RESEARCH ARTICLE

Clinical and radiographic assessment of autogenous dentin nanoparticles in treatment of stage iii periodontitis: a split-mouth clinical study

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Abstract

Objective: To compare autogenous dentin nanoparticles with allograft bone grafts in the treatment of stage III periodontitis.

Method: The randomised study was conducted at the Department of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Kafrelsheikh University Hospital, Egypt, from January 2021 to January 2022, and comprised adult patients of either gender with stage III periodontitis. Each patient's bilateral intrabony defect was randomly treated with an allograft on one side and a graft made of dentin nanoparticles on the test side. Each patient's removed tooth was ground into these nanoparticles. Both groups had their probing pocket depth and clinical attachment loss evaluated at baseline and six months after surgery. Additionally, digital periapical films were collected in both groups at baseline and six months after therapy to assess vertical bone loss. Data was analysed using SPSS 20.

Results: Of the 20 patients, 8(40%) were males and 12(60%) were females with overall mean age 31.00±4.06 years (range: 18-50 years). Of the 40 sites, 20(50%) each were in test and control groups. Compared to baseline values, both groups showed significant improvement in probing pocket depth, clinical attachment loss and vertical bone loss post-intervention ($p<0.05$). There was no significant difference between the postoperative outcomes of the two groups ($p>0.05$).

Conclusion: Autogenous dentin nanoparticles were found to be an effective and promising biomaterial for bone regeneration in intrabony defects.

Clinical Trial: NCT05258006 link: https://clinicaltrials.gov/ct2/show/NCT05258006, Registration date of the Trial 10/2/2022.

Keywords: Bone transplantation, Tuberculin, Autografts, Heterografts, Periodontitis, Nanoparticles, Dentin.

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Introduction

Periodontitis was the sixth most prevalent condition in the world affecting millions of people worldwide in 2017.[1] Periodontal disease-related tooth loss disrupts a person's quality of life, especially when it has an impact on their health, looks, and nutritional condition.[2] Regeneration of structures lost to disease is the ultimate goal of periodontal therapy. Open-flap debridement is just one example of a traditional surgical technique that has limited potential for regeneration. Periodontal regeneration currently uses a variety of substances, including biological modifiers, barrier membranes, and bone replacement grafts.[3]

The main sources of bone grafting are synthetic mineral materials and allogeneic bone. Despite the need to harvest bone and the potential morbidity associated with it, a fresh autogenous bone graft is still regarded as the gold standard because it demonstrates bioactive cell instructive matrix properties, osteoinductivity, osteoconductivity, and osteogenicity, and is non-immunogenic and non-pathogenic.[4] A tooth that is extracted can be easily thrown into the yellow bag, but one must think twice before doing that. Studies have shown that patient-extracted teeth that have been cleaned, ground down, demineralised and sterilised can be a very successful graft to replace alveolar bone deficiencies in the same patient.[5,6]

Dentin and bone share a biochemical makeup, hence, they could both be used as bone graft materials. Like bone, dentin has an equal amount of organic and inorganic materials. Dentin contains 70–75% inorganic content, 20% organic content, and 10% water; the inorganic, organic, and water contents in alveolar bone are 65%, 25%, and 10%, respectively. Type *I* collagen accounts for 90% of the organic contents in dentin and plays a supporting and connecting role during bone formation.[6] The remaining 10% of the organic portion of dentin is composed of non-collagenous proteins (NCPs) that can induce bone formation, such as bone morphogenetic protein (BMP), insulin-like growth factor-*II* (IGF-*II*) and transforming growth factor-beta (TGF-β). In the relationship between non-collagen organic material and jaw bone repair, BMP is a key factor for bone repair.[7]

Nanomaterials are substances with component sizes in at least one dimension ranging from 0 to 100nm. In

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comparison to the parent material, nanomaterials have better characteristics. Nanotechnology enables interactions with cell constituents, influences cell proliferation and differentiation, and regulates the synthesis and arrangement of extracellular matrix.[8]

The current study was planned to examine the use of autogenous dentine nanoparticles as bone graft for the treatment of stage III periodontitis.

Materials and Methods

The randomised study was conducted at the Department of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Kafrelsheikh University Hospital, Egypt, from January 2021 to January 2022. After approval from the institutional ethics review committee, the clinical trial was registered at Clinical Trials.gov (NCT05258006 - Registration date of the Trial 10/2/2022). The sample size was calculated using the equation.[9]:

$$N= \frac{(Z\alpha)^2 * (SD)^2}{(d)^2}$$

with N= total sample size, Z_α = standard normal variant 7.50 at $p<0.05$, SD = standard deviation of the variable; and d = absolute error or precision.

The sample was raised from among adult patients of either gender suffering from stage III periodontitis, which was determined both clinically and radiographically. Each of the enrolled patients had at least two sites; one in each quadrant.

Patients included were those having no contraindications to periodontal surgery and those with good overall health and with the ability to practise proper oral hygiene. Besides, the patients included had at least one tooth with hopeless status. Concerning the chosen site, it had gingival thickness of >1mm and probing depth of around 6mm.

Patients with infectious diseases that were still active, such as hepatitis, tuberculosis, etc., were excluded, and so were those who had used vitamins, antibiotics or anti-inflammatory medications in the preceding 3 months, or often used mouthwash. Also, patients with poor health, smokers, heavy drinkers, sufferers of any systemic condition that affected the periodontium were excluded. In addition, participants in previous clinical trials, postmenopausal and pregnant females were also excluded.

After taking informed consent from the enrolled subjects, phase I therapy was initiated. Appropriate oral hygiene instructions were given to all patients (twice daily brushing for 2min with flossing once per day). In a single visit, both manual and ultrasonic tools were used to do full mouth scaling and root planning (SRP) on each patient. The occlusal adjustment was also carried out if there was obvious evidence of trauma. Study casts were created after alginate imprints of the upper and lower jaws were taken. All patients were assessed one month after receiving phase one therapy to determine their readiness for surgery.

Following the phase I therapy, the sites were randomly assigned using the sealed envelopes technique to two equal groups. Group I received open-flap debridement + allograft bone (Maxgraft, botiss biomaterials GmbH Hauptstr 28, 15806 Zossen, Germany). Group II received open-flap debridement + Autogenous dentin nanoparticles graft.

Patients were clinically assessed by measuring Plaque Index (PI)[10], Gingival Index (GI)[11], Probing Pocket Depth (PPD) and clinical attachment level (CAL)[12] All clinical parameters were recorded at baseline, and at 3 and 6 months post-treatment. Maintenance was also ensured during the follow-up visits.

Digital periapical radiographs were obtained from each patient before treatment and at 6 months after surgery. All radiographs were obtained using the same X-ray machine (de Gotzen Xgenus intraoral X-ray unit). Digital X-ray was done by image plate technology (DÜRR DENTAL Ltd., United Kingdom). At each site in both groups, vertical bone loss (VBL) was calculated from the cementoenamel junction (CEJ) to the base of the bone defect.

Baseline VBL was assessed as the distance from the base of the bone defect to CEJ. Postoperative VBL was measured as the distance from CEJ to the base of the bone development 6 months after surgery, and it was deducted from the baseline reading to measure bone growth (BG).

Immediately after extraction, any crowns, composite, gutta perca, amalgam, carious lesions, and discoloured dentin, or remnants of the periodontal ligament, calculus and enamel was removed by high-speed tungsten carbide burs. Clean teeth, including crown and root dentin, were dried by air syringe and put into the grinding sterile chamber of the dentin grinder. The dentin grinder (MediaTech Blender MT-B16, 400watt, 1.5L) with sharp stainless-steel blades could grind the tooth in 20 seconds with high speed. The grafts thus produced received neither chemical conditioning nor physical conditioning. The particulate dentin was placed in a tiny, sterilised glass container and submerged in saline for 10min (Figure1).

The prepared nano dentin was subjected to the scanning electron microscope (Quattro S FEGSEM, Thermofischer NL, Japan). The morphological investigation of the nano dentine was performed by Field emission scanning electron microscopy (FESEM) imaging. The qualitative analysis of the elemental composition of

nano dentin measured by Scanning Electron Microscopy with Energy Dispersive X-Ray (SEM-EDX) analysis.

The crystalline nature was detected using an X-ray Diffractometer (XRD-6000 Shimadzu) operated at 40kV, and the current was adjusted at 30mA with CuKa1 radiation λ=1.54056Å, and the angle range was 10-80°. The zeta-potential examination[13,14] was performed Zetasizer Nano ZS (model type, Malvern) in a disposable cell at 25°C.

Structural analysis was done over the Fourier transform infrared (FTIR) findings in the range from 4000 to 400cm-1 on a spectrometer (JASCO, Japan). A 2 mg of nanoparticle sample was grounded with 200 mg KBr (optically transparent) and pressed to form pellets and scanned on FTIR spectrophotometer

A local anaesthetic (mepivacaine with 1:100,000 adrenaline) was injected into the sites of surgery. Sulcular incisions were made and full-thickness flaps were elevated. Granulomatous tissue was carefully removed, and root planning was performed. Randomised, allograft bone was placed in one site and autogenous dentin nanoparticles graft was placed in the other site to fill the defect. Flaps were closed using simple interrupted sutures. For 15 days, a periodontal dressing was applied on the recipient site to protect the operation (Figure 1).

Regarding post-operative care**,** doxycycline 100mg was prescribed twice daily on the first day, then 100mg once daily for 10 days. In addition, non-steroidal anti-inflammatory drugs (NSAIDs) were prescribed to control postoperative discomfort (Brufen 600mg, 1 tablet every 8 hours for 48 hours). For 10 days, a soft diet and twice-daily mouthwash with a 0.12% chlorhexidine solution were advised. The patients were instructed not to brush or floss the operated area and to avoid hard and spicy food for 2 weeks. After pack removal 15 days postoperatively, the patients were instructed to clean the surgical sites using a soft brush twice daily. From the first to the third month, every patient was called back for professional prophylaxis, plaque control, and tongue cleaning every 15 days. From the third month until the final assessment, every patient was called back once a month.

Data was analysed using SPSS 20. Intergroup differences were compared using independent sample Student's t-test and paired t-test. P<0.05 was considered statistically significant.

Results

Of the 20 patients, 8(40%) were males and 12(60%) were females with overall mean age 31.00±4.06 years (range: 18-50 years). Of the 40 sites, 20(50%) each were in test and control groups. All the 20(100%) patients finished the study and reported improvement.

Compared to baseline values, both groups should significant improvement in PPD, CAL and VBL post-intervention (p<0.05). There was no significant difference between the postoperative outcomes of the two groups (p>0.05). Compared to baseline values in both groups, there was no significant decrease in the mean PI and GI values at 3- and 6-months post-surgery (Table 1).

There was no significant difference in the mean BG at 6 months between the groups (p>0.05) (Figures 2-3).

The zeta potential value representing the net surface charges on the nano dentine was -16.21mV (Figure 4A). The XRD pattern of nano dentine exhibited diffraction peaks at 2θ values of 25.80°, 31.90°, 39.60°, 46.40°, 49.6° and 64.10°, corresponding to 002, 211, 310, 222, 213 and 420 crystal planes, respectively, (Figure 4B).

FESEM morphological findings were also noted (Figure 4C-D).

Table: Mean values of plaque index (PI), gingival index (GI), probing pocket depth (PPD in mm) clinical attachment loss (CAL in mm), vertical bone loss (VBL in mm) and bone gain (BG in mm) among the study groups at baseline, 3 and 6 months after treatment.

Variable	Time	Group 1	Group 2	*p-value*
PI	Baseline (1)	0.46±0.33	0.52±0.31	0.547
	3 months (2)	0.50±0.25	0.57±0.30	0.405
	6 months (3)	0.61±0.29	0.62±0.29	0.895
p-value		1 vs 2= 0.603	1 vs 2= 0.330	
		1 vs 3= 0.117	1 vs 3= 0.088	
GI	Baseline (1)	0.53±0.32	0.65±0.31	0.277
	3 months (2)	0.51±0.28	0.61±0.31	0.302
	6 months (3)	0.48±0.27	0.62±0.26	0.114
p-value		1 vs 2= 0.330	1 vs 2= 0.666	
		1 vs 3= 0.297	1 vs 3= 0.776	
PPD	Baseline (1)	7.40±0.59	7.5±0.68	0.627
	6 months (2)	3.65±0.56	3.40±0.52	0.156
p-value		1 vs 2 0.000	1 vs 2 0.000	
CAL	Baseline (1)	7.25±0.71	7.35±0.74	0.668
	6 months (2)	2.62±0.53	2.47±0.54	0.387
P-value		1 vs 2 0.000*	1 vs 2 0.000*	
VBL	Baseline (1)	7.10±0.71	7.00±0.56	0.627
	6 months (2)	2.72±0.49	2.65±0.48	0.634
		1 vs 2 0.000	1 vs 2 0.000	
BG	6 months	4.37±0.95	4.35±0.67	0.924

Group I: Open flap debridement + allograft bone (Maxgraft)
Group II: Open flap debridement + Autogenous dentin nanoparticles graft.

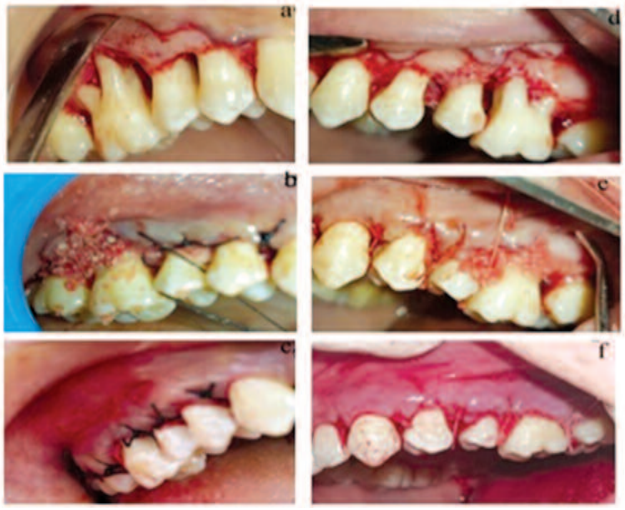

Figure 1: (a) A full thickness flap was elevated, (b) Allograft bone was placed in one site, (c) Simple interrupted sutures, (d) A full thickness flap was elevated, (e) Autogenous dentin nanoparticles graft was placed in one site, (f) Simple interrupted sutures.

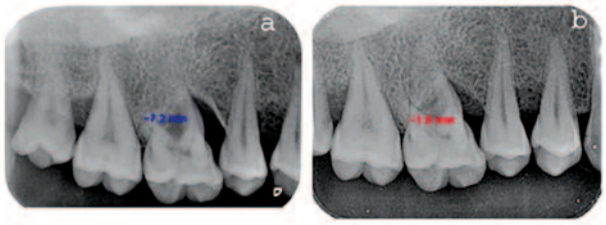

Figure 2: (a) Measurement of vertical bone loss (VBL) at baseline, (b) Measurement of VBL at 6 months post-operative in group I.

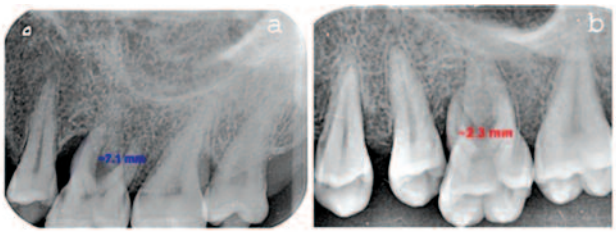

Figure 3: (a) Measurement of vertical bone loss (VBL) at baseline, (b) Measurement of VBL at 6 months post-operative in group II.

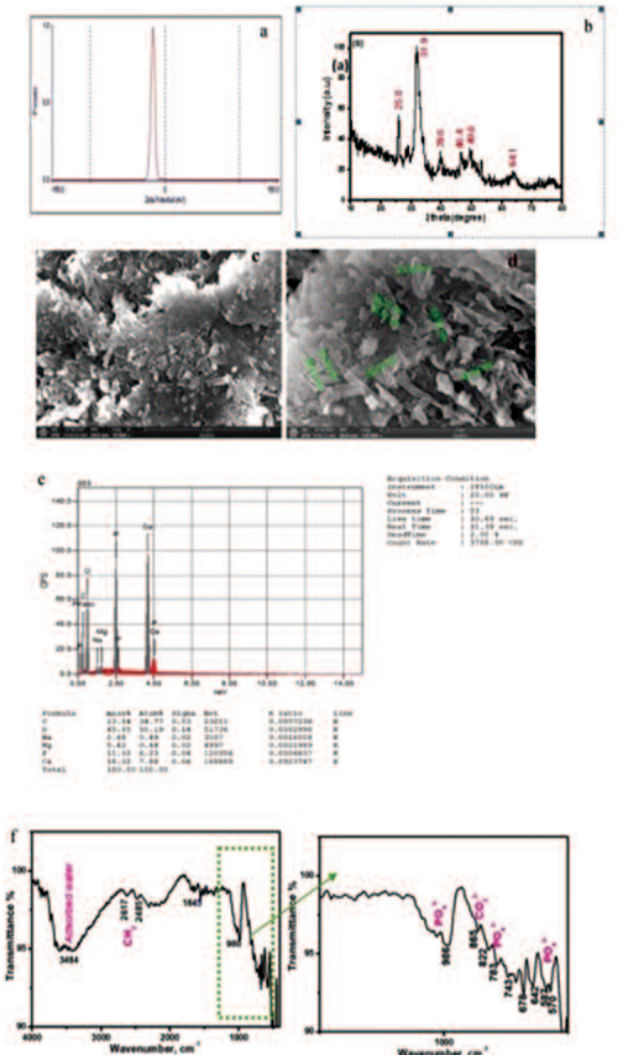

Figure 4: (a) The zeta potential of nano dentine, (b) The X-ray diffractometer (XRD) pattern of the nano dentine. (c, d) Field emission scanning electron microscopy (FESEM) images for the dentine nanoparticles from different spots at different magnification scales, (e), scanning electron microscopy with energy dispersive X-ray (SEM-EDX) analysis, (f) Fourier transform infrared (FTIR) spectrum of nano dentine.

SEM-EDX analysis and the recorded XRD chart demonstrated the presence of several peaks attributed to carbon, oxygen, nitrogen, phosphorous, calcium and traces of sodium and magnesium elements (Figure 4E).

FTIR spectrum showed several peaks centred at 3484cm^{-1}, 2617cm^{-1}, 2485cm^{-1}, 1645cm^{-1}, 980cm^{-1}, 865cm^{-1}, 822cm^{-1}, 783cm^{-1}, 743cm^{-1}, 678cm^{-1}, 642cm^{-1}, 587cm^{-1} and 570cm^{-1} (Figure 4F).

Discussion

The zeta potential findings of the dentin were -16.0mV, suggesting the formation of highly stable dentin nanoparticles. The obtained value for the nano dentine has been reported to be greater than the dentine in the bulk size that measured earlier (-11.0 mV).[15] Of note, the agglomeration is influenced by the surface charges, and as charges increase, the nanoparticle's stability increases while being prevented from aggregation.[16]

The XRD pattern of nano dentine showed the appearance of peaks at diffraction angles and positions which are characteristic of the standard card of dentine. A study[17] confirmed that the presence of two peaks indicates the formation of healthy dentin. Generally, most of the prominent peaks in the XRD pattern resemble those detected

in later reports for bulk dentine, which means that the composition of dentine was not affected by the grinding method that decreased the dentine size into the nanoscale. Furthermore, the sharpness of the main peaks in the XRD pattern indicates dentine's existence in a crystalline phase.[18]

The determined FESEM photomicrographs showed a formation of particles ranging 50-80nm, with rods-like shape that confirmed dentine formation in the nanoscale.

The current EDX data revealed that the nano dentine surfaces' elemental composition was broadly in agreement with previous elemental compositions.[19]

The current FTIR spectrum obtained for the nano dentine was quite similar to the spectrum reported earlier.[18]

Compared to the peaks ranging from 822cm^{-1} to 570cm^{-1} In the current study, Brink,

K et al.[20] reported shoulder peak at 2485cm^{-1}.

The evident significance of regeneration in enhancing therapeutic outcomes has drawn a lot of interest. However, patients are generally hesitant to consent to the use of autografts due to the disadvantages of this graft, including second surgical-site morbidity and the amount of graft material available.[21]

Biomedical waste includes extracted teeth. It is a straightforward and widely accessible bone substitute. In the literature, there are different methods for turning the excised teeth into a bone-augmentation substance, including demineralized dentin, freeze-dried dentin, dentin grinding using an exclusive dentin grinder and chemical disinfecting, grinding with a domestic grinder, using a standard bone mill without chemical conditioning, and crushing without chemical or thermal treatment.[22-30]

Dentin that is nano-sized might have unique features because of its small size and large specific surface area. On the nanosized dentin, protein adsorption and osteoblast adhesion are significantly increased. The current study used a grinder to grind the dentine into various sizes of nanoparticles without subjecting these teeth to any chemical or physical treatment. This was in agreement with Binderman et al.[24]

The present study used bone allografts as graft material. Allografts are frequently utilised in periodontology, but many doctors are not aware of how to prepare and process them or how to use them as secure and reliable graft materials.[31] The current study chose the freeze-dried bone allograft (FDBA) to be compatible with mineralised dentin particulate.

By excluding patient-specific characteristics that might have an impact on the outcomes of regeneration surgeries, the current study was planned as a split-mouth investigation to facilitate the comparison of two treatment options. [31,32] A recent systematic evaluation found that the split-mouth design was suitable for examining regenerative processes. The fact that Wenzel et al.[33] found no evidence of increasing BG between 6 and 12 months may help to explain why the 6-month radiography analysis was used in the current investigation.

The findings of the current investigation revealed no appreciable changes between the tested groups' PI and GI at baseline and throughout the follow-up. The findings indicated that compared to baseline values, the mean PI and GI scores in both the groups did not significantly change over the course of the study. The phase 1 therapy, which included SRP and adequate maintenance of oral hygiene, may be responsible for the reduction of PI and GI in both the groups. The removal of granulation tissue, open-flap debridement (OFD), and oral hygiene training may have contributed to the reduction of PI and GI in both the groups at all evaluation intervals.

The PPD, CAL and VBL results showed that both groups had decreased levels at the end of the 6-month evaluation period compared to the mean baseline values. The improvement in group I's PPD, CAL and VBL is consistent with Camelo et al.[34]

Arabadzhiev et al., who employed crush procedure for teeth grinding, reported improvement in line with the current study's group II.[35]

Schwarz et al.[36] reported that autogenous tooth roots might be used as a substitute graft to support the augmentation of the lateral alveolar ridge and the implantation of two implants at once. Del Canto-Daz et al.[37] found that autologous dentine may be considered a viable material for use in socket preservation procedures. The BG in group II of the current study was in keeping with the finding.

Further studies are required to validate the findings of the current study.

Conclusion

Autologous dentin nanoparticles were found to be safe, easily available, and low-cost solution for the regeneration of intrabody defects. Autologous dentin nanoparticles showed promising results compared to the more expensive commercial materials.

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Conflict of Interest: None.

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