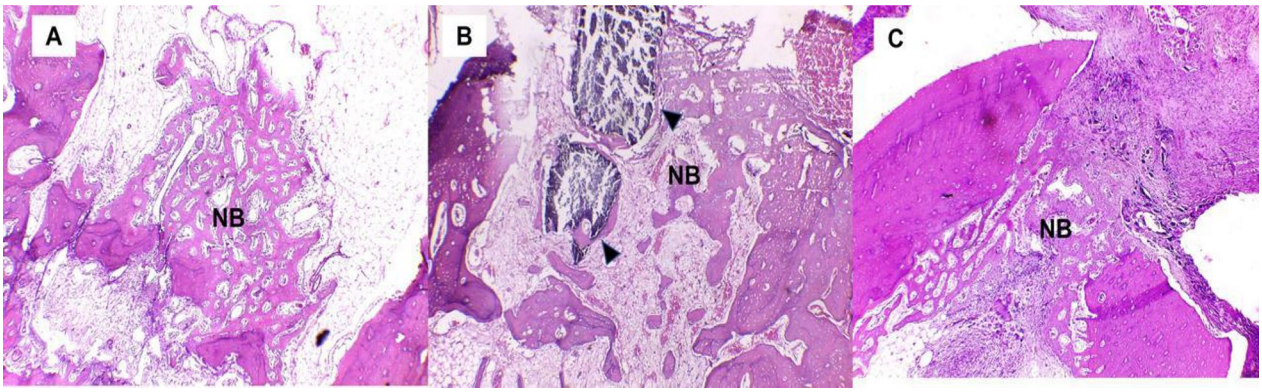

Fig. 5 Microphotograph of the bony defect of group I (control group) at 8 weeks showing no complete filling of the defect area **(A)**. In group II (micro-dentin) remnants of the graft material (arrow heads) is still evident with new bone trabeculae extending toward the center of the defect and around the graft, with evident reversal lines (**B**). In group III (**C**) the newly formed bone is almost filling the defect area, NB; new bone (H&E, orig. mag., A, B, C × 40)

Table 3 Analyses measurement of newly formed bone through different durations in all groups

Newly formed bone using the ImageJ analysis system					
Duration	**Group I (control)**	**Group II (Micro dentin P.)**	**Group III (Nano dentin P.)**	**F**	**p-value**
After two weeks	127.50 ± 24.24	120.33 ± 12.82	223 ± 11.12	67.897	0.000**
After 4 weeks	226.50 ± 20.41	475 ± 18.95	526.33 ± 15.55	454.782	0.000**
After 8 weeks	569 ± 16.19	575.83 ± 15.25	819.83 ± 12.61	562.328	0.000**
p-value	0.000**	0.000**	0.000**	–	

There is a significant at P-value < 0.05 (*), and highly significant at P-value < 0.001 (**)

Table 4 Differences between each type and two groups in all durations for measurement of newly formed bone using the Tukey test

Multiple comparisons (Tukey test)			
Durations	**GI vs. GII**	**GI vs. GIII**	**GII vs. GIII**
After two weeks	0.751	0.000**	0.000**
After 4 weeks	0.000**	0.000**	0.001*
After 8 weeks	0.708	0.000**	0.000**

There is a significant at P-value < 0.05 (*), and highly significant at P-value < 0.001 (**)

Table 5 Differences between each two durations in all groups for measurement of newly formed bone using the Tukey test

Multiple comparisons (Tukey test)			
Groups	**W2 vs. W4**	**W2 vs. W8**	**W4 vs. W8**
Group I (control)	0.002*	0.000**	0.000**
Group II (Micro dentin P.)	0.000**	0.000**	0.000**
Group III (Nano dentin P.)	0.000**	0.000**	0.000**

There is a significant at P-value < 0.05 (*), and highly significant at P-value < 0.001 (**)

Table 6 Analyses measurement of graft particle remnant through different durations in groups II and III

Remnant of graft particles using the ImageJ analysis system				
Duration	**Group II (Micro dentin P.)**	**Group III (Nano dentin P.)**	**t**	**p-value**
After two weeks	358 ± 2.09	31.83 ± 3.19	209.332	0.000**
After 4 weeks	261.50 ± 5.89	28.67 ± 3.01	86.208	0.000**
After 8 weeks	253.50 ± 3.73	23.17 ± 3.73	132.737	0.000**
p-value	0.288	0.001*	–	

There is a significant at P-value < 0.05 (*), and highly significant at P-value < 0.001 (**)

its unique inorganic composition, comprising a natural composite of calcium phosphate phases such as TCP, HA, octacalcium phosphate, dicalcium phosphate dihydrate, and amorphous calcium phosphate. Additionally, dentin's organic matrix predominantly comprises type I collagen and various growth factors, including BMPs, TGF-β, IGF, and IGF2 [42, 43].

Despite these advantages, dentin contains higher mineral and crystalline content than bone, which resists

Table 7 Multiple comparisons (Tukey test) is used to show the difference between each two durations for the remnant of graft particles in groups II and III

Multiple comparisons (Tukey test)			
Groups	**W2 vs. W4**	**W2 vs. W8**	**W4 vs. W8**
Group II (Micro dentin P.)	0.000**	0.000**	0.061
Group III (Nano dentin P.)	0.112	0.001*	0.002*

There is a significant at P-value < 0.05 (*), and highly significant at P-value < 0.001 (**)

osteoclastic degradation. This hinders the release of entrapped growth factors, leading to slow resorption and suboptimal osteoconductivity [44]. Non-decalcified dentin blocks have shown delayed new bone formation, typically between 8–12 weeks post-implantation, necessitating dentin demineralization before use as a graft material. This aligns with findings by Mordenfeld et al. who reported a proportional relationship between the degree of dentin demineralization and the quantity of newly formed bone [45]. However, excessive exposure to decalcifying agents may compromise the bioactivity of growth factors essential for bone regeneration. Interestingly, fine-sized calcified dentin particles have been shown to induce bone formation within two weeks without osteoconductive delays, comparable to a completely demineralized dentin matrix [18].

Nanotechnology has been increasingly employed to enhance the physicochemical properties of scaffolds used in bone regeneration. By reducing material size to the nanoscale, the surface area-to-volume ratio is significantly increased, mimicking the hierarchical nanostructure of native bone tissue. Nanophase ceramics, such as nano-HA, are widely used bone substitutes with a high capacity to induce mineralization [46].

The present study aimed to evaluate the effects of nano-dentin versus micro-dentin particles on the healing of mandibular bony defects in New Zealand white rabbits. Nano-sized particles were chosen to emulate the nanoscale mineral crystals of bone, as their configuration and absorption mechanisms differ from those of larger crystals [47, 48]. SEM analysis confirmed particle sizes of 2.68 ± 0.35 µm and 109.45 ± 11.25 nm for micro- and nano-dentin particles, respectively.

Bone formation in the control group was minimal throughout the study, with incomplete defect healing observed at eight weeks, consistent with prior reports [49]. At two weeks, both micro- and nano-dentin groups demonstrated new bone trabeculae formation, predominantly at defect margins extending toward the center. By four weeks, graft material resorption had occurred, enhancing osteogenic cell proliferation and differentiation due to the release of growth factors from the resorbed dentin matrix [25, 50]. The resorption process also exposed collagen fibers, which served as scaffolds for cellular attachment, further promoting proliferation and differentiation [25]. Additionally, the sustained dissolution of graft materials released calcium and phosphate ions into the defect area, fostering osteogenic differentiation through ATP synthesis [49]. ATP metabolism into adenosine subsequently induced mesenchymal progenitors to differentiate into osteogenic cells via A2b adenosine receptors.

At eight weeks, the presence of reversal lines indicated advanced bone maturation, reflecting an active remodeling process [51]. Both micro- and nano-dentin groups exhibited minimal inflammatory cell infiltration across intervals, consistent with prior study demonstrating the low immunogenicity of dentin as a graft material [52]. The reduced inflammation likely contributed to favorable bone regeneration, scaffold resorption, and remodeling, mimicking a physiological rather than pathological healing process [53].

New bone deposition was observed around the graft material and defect margins in both experimental groups, demonstrating osteoinductive and osteoconductive properties. These findings align with those of Elkady et al. 2023 [35]. Quantitative analysis revealed significantly greater bone formation and density in the nano-dentin group than in the micro-dentin and control groups across all intervals. This enhanced osteoinductivity is attributed to the nano-calcium phosphate composite, which mimics the natural nanoscale of bone crystals. Nano-sized particles promote osteoblast adhesion, proliferation, and differentiation due to their unique surface properties, including increased surface area, phase content, roughness, and energy [54]. Additionally, the adsorption of vitronectin, which enhances osteoblast adhesion, is optimized at surface roughness below 100 nm [55]. The smaller particle size also facilitates cellular uptake via endocytosis, with lysosomal degradation releasing calcium ions that stimulate osteogenic differentiation [56].

Elgendy et al. [57] examined autogenous dentin nanoparticles versus allograft bone grafts in managing stage III periodontitis. Their findings indicated that nano-sized dentin exhibits unique characteristics attributed to its small dimensions and extensive specific surface area. Notably, this form of dentin enhances protein adsorption and promotes osteoblast adhesion. They concluded that autogenous dentin nanoparticles represent an effective and promising option for bone regeneration in intrabony defects.

The micro-dentin group showed insignificant graft remnant reduction over time, delaying decomposition due to the high crystalline content of dentin particles, as Kamal et al. 2017 noted [36]. Conversely, the nano-dentin group exhibited significantly reduced graft remnants due to the higher dissolution rate associated with smaller particle size and lower Ca/P ratio (1.87 vs. 2.08 for nano- and micro-dentin, respectively) [36, 58]. Smaller particles with higher solubility enhance ion exchange with the surrounding medium, accelerating recrystallization on the bone surface [58].

The accelerated degradation of nano-dentin facilitated the rapid release, absorption, and reconstruction of calcium and phosphate ions, promoting bone formation and calcification [59]. Calcium ions activate ERK1/2 and PI3K/Akt pathways, enhancing osteoblast function and lifespan [60, 61]. Furthermore, calcium ions regulate osteoclast activity, and phosphate ions enhance BMP expression, IGF-1 activation, and ERK1/2 signaling, promoting osteogenic differentiation [62]. Phosphate also inhibits osteoclast differentiation via RANK-ligand signaling feedback [63].

Mohammed and Rejeb [64] researched using milled teeth in nanoparticle form as a substitute for bone grafts in rabbits. Histological examinations revealed that the nanoparticle group displayed moderate vascularity and granulation tissue after three days, with new blood vessel formation by the seventh day. By the 14th day, the inflammation had significantly decreased, and by the 28th day, there was notable angiogenesis and moderate development of osteoid tissue. The study concluded that nanoparticles derived from milled teeth promote bone repair.

Limitations

Despite the strengths of this study, several limitations should be acknowledged:

- Animal model: The use of a rabbit model, while providing valuable insights into bone regeneration, may not fully replicate human biological responses, potentially limiting the generalizability of the findings. However, rabbits are widely used in bone regeneration research due to their rapid bone turnover and anatomical suitability for controlled defect studies [65, 66].
- Sterilization process: Gamma radiation was employed to sterilize the graft material, but its potential impact on bioactivity cannot be entirely ruled out. Although previous studies suggest minimal effects on regenerative properties, further investigation is warranted to confirm its influence on clinical applications [67–69].

These limitations should be considered when interpreting the study results and designing future research.

Conclusion

The findings of this study demonstrate that nano-dentin particles effectively address the limitations associated with dentin as a bone graft material, such as low solubility and poor osteoinductivity. The application of nanotechnology offers a promising avenue for developing advanced biomaterials with enhanced efficiency and functionality. These nanostructured biomaterials can respond dynamically to changes in their immediate environment, optimizing bone regeneration. Such advancements have significant potential for improving clinical outcomes of regenerative medicine and bone tissue engineering.

Future research should optimize nano-dentin grafts for better osteogenesis and clinical use. Testing in larger models and enhancing bioactivity with coatings or growth factors will aid translation to human applications. Further studies on immunogenicity and sterilization refinements will improve biocompatibility and effectiveness.

Abbreviations

Ca/P	Calcium-to-Phosphorus Ratio
CBCT	Cone Beam Computed Tomography
DICOM	Digital Imaging and Communications in Medicine
EDX	Energy-Dispersive X-ray Analysis
H&E	Hematoxylin and Eosin
HU	Hounsfield units
LM	Light Microscopy
SEM	Scanning Electron Microscopy
SEM–EDX	Scanning electron microscopy with energy-dispersive X-ray spectroscopy
SPSS	Statistical Package for the Social Sciences

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Not applicable.

Author contributions

Sarah Yasser, Altaib Abd elrazik Mohammed , Samy El-Safty, Hasnaa Fouad and Reda G. Saleh; Conception, Design of the Study and Acquisition of Data. Ahmed Shon , Radhwan Saleh Algabri and Ahmed Yaseen Alqutaibi; Analysis and Interpretation of Data and Drafting the Manuscript.

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Data availability

All data generated or analysed during this study are included in this published article

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Dentistry, Tanta University, (Approval No. R-OB-11–22-3).

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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