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Abstract

Intramarrow penetration (IMP) is often incorporated in regenerative periodontal surgical procedures. However, the actual benefits of adding IMP to such a procedure remain undocumented. The aim of the current study was to investigate the contribution of intramarrow penetration (IMP) to the outcomes of open flap debridement (OFD) treatment of intrabony defects. Thirty intrabony periodontal defects (ID) in patients of both sexes suffering from moderate to severe chronic periodontitis, each patient had three ID with a probing pocket depth \geq 5 mm and defect depth \geq 3 mm deep. The following clinical parameters; probing pocket depth (PPD), clinical attachment level (CAL), bleeding on probing (BOP), tooth mobility (M) were assessed for each patient at baseline, 3 months and 6 months. Radiographic parameters including bone level gain and density were assessed at baseline and 6 months. Sites were randomly assigned into one of three groups: negative control group (OFD alone), test group (OFD+IMP), positive control group (Nano-crystalline hydroxyapatite granules NcHA). At baseline, there were no statistically significant differences between groups. At 6 months, all groups showed improvements, in terms of clinical and radiographic parameters. The test group showed greater clinical and radiographic improvement compared to the negative control group. Therefore, the addition of IMP to an OFD procedure used to treat ID could result in significant improvement of clinical outcomes in well maintained patient.

Keywords: Intramarrow Penetration; Open Flap Debridement; Intrabony Defects; Chronic Periodontitis

Introduction

Chronic periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth resulting in progressive destruction of periodontal ligament and alveolar bone [1] that lead to damage of the periodontal tissues, formation of intrabony defects (ID) and subsequently tooth loss [1,2].

Treatment includes reducing the bacterial load on the tooth by mechanical debridement of supra and sub gingival calculus (scaling and root planning), pharmacologic treatment (local and\or systemic) when needed [3].

However deep ID is considered a major challenge for the clinician due to the high risk of disease progression so that periodontal therapy needs for additional surgical treatment to eliminate and deride these defects to create optimal conditions for new attachment [3-5].

Open flap debridement (OFD) was among the earliest procedures used to achieve ideal biological conditions for treating ID providing high tooth retention and improvement of periodontal clinical parameters [6,7].

Bone grafting materials provide a structural framework for clot development, maturation and remodeling that supports bone regeneration In general, they can be categorized into autogenous, allografts, xenograft and alloplastic synthetic materials such as hydroxyapatite products and tricalcium phosphate [8].

Nanocrystalline hydroxy-apatite bone graft (NcHA) combines the advantages of hydroxyapatite and the nano-material. Hydroxy-apatite as a biocompatible and osteoconductive ,nontoxic, non-immunogenic material that has a chemical and structural similarity to the mineral component of bone aids in forming strong chemical bond with host bone tissue [9].

Moreover, the nanomaterials have significant surface, size, quantum effects and self-assembly properties so that they exhibit much better performance properties. Also, they are resorbed completely within 3 months and integrated with host bone and have a powerful effect on re-organization of periodontal ligament and acceleration of periodontal healing through fibroblast proliferation and collagen formation [10].

Intramarrow penetration (IMP) or intentional drilling of holes through the cortical bone into the cancellous bone or the removal of cortical bone to expose cancellous bone is often used in many regenerative periodontal surgical procedures, the biologic rationale for decortication of bone is to induce bleeding that immerses the defect in blood, as the clot organizes, it releases cytokines and growth factors to attract progenitor cells, osteoblasts, and blood vessels, also provide passageways for blood vessels and progenitor cells to have rapid access to the treated site for healing enhancing revascularization and new bone regeneration [11-13].

Therefore, the purpose of this study was to investigate the contribution of IMP to the clinical and radiographic outcomes of OFD procedure in treatment of ID and determining the effectiveness of IMP as a treatment protocol when regenerative treatment is not feasible.

Materials and Methods

The present study was explained to the patients and informed consents were obtained according to the guidelines on human research published by the research Ethics Committee at Faculty of Dentistry, Tanta University.

Thirty intrabony periodontal defects in \geq 28 years old patients of both sexes suffering from moderate to severe chronic periodontitis will be selected without stratification by tooth type or location from the Periodontology Clinic, Faculty of Dentistry, Tanta University.

Patients were selected according to following criteria

- 1. Each patient had three intrabony defects with a probing pocket depth \ge 5mm, ID \ge 3mm deep
- 2. Absence of relevant medical conditions that contraindicate periodontal surgery.
- 3. Optimal compliance as evidenced by no missed treatment appointments and a positive attitude towards oral hygiene.
- 4. Materials: Nano-crystalline hydroxyapatite granules¹

Study design: (Table 1)

Clinical assessment	Baseline	One month	3 months	6 months
Types of assessments				
PD*, CAL∞& BOP•	+		+	+
panoramic X-ray	+			+
Phase I therapy	+			
Re-evaluation		+		
SPT#	Recall appointments were scheduled			
	2 weeks after surgery, and once a			
	month for the remaining observation			
	period			

Table 1: The study design for patients.

*PD: Probing Depth; ∞CAL: Clinical Attachment Level; •BOP: Bleeding on Probing; # SPT: Supportive Periodontal Treatment

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Clinical assessment

The following parameters were measured at baseline, 3 and 6 months following surgery.

- Probing pocket depth [PPD] [14]
- Clinical attachment level [CAL] [14]
 - Both clinical parameters were measured by UNC-15 periodontal probe from a fixed point to the deepest probing depth; the periodontal probe was inserted into the crevicular space at the angle necessary to reach the deepest portion.
- Bleeding on probing [BOP] [15]
 - The bleeding at each site was observed after probing. It was recorded positive if bleeding is observed within 30 seconds, if not it was recorded as negative.
- Tooth mobility [M]
 - The tooth is held firmly between the handles of two metallic instruments.

Radiographical assessment

1. Standardized digital panoramic radiographs were taken at baseline (Figure 1) and 6 months post therapy, using Orthophos XG DS\Ceph, Sirona^{**} to measure alveolar bone level using SIDEXIS software delivered with the x-ray machine (Figure 2).



Figure 1: Showing the preoperative radiograph for the selected area.

**Orthophos XG DS\ Ceph, Sirona (Software: SIDEXIS)

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Figure 2: Showing bone level measurement by SIDEXIS program.

2. Bone density was assessed at base line and 6 months (Figure 3 and 4) by a specially developed Visual Basic program instead of the SIDEXIS program which measures linear density and not area density, the software contained four buttons named according to their functions. The "Load" button was assigned to upload the images to the computer memory and to display them on the screen, the "Select" button was assigned to select the area of interest at which bone density will be measured, the "Gray" button was assigned to measure and display the mean gray level of the selected area and the "Exit" button was assigned to terminate the program and to remove it from the computer memory [16,17] (Figure 5).



Figure 3: Showing preoperative measurement of the mean gray level using the Visual Basic program.

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Figure 4: Showing postoperative measurement of the mean gray level using the Visual Basic program.



Figure 5: Showing the Visual Basic program used to measure the mean area gray level.

Phase I therapy

Scaling and root planning were carried out for all patients as well as comprehensive oral hygiene instructions. Re-evaluation was conducted after one month.

Surgical procedure

All surgical procedures were performed by the same operator. Prior to surgery, patients were instructed to rinse with 0.1% chlorohexidine gluconate² for 30 seconds.

After administration of local anesthesia buccal and lingual or palatal intrasulcular incisions were performed, and mucoperiosteal flaps on the facial and lingual or palatal aspects of each tooth, segment, or area involved were reflected. Incisions were designed to preserve as much of the interproximal tissue as possible. Soft tissue debridement was performed as well as thorough scaling and root planning of the exposed root surfaces.

The defects were randomly treated as follow

- Group I defects were treated by OFD only.
- **Group II** was treated by OFD and IMP as follows, the intrabony defects cortical walls were penetrated using a round carbide bur (1mm diameter) to reach marrow spaces, multiple perforations were done not closer than 1 mm from each and deep enough to obtain bleeding from spongiosa.
- Group III was treated by OFD and NcHA granules packed in the ID.

Flap closure was accomplished using 4-0 silk sutures and a periodontal dressing was placed.



Figure 6: A. Showing group I (open flap debridement between lower right 1st and 2nd premolars).
B. Showing group II (intermarrow penetration between lower left 2nd premolar and first molar) and group III (NCHA granules between lower first and second molar).

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Postoperative care

All subjects received postoperative instructions, including rinsing with 0.1% chlorohexidine (twice daily for 2 weeks), a combination antibiotic therapy, Amoxicillin/clavulanic acid 375 mg tabs and Metronidazole 250 mg tabs both 3/day, and anti-inflammatory systemic medications for 1 week were administered. Sutures removal will be performed after 14days. Supportive periodontal therapy were performed monthly which includes, periodontal evaluation (except for periodontal pocket depth which will be measured beginning 3 months post-operative), reinforcement of plaque control, scaling and root planning were carried out if needed and medical history was updated.

Statistical analysis

The collected data were organized, tabulated and statistically analysed using computer software Statistical Package for Social Science (SPSS version 16), comparison between the studied groups was performed using student t-test.

Results

All patients were followed up over the entire 6 months study period showing good healing with no observable complications. The baseline measurements showed no statistically significant differences among the different treatment modalities for any of the measured clinical and radiological parameters as evident by their mean baseline values (P > 0.05).

Intergroup comparison for the mean difference of PPD, BOP, and M between baseline and 3 months also between baseline and 6 months showed no statistically significant difference between all groups at the 5% level of significance. Regarding to the mean difference of CAL between baseline and 3 months, results showed no statistically significant difference between all groups. On the other hand, the mean difference of CAL between baseline and 6 months showed no statistically significant difference between group II and group III; however a statistically significant difference was noted between group I and group II, also between group I and group III at the 5 % level of significance (Table 2).

Parameter	Group (Mean ± SD)		t-value	P-value
PPD at 3 months	GI (3 ± 1.24)	G II (3.6 ± 1.57)	0.943	p-value > 0.05
	GI (3 ± 1.24)	G III (3.4 ± 1.35)	0.688	p-value > 0.05
	G II (3.6 ± 1.57)	G III (3.4 ± 1.35)	0.305	p-value > 0.05
PPD at 6 months	GI (3.3 ± 0.82)	G II (3.7 ± 1.4)	0.77	p-value > 0.05
	GI (3.3 ± 0.82)	G III (3.4 ± 1.43)	0.19	p-value > 0.05
	G II (3.7 ± 1.4)	G III (3.4 ± 1.43)	0.47	p-value > 0.05
CAL at 3 months	GI (1.3 ± 2)	G II (2.7 ± 1.4)	1.772	p-value > 0.05
	GI (1.3 ± 2)	G III (2.8 ± 1.5)	1.874	p-value > 0.05
	G II (2.7 ± 1.4)	G III (2.8 ± 1.5)	0.147	p-value > 0.05
CAL at 6 months	GI (1.6 ± 1.71)	G II (3.6 ± 1.57)	2.716*	p-value < 0.05
	GI (1.6 ± 1.71)	G III (3.5 ± 1.26)	2.818*	p-value < 0.05
	G II (3.6 ± 1.57)	G III (3.5 ± 1.26)	0.156	p-value > 0.05
BOP at 3 months	GI (2.2 ± 0.78)	G II (2.2 ± 0.78)	0.00	p-value > 0.05
	GI (2.2 ± 0.78)	G III (2.2 ± 0.78)	0.00	p-value > 0.05
	G II (2.2 ± 0.78)	G III (2.2 ± 0.78)	0.00	p-value > 0.05
BOP at 6 months	GI (2.8 ± 1.03)	G II (2.8 ± 1.03)	0.00	p-value > 0.05
	GI (2.8 ± 1.03)	G III (2.8 ± 1.03)	0.00	p-value > 0.05
	G II (2.8 ± 1.03)	G III (2.8 ± 1.03)		p-value > 0.05
Mobility at 3 months	GI (0.9 ± 0.3)	G II (1 ± 0.0)	1.00	p-value > 0.05
	GI (0.9 ± 0.3)	G III (1 ± 0.0)	1.00	p-value > 0.05
	G II (1 ± 0.0)	G III (1 ± 0.0)	0.00	p-value > 0.05
Mobility at 6 months	GI (1.2 ± 0.42)	G II (1.3 ± 0.48)	0.493	p-value > 0.05
	GI (1.2 ± 0.42)	G III (1.4 ± 0.51)	0.948	p-value > 0.05
	G II (1.3 ± 0.48)	G III (1.4 ± 0.51)	0.447	p-value > 0.05

Table 2: Shows the mean difference in the clinical parameters (PPD, CAL, BOP and M) ± SDfor the study group (baseline, 3 months and 6 months).

*Statistically significant at the 5% level of significance.

SD: Standard Deviation

Due to variability of the alveolar bone density in the anterior region, middle and posterior regions of mandible and maxilla, the difference between baseline and 6 months results were taken for comparison. Regarding to bone density, the mean difference of grey level gain between baseline and 6 months showed statistically significant difference between group I and Group II, also statistically significant difference between group I and Group III but statistically non-significant difference between group II and Group III at the 5 % level of significance. The mean difference of bone level gain between baseline and 6 months showed no statistically significant difference between all groups at the 5 % level of significance (Table 3).

Parameter	Group (Mean ± SD)		t-value	P-value
Bone density at 6 months	GI (5.4 ± 2.63)	G II (13.6 ± 2.79)	6.75*	p-value < 0.05
	GI (5.4 ± 2.63)	G III (22.2 ± 15.9)	3.28*	p-value < 0.05
	G II (13.6 ± 2.79	G III (22.2 ± 15.9)	1.68	p-value > 0.05
Bone level at 6 months	GI (0.9 ± 1.09)	G II (13.6 ± 2.79)	2.06	p-value > 0.05
	GI (0.9 ± 1.09)	G III (1.6 ± 1.11)	1.421	p-value > 0.05
	G II (2.06 ± 1.4)	G III (1.6 ± 1.11)	0.806	p-value > 0.05

Table 3: Shows the mean difference of bone density and the mean difference of the bone level ±SD for the study group (baseline and 6months).*Statistically significant at the 5% level of significance.

SD: Standard Deviation

Discussion

To the best of our knowledge, up till this point of time the current human study was the second to investigate whether it is possible to replace the synthetic bone graft with other treatment options such as intramarrow penetration in the treatment of intrabony defects in sever chronic periodontitis.

Intrabony defects (ID) associated with periodontal pockets represent the anatomic sequelae of the apical spread of plaque in the course of periodontitis; these defects are risk factors for periodontitis progression and additional loss of attachment if left untreated. This representing a major challenge for the clinician to treat such defects [5,18].

Multiple surgical approaches for treating intrabony defects have shown effectiveness in improving clinical and radiographic parameters, such as clinical attachment level and defect depth. However, there are associated drawbacks and limitations to their use and availability, and even controversial reports about their efficacy and cost-effectiveness. Furthermore, at present there are no heterologous or synthetic bone substitutes available that have superior or even have the same biological or mechanical properties compared with natural bone. Therefore, there is a necessity to develop a novel treatments as alternatives or adjuncts to the standard methods used for bone regeneration, in an effort to overcome these limitations, which has been a goal for many decades [19].

Conventional surgical approaches such as open flab debridement provide surgical elimination of pockets and direct access to root surfaces and bony walls to create optimal conditions for periodontal regeneration by formation of new cementum, periodontal fibers and alveolar bone [4,5]. While, intramarrow penetration was used as a treatment modality as it is considered a simple and effective osteogenic procedure to enhance the healing process by promoting bleeding and allow progenitor cells and blood vessels to reach treatment area enhancing revascularization and new bone regeneration [12].

Moreover, nano-crystalline hydroxy-apatite (NcHA) bone graft was selected as it is considered a novel biocompatible bone graft that combines the nano-crystalline structure and the hydroxyapatite composition that resembles structurally and chemically the natural bone structure favoring the osteoconductive capacity, also biodegraded by osteoclasts the same as the natural bone remodeling process [20].

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Standardized digital panoramic x-ray was used in this study for assessment of bone level and bone density in view of time efficiency, greater patient tolerance and lower radiation exposure by 80%. Also, the ability to manipulate the panoramic images, such as zooming in and changing contrast, has proved a very useful feature in assessment of the shape, depth and extent of bony defects and root morphologies when compared to viewing the periapical or the conventional panoramic oral radiographs [10,21].

Regarding group II, Our results agree with De Carvalho., *et al.* (2000) [22] who studied the influence of bed preparation on the incorporation of autogenous bone grafts in dogs mandible using three different types of receptor bed: cortical, perforated, and decorticated. Histological results showed that the autogenous bone grafts were integrated with the receptor bed, mainly in the perforated and decorticated groups. The poorest results were found in the cortical group. These results could be attributed to initiation of local tissue repair and release of osteoprogenitor cells (signaling and angiogenesis) and osteoinductive agents as vascularity peaked after trauma to the bone tissues which followed by high tissue turnover that are functionally normal [23].

Moreover, Saudi., *et al.* (2000) [13] conducted a split mouth study to evaluate the use of bioglass bone graft in the treatment of ID with bone holes and without bone holes clinically by means of PPD, CAL and mobility, also radiographically by quantitative subtraction and gray level in digitalized standard periapical radiographs using a specially designed visual basic program. Results showed that there was no significant difference between the two groups regarding to clinical parameters, however radiographic results showed significant more bone gain and higher density in holes associated bioglass graft site, such results agree with our study.

The positive influence of the IMP to initiate local tissue repair and enhancing new bone formation in the present research agree with those of Min., *et al.* (2007) [24] and Issa., *et al.* (2008) [25] who evaluated the effect of IMP histologically and showed significantly increased bone neogenesis and higher percent area of mineralized bone in the test group. Therefore, decortication surgical technique was able to optimize the osteoinduction properties independently of the material carrier used and the period of time.

Recently, Crea A., *et al.* (2014) [26] evaluated the addition of IMP to an OFD procedure used to treat intrabony defects in patients with moderate to severe chronic periodontitis clinically in terms of (PPD, CAL and BOP) and radiographically measuring (defect depth and width). Results showed significant enhancement of both clinical and radiographic outcomes in the test group, that were agree with our results.

On the other view, our results disagree with those of Lundrgen., *et al.* (2000) [11] who evaluated the influence of decortication of the donor bone on guided bone augmentation in rabbits. The authors revealed that decortication of the donor bone did not result in more bone formation beyond the skeletal envelop or increasing density than the control group, this may be explained by the usage of animal model compared to humans in our study also the different design of the titanium cylinders used in their research compared to the animal studies mentioned before.

Results of group III were in agreement with those of Heinz., *et al.* (2010) [27] who studied clinical effects of NcHA paste in the treatment of intrabony periodontal defects, the authors had measured PPD and alveolar bone level gain using long cone paralling intra oral radiograph. Results showed significantly improved PPD reduction and BL gain in the test group.

Similarly we are agree with Pietruska, *et al.* (2012) [28] and Al Machot., *et al.* (2014) [29] who showed improvement in all clinical and radiographic parameters in the test group when compared the nano-crystalline hydroxyapatite with open flap debridement or enamel matrix derivatives (Emdogain) respectively in the treatment of periodontal intrabony defects.

Additionally, Our results considering PPD, CAL and grey level agree with those of Elgendy., *et al.* (2015) [30] who evaluated the use of NcHA bone graft with or without platelet rich fibrin in the treatment of intrabony defects. The gray levels were carried out using the computer graphic software Adobe Photoshop on periapical dental radiographs taken by long cone paralleling technique showed significant improvement of NcHA group over baseline values.

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Conclusion

In conclusion, all treatment modalities improved clinical and radiographic outcomes however our findings strongly suggest that NcHA and IMP provide more clinical and radiographic improvement that OFD. Therefore IMP alone can be used as a promising treatment protocol in the surgical treatment of intrabony defects as it adds minimal time and cost-effective protocol. Additional studies with a longer time course evaluation and volumetric assessment of the newly formed bone following this treatment protocol by 3D radiographic analysis are needed.

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